Effects of IV-Administered Ca-DTPA and Zn-DTPA To Treat Patients With Gadolinium Deposition Disease

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INVESTIGATOR-INITIATED INVESTIGATIONAL NEW DRUG (IND) APPLICATION TO STUDY THE EFFECTS OF INTRAVENOUSLY ADMINISTERED CA-DTPA AND ZN-DTPA TO TREAT PATIENTS WITH GADOLINIUM DEPOSITION DISEASE

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Protocol Number: *GDD-001*

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List of Abbreviations

DTPA—Diethylene Triamine Penta-acetic Acid

Gd—Gadolinium

GBCA—Gadolinium Based Contrast Agent

MRI—Magnetic Resonance Imaging

Study Summary

Title	INVESTIGATOR-INITIATED INVESTIGATIONAL NEW DRUG (IND) APPLICATION TO STUDY THE EFFECTS OF INTRAVENOUSLY ADMINISTERED CA-DTPA AND ZN-DTPA TO TREAT PATIENTS WITH GADOLINIUM DEPOSITION DISEASE
Short Title	Ca/Zn DTPA for GDD
Protocol Number	GDD-001
Phase	Feasibility study
Methodology	Open label, non-randomization
Study Duration	1 year
Study Center(s)	Single center
Objectives	To see an increase in the elimination of Gd as measured by analysis of 24 hour urine collections.
Number of Subjects	20
Diagnosis and Main Inclusion Criteria	Gadolinium Deposition Disease (GDD)— *Symptoms of pain, depressed levels of consciousness, and skin induration post administration of a GBCA as well as elevated levels of gadolinium in 24 hr urine, greater than 30 days post administration of a GBCA
Study Product, Dose, Route, Regimen	Ca –DTPA (0.5 gm) administered IV push over 1 minute at the beginning of infusion, along with normal saline (over a 90 minute period), ending with another 0.5 gm DTPA dose with 10 minutes left of saline infusion. This will be followed the next day with Zn-DTPA (0.5 gm) administered IV push over 1 minute with normal saline (over a 90 minute period), ending with another 0.5 gm DTPA dose with 10 minutes left of saline infusion. A total of 1 gