Safety and Efficacy of Chlorthalidone to Reduce Urinary Calcium Excretion in Adolescents with Type 1 Diabetes

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1. PURPOSE OF THE STUDY AND BACKGROUND

1.1. Purpose of the study

The purpose of this single-center, open-label study is to gather short-term safety and efficacy data on the use of chlorthalidone (long acting thiazide-like diuretic) to reduce urinary calcium excretion in adolescents and young adults with type 1 diabetes (T1D) complicated by hypercalciuria. The objectives are:

Objective 1: To examine the safety and tolerability of chlorthalidone in adolescents and young adults with T1D complicated by hypercalciuria.

Objective 2: To examine the effect of chlorthalidone to reduce urinary calcium excretion in adolescents and young adults with T1D complicated by hypercalciuria.

Safety outcomes:
1. Changes from baseline in laboratory tests (serum calcium, serum potassium, serum fructosamine)
2. Changes from baseline in clinical measures (blood pressure, insulin dose)
3. Occurrence of serious adverse events
4. Occurrence of non-serious adverse events

Efficacy outcome:
1. Change from baseline in 24-hour urine calcium excretion

1.2. Background

T1D is a chronic, incurable disease characterized by absolute insulin deficiency and hyperglycemia.[1] Low bone mineral density and increased fracture risk are known complications of T1D, however the underlying mechanism is not understood.[2-7] Hypercalciuria (excess urinary calcium excretion) is a known risk fracture for low bone mineral density and fracture risk in other conditions,[8-13] and has been reported to occur with increased frequency in T1D.[14-20] Adolescence is the critical period of bone accrual, with up to 1/3 of lifetime bone mineral accrual occurring during the few years surrounding the pubertal growth spurt.[21] The majority of patients with T1D are diagnosed in childhood[22] and will therefore be exposed to the potentially deleterious effect of hyperglycemia, insulin deficiency and hypercalciuria during the critical years for skeletal development.

Hypercalciuria may be a modifiable contributor to impaired bone accrual in T1D, as treatment options exist in the form of thiazide and thiazide-like diuretics, which can be used to reduce urinary calcium excretion.[23-32] These agents stimulate renal calcium reabsorption in the distal tubule through their action to inhibit sodium and chloride transport via the NCCT and possibly in the proximal tubule due to volume contraction.[33] The rationale for a trial of

thiazides in the T1D population is further supported by evidence for increased abundance of the thiazide-responsive NCCT in T1D rodents.[34, 35] A finding that thiazides safely reduce urinary calcium excretion in T1D would have important implications for further research. Thiazides have been associated with decreased risk of hip fracture in observational studies in adults[36, 37] and were shown to preserve BMD in an RCT.[38] There is currently no standard approach to the prevention or treatment of T1D related bone disease and current therapies may be inadequate. Improved glycemic control is difficult to achieve and may not be sufficient to normalize urinary calcium excretion. T1D rodents treated with insulin showed normalization of intestinal absorption but not urinary calcium excretion. [39-41] A study in humans found that 50% of T1D participants continued to have excess urinary calcium despite decreases in blood glucose. [14]

Chlorthalidone, a thiazide-like diuretic, is most appealing drug in this therapeutic class for clinical use because it has a long half-life and can be dosed once daily (as opposed to hydrochlorothiazide, which requires multiple daily doses). Chlorthalidone is commonly used off-label to treat idiopathic hypercalciuria and nephrolithiasis in both adult and pediatric populations,[42, 43] but to our knowledge, its safety and efficacy have not been specifically studied in the T1D population.

2. STUDY DESIGN

2.1. Overview

The study design is a single-center, investigator-initiated, open-label pilot study designed to evaluate short-term safety, efficacy, and feasibility for the use of chlorthalidone to reduce urinary calcium excretion in adolescents and young adults with T1D. Adolescents and young adults with T1D and hypercalciuria will be identified from the patient population served by the Division of Pediatric Endocrinology at URMC. Hypercalciuria will be defined as 24-hourr urine calcium excretion $\geq$ 4 mg/kg/day. Eligible subjects who consent to participate in the 4-week study will be started on oral chlorthalidone tablets with follow up study visits at 1-,2-,and 4-weeks. Safety outcomes will include changes in laboratory values including calcium, potassium, and fructosamine (to assess glycemic control); clinical outcomes including blood pressure (assessed by sphygmomanometer at study visits) and insulin dose (assessed by subject interview), and frequency of adverse events. The efficacy outcome with be change in 24-hr urine calcium excretion from baseline to 4 weeks. The protocol will allow for adjustment of chlorthalidone dose based upon interim safety and efficacy outcomes and addition of potassium chloride (in the form of Klor-Con extended release tablets) to treat hypokalemia, as needed. There will be no control group. The FDA reviewed this study and determined that chlorthalidone and Klor-Con were exempt from IND regulations on 2/21/2017 (IND # 134281)

2.2. Rationale for Study Design

The study was designed to evaluate short term safety, efficacy, and feasibility for the use of chlorthalidone in adolescents and young adults with T1D and hypercalciuria. The safety and efficacy of chlorthalidone to reduce urinary calcium excretion in the T1D population has not been previously reported. The results of this study will allow us to determine whether to proceed with a larger and more costly randomized, placebo-controlled trial designed to determine if chlorthalidone can be used to reduce urine calcium excretion and improve bone mineral content accrual in T1D participants. Limitations of an open-label study include possibility of investigator and participant bias influencing results. Limitations of an uncontrolled study include greater uncertainty as to whether observed outcomes were attributable to the intervention. The findings of this study will therefore serve to guide future research, in keeping with the study objective.

The primary safety outcome measures are serum calcium (to monitor for hypercalcemia),

serum potassium (to monitor for hypokalemia), serum fructosamine (to monitor for an effect on glycemic control), blood pressure (to monitor for hypotension) and insulin requirement (to monitor for an effect on glycemic control). Safety outcomes were chosen from relevant possible adverse events listed in the package insert for chlorthalidone and from safety outcomes reported in previous studies that evaluated the effect of chlorthalidone on urinary calcium excretion, bone accrual, or kidney stones. [25, 26, 44, 45] We hypothesize that the side effect profile of chlorthalidone will be acceptable and comparable to that previously reported for chlorthalidone in children and adults. The primary efficacy outcome is 24-hour urine calcium excretion. We hypothesize that chlorthalidone will be effective in reducing 24-hour urine calcium over the study period.

2.3. Rationale for Dosage

Chlorthalidone will be given orally as a tablet at a starting dose of 12.5 mg/d, with a plan to increase by 12.5 mg/d to a max of 50 mg/day at weeks 1-3 if hypercalciuria (defined as spot urine Ca/Cr >0.21) persists. A previous study in children with hypercalciuria reported safety and efficacy with a chlorthalidone dose of 25 mg/d;[26] studies in adults have reported safety and efficacy with doses ranging from 25 to 50 mg/d.[25, 43-45] We are proposing to start with a lower starting dose to allow for early monitoring for adverse events. Thiazide diuretics have been shown to rapidly reduce urine calcium excretion within three days after starting therapy,[23] therefore we will not compromise our ability to detect an effect of chlorthalidone by starting at a potentially sub-therapeutic dose.

Klor-Con (extended release potassium chloride) tablets will be started orally at a dose of 1 mEg/kg/day up to a max starting dose of 20 mEq/day as needed in participants who develop hypokalemia (serum potassium <3.5 mmol/L) and increased by up 1 mEq/kg/day (up to 20 mEq/day) weekly as needed for persistent hypokalemia (<3.5 mmol/L). Doses > 20 mEq/day will be divided so that no single dose will exceed 20 mEq. Doses were determined from prescribing guidelines for adults provided in the package insert and from recommended prescribing information for the use of potassium chloride in children provided in the URMC formulary/Lexicomp.

3. CHARACTERISTICS OF THE RESEARCH POPULATION

3.1. Subject Characteristics

a) **Number of Subjects:**
Up to 100 subjects are expected to be consented in order to achieve the goal of enrolling 20 evaluable subjects. Potentially eligible subjects will have a screening visit to include informed consent, review and documentation of inclusion/exclusion criteria, spot urine sample, blood test, and completion of a 24-hour home urine collection (in potential subjects without record of a 24-hour urine calcium ≥ 4 mg/kg/day in the past year). Subjects found meet inclusion/exclusion criteria will then be enrolled into the study. Currently, 20-30% of adolescents with T1D participating in studies of bone health at URMC have been found to have 24-hour urine calcium excretion of ≥ 4 mg/kg/day. We are anticipating a 10% drop out rate; if more than 2 subjects withdraw prior to completion of study procedures, we plan to replace them to achieve the recruitment goal of 18 participants completing study procedures.

b) **Gender and Age of Subjects:**
Males and females aged 12-21 years will be eligible for participation. This age range was chosen to encompass the earliest accepted normal age of onset of adolescence[46] on the low

end to the mean age of attainment of peak bone mass on the high end.[47] Enrollment is intended to match the gender distribution of the underlying T1D population, therefore we expect to enroll approximately 25% more males than females.[48]

c) **Racial and Ethnic Origin:**
Race and ethnicity will not serve as an inclusion or exclusion criteria, therefore the racial ethnic distribution of the study sample is intended to match the racial ethnic distribution of English speaking T1D patients in our clinic. Based on the higher prevalence of T1D among whites, and the higher population of whites in our clinic population, we expect the majority of the participants in this study to be white.

d) **Vulnerable Subjects:**
The study will include subjects <18 years of age. Adolescence is the critical period for skeletal development, therefore this study must include pediatric subjects. Study risks and benefits will be explained directly to potential subjects as well as their parents/legal guardians. Assent will be obtained in addition to parental/legal guardian consent for all participants. The PI is a pediatrician experience with clinical research in children and all study procedures will be conducted by study staff skilled in the care of pediatric subjects.

3.2. **Inclusion and Exclusion Criteria**

a) **Inclusion Criteria:**
- Diagnosis of T1D
- Age 12-21 years
- Tanner Stage 2 or greater pubertal development
- Urine calcium excretion ≥ 4 mg/kg/day
- Able to swallow pills

b) **Exclusion Criteria:**
- BMI > 99th percentile for age (<18 years) or BMI >35 kg/m^2 (≥ 18 years)
- Coexistent conditions that may affect calcium metabolism including:
  - celiac disease
  - Graves' Disease
  - Addison's disease
  - hypo- or hyperparathyroidism
- History of diabetes related complications including:
  - neuropathy
  - retinopathy
  - nephropathy
  - gastroparesis
- History of oral or inhaled corticosteroid use for ≥ 5 consecutive days within the past month
- History of any diuretic use within the past month
- Laboratory abnormalities on screening bloodwork including:
  - eGFR <90 mL/min per 1.73 m2 BSA[49]
  - serum calcium >10.5 mg/dL
  - serum potassium <3.5 mmol/L
- Systolic or diastolic blood pressure <5th percentile for age and sex[50] for age <18 years or systolic <90 mmHg or diastolic blood pressure <60 mmHG for age ≥18 years

- Pregnant
- Known allergy to sulfonamide drugs
- Parents/guardians or participants who, in the opinion of the Investigator, may be non-compliant with study schedules or procedures.

3.3. Discussion of Subject Population

Inclusion criteria are designed to identify participants with T1D complicated by excess urinary calcium excretion during the critical adolescent years for bone accrual. Exclusion criteria are designed to reduce risk of misclassification bias (by excluding obese individuals who might have type 2 diabetes and excluding individuals with conditions and/or medications known to affect urine calcium excretion). Additional exclusion criteria related to blood chemistry and blood pressure are designed to reduce risk of harm to participants who may be at greater risk of chlorthalidone or potassium-chloride related side effects.

4. SUBJECT IDENTIFICATION, RECRUITMENT AND CONSENT

4.1. Method Of Subject Identification And Recruitment

Subjects will be identified and recruited from the T1D patient population served by the Division of Pediatric Endocrinology at URMC. The PI is a member of this division, and has routine access to this population. Potentially eligible subjects will be identified during routine clinic visits, and approached in person by a member of the study staff. Additionally, subjects will be identified from potentially eligible subjects from the URMC Pediatric Endocrine practice who have participated in previous research studies and have agreed to be contacted for future research. The consent form will be used as the basis of the recruitment script. In order to limit the possibility of coercion, potential participants/guardians will be informed that participation is voluntary and will not affect clinical care. Eligible participants will be free to take study consent/assent forms with them for review outside of the clinic visit.

4.2. Process of Consent

a) Potentially eligible participants and parents/guardians (if applicable) will be approached by a member of the study team. Potential participants and their parents/guardians interested in hearing more about the study will then be screened for eligibility. Eligible participants will then be given a copy of the consent and assent forms for review. They will be given the time to review the forms alone to safeguard against potential coercion. Informed consent/assent will take place in a private space with the Department of Pediatrics. The study team member obtaining consent/assent will answer all questions that the participant and/or parent/guardian may have. The participant and parent/guardian will be asked if they comprehend the nature of the study and will be reminded that participation in the study is optional and that their care at URMC will not be affected if they decide not to participate. It is not expected that participants and/or parents/guardians will lose capacity to comprehend as the study progresses as this is a short term study T1D is not a progressive condition nor associated with mental impairment.

b) Vulnerable Populations: Participants will be asked directly if they comprehend the nature of the study. Participants who do not provide assent and/or do not verbally confirm that they comprehend the nature of the study will not be allowed to enroll.

Subject and/or parent/guardian consent and assent will be documented in writing on RSRB approved and watermarked paper forms. Original signed documents will be maintained by

the study team in subject binders stored in a locked cabinet in a locked office in the Department of Pediatrics at URMC.

5. METHODS AND STUDY PROCEDURES

An overview of the schedule of activities is provided in **Table 1.** Following informed consent, subjects will have a screening visit. Participants who meet all eligibility requirements will be enrolled and complete study visits at baseline, 7-days, 14-days, 21-days, and 28-days. Throughout the study, participants will receive weekly phone calls from a study team member to address any study-related difficulties. All subjects will be followed for the full 4-weeks, if willing, regardless of protocol violations or withdrawal of study drug.

Table 1: Schedule of Study Activities

Visit	0 (Screening)	1	2	3	4	5
Visit Window	**-30 days**	**0 days**	**7 days**	**14 days**	**21 days**	**28 days**
Obtain informed consent / assent	X					
Confirm eligibility / enroll		X				
Medical history / demographics	X					
Medical record review	X					
Vital signs	X	X	X	X	X	X
Physical exam for pubertal status	X					
Blood draw	X	X	X	X	X	X
24-hr urine sample	X	X				X
Spot urine sample	X	X	X	X	X	X
Side effect questionnaire		X	X	X	X	X
Concomitant medication review	X	X	X	X	X	X
Study medication log review			X	X	X	X
Dietary questionnaire		X				X
Blood glucose log review			X	X	X	X
Study drug dispensed		X	X	X	X	
Study drug returned			X	X	X	X
Dose adjustment (as			X	X	X	

needed, see Figure 1)						
Addition of potassium chloride supplement (as needed, see Figure 1)			X	X	X	

a) **Screening visit (Visit 0):**
During this visit, the subject will be informed about all aspects of the study, including eligibility requirements and the schedule of study visits and activities. Subjects will be requested to sign and date the informed consent prior to performing any study-related procedures. Subjects will be assessed for eligibility by a member of the study team. The following procedures will be performed at the screening visit

- Obtain written informed consent/assent
- Study ID number assigned
- Medical history and demographics interview
- Review of medical record
- Review of concomitant medication usage
- Vital signs (height, weight, BMI, blood pressure)
- Physical exam for pubertal status
- Obtain blood sample for laboratory testing (basic metabolic panel)
- Obtain spot urine sample for pregnancy test (females only)
- Obtain 24-hour urine sample if no record of 24-hour urine calcium $\geq$ 4mg/kg/day in the past year (subjects will be provided with urine collection materials and instruction at visit, they will perform the collection at home and return sample to lab for analysis).

b) **Baseline visit (Visit 1):**
During this visit, eligibility will be confirmed by PI and subjects will be enrolled into the study. All the inclusion criteria must be met and none of the exclusion criteria may apply. All results from screening procedures must be available before eligibility can be confirmed. The following procedures will be performed at the baseline visit. Baseline visit must be conducted within 30 days of screening visit. Subjects not presenting for baseline visit within 30 days will need to have eligibility re-confirmed (vital signs, blood sample, medication review)

- Confirmation of eligibility and enrollment
- Vital signs (height, weight, BMI, blood pressure)
- Complete side effect questionnaire
- Review of concomitant medication usage
- Complete dietary questionnaire
- Instruction in blood glucose log record keeping
- Instruction in correct administration of study drug and drug dispense
- Instruction to return unused study drug to each visit and to immediately report any adverse events to a study team member
- Obtain blood sample (comprehensive metabolic profile, fructosamine, intact PTH, 25-OH vitamin D, 1,25-OH vitamin D, magnesium, phosphorus, bone

specific alkaline phosphatase, beta-CTX, uric acid)
- Obtain spot urine sample (calcium, creatinine, glucose)
- Obtain 24 hour urine sample (calcium, creatinine, glucose, sodium, phosphorus)

c) **Visit 2 (7 days):**
The following procedures will be performed at an in-person study visit 1 week after the baseline visit. Visit may be conducted ± 3 days of target date.

- Vital signs (weight, blood pressure)
- Complete side effect questionnaire
- Review of concomitant medication usage
- Assessment for adverse events
- Review of blood sugar log by safety monitor, with recommendations to adjust insulin doses as needed
- Obtain blood sample (basic metabolic profile)
- Obtain spot urine sample (calcium, creatinine, glucose)
- Review and documentation of unused study drug
- Chlorthalidone dose adjustment and/or addition of potassium chloride, as needed

d) **Visit 3 (14 days):**
The following procedures will be performed at an in-person study visit 1 week after Visit 2. Visit may be conducted ± 3 days of target date.

- Vital signs (weight, blood pressure)
- Complete side effect questionnaire
- Review of concomitant medication usage
- Assessment for adverse events
- Review of blood sugar log by safety monitor, with recommendations to adjust insulin doses as needed
- Obtain blood sample (basic metabolic profile)
- Obtain spot urine sample (calcium, creatinine, glucose)
- Review and documentation of unused study drug
- Chlorthalidone dose adjustment and/or addition of potassium chloride, as needed

e) **Visit 4 (21 days):**
The following procedures will be performed at an in-person study visit 1 week after Visit 2. Visit may be conducted ± 3 days of target date.

- Vital signs (weight, blood pressure)
- Complete side effect questionnaire
- Review of concomitant medication usage
- Assessment for adverse events
- Review of blood sugar log by safety monitor, with recommendations to adjust insulin doses as needed
- Obtain blood sample (basic metabolic profile)
- Obtain spot urine sample (calcium, creatinine, glucose)
- Review and documentation of unused study drug
- Chlorthalidone dose adjustment and/or addition of potassium chloride, as needed
- Provide urine collection kit to be completed at home and brought to final study

visit

f) **Visit 5 (28 days):**
The following procedures will be performed at an in-person study visit 2 week after Visit 3. Visit may be conducted ± 3 days of target date.

- Vital signs (weight, blood pressure)
- Complete side effect questionnaire
- Review of concomitant medication usage
- Assessment for adverse events
- Complete dietary questionnaire
- Review of blood sugar log by safety monitor, with recommendations to adjust insulin doses as needed
- Obtain blood sample Obtain blood sample (comprehensive metabolic profile, fructosamine, intact PTH, 25-OH vitamin D, 1,25-OH vitamin D, magnesium, phosphorus, bone specific alkaline phosphatase, beta-CTX, uric acid)
- Obtain 24-hr urine sample (calcium, creatinine, glucose, sodium, phosphorus)
- Obtain sport urine sample
- Review and documentation of unused study drug

g) **Telephone-based follow-up evaluations:**
Subjects will receive a weekly phone-call from a member of the study team to evaluate for any side effects or interval changes in medical health. Each participant will be asked if they have any questions regarding the study or the study drug(s). Participants will be asked if they have been compliant with the study drug(s). Reasons for non-compliance will be discussed as will scheduling for upcoming in-person study visits.

h) **Unscheduled visits:**
Unscheduled in-person visits may occur at any time during the study. Assessments listed below will be completed and any data generated, including that from unscheduled telephone contacts, will be documented on appropriate study evaluation forms.

- Vital signs (weight, blood pressure, other as deemed necessary by PI)
- Complete side effect questionnaire
- Review of concomitant medication usage
- Assessment of adverse events
- Blood and/or urine sample collection (if deemed necessary by the PI to assess adverse events

If a subject has an Unscheduled Visit between the scheduled visits he/she will be instructed to attend his/her next visit according to the study schedule as planned.

i) **Early withdrawal visits:**
In the event a subject is unwilling or unable to continue to be followed off study drug, when possible, an Early Withdrawal visit will be conducted and all unused study drug returned to the study team. The following procedures will be performed at an in-person Early Withdrawal visit:

- Vital signs (weight, blood pressure)
- Complete side effect questionnaire
- Review of concomitant medication usage

- Assessment for adverse events
- Complete dietary questionnaire
- Review of blood sugar log by safety monitor, with recommendations to adjust insulin doses as needed
- Obtain blood sample Obtain blood sample (comprehensive metabolic profile, fructosamine, intact PTH, 25-OH vitamin D, 1,25-OH vitamin D, magnesium, phosphorus, bone specific alkaline phosphatase, beta-CTX, uric acid)
- Obtain 24-hr urine sample
- Review and documentation of unused study drug
- Review and documentation of reason for withdrawal

If a subject does not return for an in-person evaluation, adverse events, concomitant medications, reason for withdrawal and method of study drug return will be reviewed by phone, when possible.

j) **Milestone and Study Calendar**
The proposed clinical trial will be completed within three years of obtained funding. Funding from NIH was received July 2017, we will 1) have completed all regulatory approvals by January of 2018; 2) trained clinical site staff by March of 2018; 3) enrolled the first subject by June of 2018; 4) enrolled 33% participants enrolled by December 2018, 75% by July 2019 and 100% by January of 2020; 5) completed all data collection by February of 2020 and 5) completed all study analysis and final report/manuscript by June of 2020.

5.1. Treatment Dosage and Administration

5.1.1. Summary of treatment plan:

This study will be an open label study designed to evaluate the short term safety and efficacy of chlorthalidone. Drug exposure will be limited to a maximum of 4 weeks. The study protocol will allow for adjustment of chlorthalidone based upon results of safety and efficacy outcomes over the study period. Potassium chloride supplements will be added as needed for subjects who are found to develop hypokalemia over the study period.

5.1.2. Description of treatment plan:

Subjects will be dispensed the study drug (chlorthalidone) at Baseline and each Follow-up visit for home administration. Compliance with chlorthalidone and potassium chloride (if needed) will be assessed by questionnaire, review of medication administration log, and by pill-count of unused drug(s) returned at study visits. Specific details of the treatments are as follows:

a) **Chlorthalidone:**
All subjects will be started at chlorthalidone tablets at an initial dose of 12.5 mg, to be taken once per day by mouth at home. Subjects will be instructed to take the tablet in the morning with food. Dose will be adjusted at Study Visit 2, 3, and/or 4 based upon results of safety and efficacy measures (dose titration described in Section 8 below).

b) **Klor-Con (potassium chloride, extended release)**
Subjects who develop hypokalemia (defined as serum potassium <3.5 mmol/L will be started on Klor-Con tablets at an initial dose of 1 mEq/kg (maximum 20 mEq/dose), to be

given once per day by mouth at home. Subjects will be instructed to take the tablet in the morning with food and a full glass (8 oz or more) of liquid. Dose will be adjusted at follow-up visits as needed based upon results of safety measures (dose titration described in Section 8 below).

5.1.3. **Study Agent(s)**

a) **Chlorthalidone**
Chlorthalidone should be administered with food. Relevant precautions include monitoring for the development of hypokalemia, hypercalcemia, hyperglycemia, and hypotension all of which are potential side effects of chlorthalidone relevant to the study population.

b) **Klor-Con (potassium chloride, extended release)**
Klor-Con should be administered with food and a full glass of liquid (8 oz). Tablets should be swallowed intact. Hyperkalemia is a potential side effect of Klor-Con and will be monitored for by biochemistry. Klor-Con is contraindicated in patients taking potassium-sparing diuretics and angiotensin converting enzyme inhibitors (use of either of these medication classes would have excluded subjects from enrollment in this study) because of increased risk of hyperkalemia. Subjects will be advised to stop taking Klor-Con if they should develop diabetic ketoacidosis (DKA) over the course of this study, as DKA may be another risk factor for hyperkalemia. Solid dosage forms of potassium chloride increase risk of ulcerative and/or stenotic lesions of the gastrointestinal tract. Klor-Con tablets were chosen for this study because they are wax matrix tablets formulated to provide an extended rate of release of potassium chloride and thus minimize the possibility of high local concentrations of potassium near the gastrointestinal wall. Per the product insert, the incidence of gastrointestinal adverse events with extended release wax matrix potassium chloride preparations is less than one per 100,000 patient years.

5.2. **Efficacy Assessments**

- Urine calcium excretion: The primary efficacy outcome will be change in 24 hour urine calcium from baseline to last follow up visit. Subjects will perform urine collection at home and bring to study visit for analysis by the URMC lab. Subjects will be given verbal and written instructions for proper 24 hour urine collection technique. They will be asked to record the start and stop time/date of the collection period and to report if any urine was missed. Urine creatinine and volume will be used to assess adequacy of collection. 24-hr urine creatinine values of <10 mg/kg102 and volumes < 0.5 cc/kg/hr will be flagged as concerning for incomplete collection and excluded in sensitivity analyses. The secondary efficacy outcome will be spot urine calcium/creatinine ratio (Uca/Ucr). Spot Uca/Ucr is an established screening test for hypercalciuria in children, with a level greater than 0.2 used to define hypercalciuria.[51] Because it is less burdensome than a 24 hour collection, spot Uca/Ucr will be used to assess efficacy for chlorthalidone dose adjustments at Visit 2 and Visit 3. Uca/Ucr will also be performed at study visit 4 in the case that the subject does not complete 24 hour urine collection as requested.

5.3. **Safety Assessments**

- Adverse Event Assessment: Adverse events, attribution of adverse event to the study drug, actions take with respect to study drug (i.e. dosage change), and classification of

seriousness will be documented.

- Blood samples: The following laboratory assessments will be performed from blood samples taken at time of study visit (**Table 2)**:
    - Serum potassium: Serum potassium will be assessed for development of hypokalemia (defined as serum potassium < 3.5 mEq/L)
    - Serum calcium: Serum calcium will assessed for development of hypercalcemia (defined as serum calcium >10.5 mg/dL)
    - Fructosamine: Serum fructosamine will performed to assess for an effect of chlorthalidone on glycemic control over the study period

Additional blood and urine labs will be collected at baseline and study end to include CMP, urine glucose and electrolytes, markers of bone mineral metabolism (magnesium, phosphorus, intact PTH, 25-OHvitamin D, 1,25-OHvitamin D, bone specific alkaline phosphatase, beta CTX) to look for an effect of chlorthalidone on other electrolytes or markers of bone mineral metabolism

a) Clinical Safety Assessments:
    - Blood pressure: Blood pressure will be assessed for hypotension at study visits
    - Blood sugar log: Subjects will record daily blood sugars in a log provided by study team. Blood sugar logs will be reviewed at each study visit by an independent safety monitor who is a T1D practitioner who will adjust subject insulin regimen, as needed
    - Side effect questionnaire: Subjects will complete a questionnaire at each study with questions regarding the development of anticipated or unanticipated side effects from study drug. Separate questionnaires will be used for chlorthalidone and Klor-Con.

5.4. **Assessment of Subject Compliance**

Compliance will be measured as the percentage of dispensed tablets used out of those scheduled to be used. Additionally, subjects will be asked to record missed doses on a log and will be asked questions about compliance at study visits.

5.5. **Data & Specimen Banking for Future Research Use**

Blood and urine will be stored for future analysis. There are a number of potential contributors to the effects of T1D and chlorthalidone and on calcium metabolism that are beyond the scope and budget of this study, but may be of interest once further funding is obtained. No identifiable data will be used for future study without first obtaining RSRB approval. As a part of the consent process, participants will be asked if their blood and samples can be stored for future research and also if they are interested in being contacted regarding potential future studies of diabetes and bone health. Participants will be eligible to complete the study even if they do not consent to having samples stored for future research. Samples for future research will be de-identified using the unique participant ID, frozen, and stored in a -80C freezer at URMC. Samples will be stored for a period of 10 years following completion of all study procedures or until which time the investigator is no longer involved in research (if sooner). An extension may be requested after 10 years. Only members of the study team will have access to stored samples. No requests for release of data/specimens will be granted without first obtaining RSRB approval and the development of an appropriate data sharing plan. Participants may withdraw their consent for the use of stored specimens at any time by asking the PI in writing. Participants will not be re-contacted regarding stored data/specimens.

5.6. **Costs to the Subject**

There will be no costs to subjects.

5.7. **Payment for Participation**

Subjects will be compensated for participation. Subjects will be paid $25 in the form of a VISA gift card after completion of each scheduled study visit for a total of $150 for subjects completing all study visits. Payment will be prorated such that participants will be paid after completion of each study visit. Subjects will be required to complete all study procedures for a given visit to receive payment.

5.8. **Return of Individual Research Results**

Results of laboratory safety assessments (potassium, calcium, and fructosamine) will be returned to subjects at the time they are received and reviewed by the PI as they are deemed potentially clinically relevant. The clinical relevance of efficacy outcomes (urine calcium) and other labs including urine electrolytes and markers of bone and mineral metabolism are unclear and therefore will not be returned. 25-OHvitamin D is used clinically to assess vitamin D status, however we will not analyze this result until all subjects have completed the study in order to ensure that all samples are performed on the same assay. This may be months to years after the sample was initially drawn, therefore we will not report this result to subjects or providers as it will no longer be clinically meaningful.

6. CONCOMITANT AND DISALLOWED MEDICATIONS

Subjects will be allowed to continue on any medications used at baseline, provided they are not listed in the exclusion criteria. Subjects will be withdrawn from the study if they are prescribed a medication listed in the exclusion criteria by a medical provider during the course of the study. Subjects must also refrain from starting any new vitamin, mineral, or nutritional supplement while enrolled.

7. SUBJECT WITHDRAWALS

Subjects will be advised in the written informed consent forms that they have the right to withdraw from the study at any time and for any reason without prejudice. The investigator also has the right to discontinue the study drug or subject's participation due to worsening of clinical condition, adverse events related or unrelated to study drug, intercurrent illness, pregnancy, subject non-compliance with study procedures, or termination of research funding. Subjects who withdraw or are withdrawn from the study will be asked to complete an "Early Withdrawal" visit as described in Section 5, if willing. Subjects withdrawn from the study will be replaced to meet the goal of 18 subjects completing all study visits.

8. STUDY DRUG/DEVICE/BIOLOGIC ADMINISTRATION/ASSIGNMENT

8.1. **Study Drug/Device/Biologic**

a) **Chlorthalidone**:
Chlorthalidone will be purchased by the Investigational Drug Services (IDS) at URMC from Mylan Pharmaceuticals through a local vendor. Chlorthalidone will be packed, labeled, and dispensed by the IDS at URMC. Active ingredients: chlorthalidone. Inactive ingredients: silicon dioxide, cellulose microcrystalline, D&C yellow No. 10, sodium starch glycolate type A potato, starch-corn, stearic acid. Dosage unit is 25 mg tablet. Tablets will be split by the IDS at URMC prior to dispensing to subjects.

b) **Klor-Con (extended release potassium chloride)**
Klor-Con will be purchased by the Investigational Drug Services (IDS) at URMC from Sandoz, Inc, through a local vendor. Klor-Con will be packed, labeled, and dispensed by t he IDS at URMC. Active ingredients: potassium chloride. Inactive ingredients:

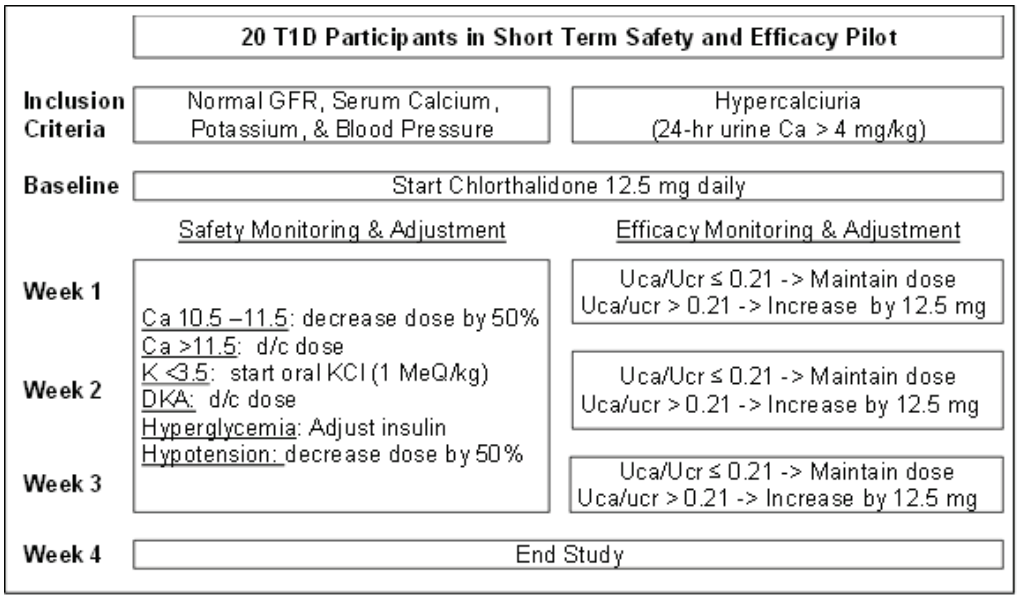
hydrogenated cottonseed oil, magnesium stearate, polyethylene glycols, polyvinyl alcohol, silicon dioxide, titanium dioxide, FD&C blue no. 1, FD&C blue no. 2. Dosage unit is 10 mEq tablet.

8.2. **Dosage of Study Drug/Biologic**

a) **Chlorthalidone**
All subjects will be started at 12.5 mg/daily. Dose titration will occur at study visits, or between study visits, as outlined in **Figure 1:**

- The dose will be increased by 12.5 mg/day at Study Visit 2, 3, and/or 4, as needed, if urine ca/cr is >0.21, to max of 50 mg.
- The dose will be decreased by 50%, as needed, for hypercalcemia (serum calcium between 10.5-11.5) or hypotension (Systolic or diastolic blood pressure <5th percentile for age and sex[50] for age <18 years or systolic <90 mmHg or diastolic blood pressure <60 mmHG for age ≥18 years)
- The study drug will be discontinued, as needed, for severe hypercalcemia (serum calcium >11.5 mg/dL) or if participant develops diabetic ketoacidosis over the course of the study.

b) **Klor-Con**
Klor-Con will be started as needed at Study Visit 2, 3, or 4 in subjects who develop hypokalemia (serum potassium <3.5 mmol/L). Starting dose will be 1 mEq/Kg to a max of 20 mEq/day. Dose will be increased, as needed, at subsequent study visits by 1 mEq/Kg (maximum increase 20 mEq/day) for persistent hypokalemia (serum potassium <3.5 mmol/L).



8.3. **Subject Enrollment/Randomization**
N/A. Open label study.

8.4. **Accountability of Investigational Supplies**
The IDS pharmacist at URMC will be responsible for receipt, storage, dispensing, collection, accountability and disposal of the study drug(s). Drug accountability logs will be completed by a member of the study team to account for dispensed and returned drugs at each study visit.

8.5. **Subject Withdrawal of Study Drug**
Subjects who stop taking the study drug will be asked to stay in the study and complete study procedures, if willing. Subjects who stop taking the study drug will be allowed to resume the study drug, provided that drug discontinuation was not due to an adverse event.

8.6. **Emergency Drug Disclosure**
N/A. Open label study.

9. SAFETY AND REPORTABLE EVENTS

9.1. **Adverse Event Definition**
An adverse event is any symptom, sign, illness, or experience which develops or worsens during the course of the study, whether or not the event is considered related to study drug.

9.2. **Serious Adverse Event**
A serious adverse event is defined as any adverse medical experience that results in any of the following outcomes:

- death;
- is life-threatening;
- requires inpatient hospitalization or prolongation of existing hospitalization;
- results in persistent or significant disability/incapacity;
- is a congenital anomaly/birth defect; or
- requires medical or surgical intervention to prevent permanent impairment or damage.

9.3. **Recording Adverse Events**
At each subject visit a member of the study team will assess adverse events by recording all voluntary complaints of the subject and by assessment of clinical and laboratory features. At each study visit, the subject will be questioned directly regarding the occurrence of any adverse experience since his/her last visit.

All adverse events, whether observed by the Investigator, elicited from or volunteered by the subject, will be documented in the adverse event log. Each adverse event will include a brief description of the experience, the date of onset, the date of resolution, the duration and type of experience, the severity, the relationship to investigational product (i.e., drug or device), contributing factors, and any action taken with respect to the study drug/device.

Adverse events will be classified by the PI as "serious" or "non-serious". The maximum intensity of adverse events will be graded as follows:

Table 2: Adverse Event Classification

Grade Level	Definition
1	Mild (awareness of sign/symptom that is easily tolerated)
2	Moderate (sign/symptom intense enough to interfere with usual activity)
3	Severe (sign/symptom significantly interferes with ability to do usual activity

With careful medical consideration at the time of evaluation, the reasonable possibility of an adverse event's relationship to study drug will be assessed. The Safety Monitoring Board's opinion may be sought in those cases in which the PI is unable to make an independent judgment.

The Safety Monitor Board may in turn consult with the PI as needed. The following definitions are general guidelines only to help assign grade of attribution:

Table 3: Adverse Event Grading

Grade Level	Descriptor	Definition
1	Unrelated	Adverse event is clearly unrelated to the study drug
2	Unlikely	Adverse event is doubtfully related to the study drug
3	Possible	Adverse event may be related to the study drug
4	Probable	Adverse event is likely related to the study drug
5	Definite	Adverse event is clearly related to the study drug

Definite
This category applies to those adverse events, which after careful medical consideration at the time they are evaluated, are felt with certainty to be related to study medication. An adverse event may be considered definitely related if or when (at least three of the following):

- It follows a direct temporal sequence from administration of the study drug.
- It cannot be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject.
- It disappears or decreases on cessation or reduction in dosage. There are important exceptions when an adverse event does not disappear upon the discontinuation of study drug, yet drug-relatedness clearly exists.
- It follows a known pattern of response to the study drug.

Probable
This category applies to those adverse events, which after careful medical consideration at the time they are evaluated, are felt with a high degree of certainty to be likely related to study medication. An adverse event may be considered probably related if or when (at least three of the following):

- It follows a reasonable temporal sequence from administration of the study medication.
- It could not be reasonably explained by the known characteristics of the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject.
- It disappears or decreases on cessation or reduction in dosage. There are important exceptions when an adverse event does not disappear upon the discontinuation of study medication, yet drug-relatedness clearly exists.
- It follows a known pattern of response to the test drug.

Possible
This category applies to those adverse events for which, after careful medical consideration at the time they are evaluated, a connection with administration of study medication appears unlikely but cannot be ruled out with certainty. An adverse event may be considered possibly related if or when (at least two of the following):

- It follows a reasonable temporal sequence from administration of the study medication.
- It could not readily have been produced by the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject.
- It follows a known pattern of response to the test drug.

Unlikely
In general, this category can be considered applicable to those adverse events, which, after careful medical consideration at the time they are evaluated, are judged to be doubtfully related to study medication. An adverse event may be considered unlikely related if or when (must have two):

- It does not follow a reasonable temporal sequence from the administration of the test drug.
- It could readily have been produced by the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the test drug.
- It does not reappear or worsen when the drug is re-administered.

Unrelated
In general, this category can be considered applicable to those adverse events, which, after careful medical consideration at the time they are evaluated, are judged to be clearly unrelated to study medication. An adverse event may be considered unrelated if or when (must have three):

- It does not follow the temporal sequence from the administration of the test drug.
- It was most likely produced by the subject's clinical state, environmental or toxic factors or other modes of therapy administered to the subject.
- It does not follow a known pattern of response to the test drug.
- It does not reappear or worsen when the drug is re-administered.

9.4. Responsibilities for Reporting Serious Adverse Events

The Investigator will record all severe adverse events that occur during the study period in the adverse event log. The study period for reporting serious adverse events is defined as the time period from when the subject signs the informed consent until 30 days following the subject's completion of the study. Any unanticipated and serious and related events that occur during the reporting period will be reported to the RSRB as soon as identified and within 10 calendar days, as defined by the policy laid out in the "Guideline for Reporting Researching Events" policy. Events that are deemed not unanticipated not serious and not related but that are notable and could involve risks to subjects will be summarized in narrative or other format and submitted to the RSRB at the time of continuing review.

10. RISK/BENEFIT ASSESSMENT

10.1. Potential Risks

- Adverse reactions associated with chlorthalidone: The primary potential clinically significant risks associated with chlorthalidone use and relevant to this population include hypercalcemia, hypokalemia, hypotension, and hyperglycemia. Additional potential adverse reactions listed in the product insert include gastrointestinal upset, jaundice, pancreatitis, dizziness, headache, paresthesias, xanthopsia, suppression of blood counts, dermatologic-hypersensitivity reactions, bronchospasm, hyperuricemia, muscle weakness/spasm, restlessness, impotence, renal insufficiency, hypomagnesemia. There is insufficient data to estimate expected frequency of adverse reactions.
- Adverse reactions associated with Klor-Con: Potential adverse reactions to Klor-Con listed in the product insert include hyperkalemia, gastrointestinal lesions (bleeding, ulceration, perforation, obstruction), gastrointestinal upset, and rash. Per the product insert, frequency

of gastrointestinal lesions is <1 per 100,000 patient years. There is insufficient data to estimate frequency of other adverse reactions.
- Pain and infection with blood draw.
- Loss of confidentiality of protected health information.

10.2. **Protection Against Risks**
- Risks associated with chlorthalidone: Steps taken to prevent and/or minimize potential adverse events associated with chlorthalidone include initiation of drug at a low dose, and monitoring of safety outcomes including laboratory tests, blood pressure, adverse event questionnaire, and blood sugar logs at follow-up study visits. Chlorthalidone dose will be adjusted or discontinued, as indicated, based upon safety outcomes. Subjects developing an adverse reaction related to chlorthalidone will be referred for further medical evaluation and/or treatment as indicated.
- Risks associated with Klor-Con: Steps taken to prevent and/or minimize potential adverse events associated with Klor-Con include initiation of drug at a low dose, and monitoring of safety outcomes including laboratory tests, and adverse event questionnaire at follow-up study visits. Klor-Con dose will be adjusted or discontinued, as indicated, based upon safety outcomes. Subjects developing an adverse reaction related to Klor-Con will be referred for further medical evaluation and/or treatment as indicated.
- Pain and infection with blood draw. Blood draw will be performed by a research nurse trained in the care of pediatric patients using sterile techniques. The blood volume will not exceed 3 mL/kg for any participants.
- Loss of confidentiality of protected health information. Steps taken to reduce this risk include the use of a unique study ID for all participant samples and study documents, the use of a secure REDCAP database to store study data, and the use of a locked cabinet in a locked office in the Department of Pediatrics to store other study documents.

10.3. **Potential Benefits to Subjects**
Subjects are not expected to benefit from this study.

10.4. **Alternatives to Participation**
Potential subjects can choose not to participate in the study; their clinical care will not be affected.

11. CONFIDENTIALIATY OF DATA AND INFORMATION STORAGE

All information that is collected for this research protocol will be kept confidential. All subjects will be assigned a unique study identification number (Study ID) at the start of the protocol by a member of the study team. A document linking study ID to participant identifiable information will be stored separately from study data in an encrypted password protected folder on the URMC server. All patient identifiers as defined under HIPAA regulation 45 CFR Subtitle A, Subchapter C, Section 164.514 will be removed to de-identify study samples and data collection instruments. All paper study records will be stored in locked cabinets in locked offices in the Department of Pediatrics. Patient identifiers will be kept separately from study data in electronic data fields. All research samples for storage or shipment to outside labs will be labeled only with the participant study ID. Study data will be maintained using a REDCap database. The REDCap system is a secure, web-based application that is flexible enough to be used for a variety of types of research. It provides an intuitive interface for users to enter data and real time validation rules (with automated data type and range checks) at the time of data entry. REDCap offers easy data manipulation with audit trails and functionality for reporting, monitoring and querying patient records, as well as an automated export

mechanism to common statistical packages (SPSS, SAS, Stata, R/S-Plus). Through the REDCap Consortium, Vanderbilt has disseminated REDCap for use around the world. Currently, over 240 academic and non-profit consortium partners on six continents with over 26,000 research end-users use REDCap (www.project-redcap.org). REDCap servers are housed in a local data center at the University of Rochester and all web-based information transmission is encrypted. REDCap was developed in a manner consistent with HIPAA security requirements and is recommended to University of Rochester researchers by the URMC Research Privacy Officer and Office for Human Subject Protection. Access to the REDCAP database and PHI will be limited to members of the study team. The investigator will retain all study essential documents until all data analysis is complete, manuscripts have been accepted for publication, and any grant proposals generated from this study have been submitted. Study results will be reported in aggregate.

12. RESEARCH INFORMATION IN MEDICAL RECORDS

Results of labs performed at the URMC lab (as listed in Section 4.1.5) will appear in the electronic medical record. Documentation of chlorthalidone and Klor-Con (if needed) will be added to the medication list in the medical record for patient safety purposes, should subject need medical evaluation either related or unrelated to study participation.

13. DATA ANALYSIS AND MONITORING

13.1. Sample Size Determination

Based upon clinical use and previous reports, we expect the frequency of hypercalcemia, hypokalemia refractory to potassium supplementation, and hypotension to be low. The effect on glycemic control is not known. A previous study in children with idiopathic hypercalciuria found that urine calcium excretion decreased by greater than 50% with thiazide use. [32] A *sample size* of 18 was chosen to provide 80% power at a 5% significance level to detect an alternate adverse event rate of 25% from a null hypothesized value of 0 and to detect an effect size of a 30% reduction in 24-hr urinary calcium. **Table 4** provides the 95% confidence intervals for a range of possible adverse event rates to quantify the precision of the point estimates we will be able to detect.

Adverse event (n)	Observed rate (%)	95% CI of the true rate
0	0	(0, 15.5)
1	5.5	(0, 27.7)
2	11.1	(1.9, 34.1)

Table 4: Precision of point estimates for adverse event detection

13.2. Planned Statistical Analysis

a) Safety outcomes:

Adverse events will be tabulated overall and by severity. For each adverse event, a 95% confidence interval will be computed for the incidence using the Wilson score method.[52] This will be repeated excluding all mild symptoms. Similar analyses will be performed after grouping adverse events by body system. Individual adverse events will be listed, with particular attention paid to serious adverse events. Laboratory tests, vital signs, and need for insulin adjustment will be summarized similarly. A complete accounting of subject disposition will be summarized, including a tabulation of subject withdrawals, dosage adjustments, and

early discontinuations of study medication (with reasons for each). Concomitant medication usage for each participant will be listed for review. Paired t-tests will be used to determine the effect of chlorthalidone on fructosamine from baseline to 4-weeks.

b) Efficacy outcomes:
Paired t-tests will be used to determine the effect of chlorthalidone on 24-hr urine calcium excretion from baseline to 4-weeks.

13.3. **Data and Safety Monitoring**

Safety monitoring will be done by PI and a Data Safety Monitoring Committee (DSMC) composed of Marc Lande MD (Pediatric Nephrologist, URMC) and Steven Wittlin MD (Adult Endocrinologist and Diabetologist, URMC). PI will directly review all laboratory values, vital signs, adverse events, and results of other safety outcomes as they become available. PI will submit a weekly report to the DSMC that includes both raw safety data and a summary of adverse events and recruitment statistics. Decisions regarding the need for dose adjustment/discontinuation of chlorthalidone and/or addition of Klor-Con will be made by PI according to the protocol (figure 1) and reviewed by DSMC at time of weekly report. The PI will additionally notify the DSMC of any serious events, any unanticipated events, or the need for any unscheduled study visits immediately as they arise. Reporting of adverse events will comply with RSRB regulations as described in Section 9.4 above. Additionally, the DSMC will meet quarterly to review study progress and safety data in aggregate and submit a report to the PI and RSRB with a recommendation to continue, modify, or discontinue the study. The DSMC will have final say in the determination as to whether the study should be discontinued as a result of any expected or unexpected adverse events. As an additional data integrity and subject safety safeguard, Jean Mack-Fogg, a pediatric nurse practitioner who treats pediatric and young adult T1D patients at URMC, will independently review blood sugar logs at time of study visits and make recommendations to subject/families regarding need for insulin adjustment.

The PI will be responsible for overseeing data management, entry, quality assurance and reporting. REDCap electronic data capture tools will be used to collect and manage data for this study. All data will be entered by one individual and checked by a second. The database will be constructed with an auditing system to track both original data entry and corrections. This auditing procedure will be carried out for 100% of outcome data points and 100% of subjects. Once data entry, review, and resolution have been completed for all enrolled subjects, the database will be considered closed. Once all data corrections have been made, the database will be considered locked. The PI can unlock the database should additional data become available and deemed critical to the success of the study. Access to the locked database will be limited to read-only. The database will be password-protected and will reside on a secure password-protected website. The PI will be responsible for retaining source documentation for all data. The database will be maintained in a 'locked' mode for a minimum of three years after the close of the project, in order to provide any unpublished data to a third party, with approval of the IRB.

14. REFERENCES

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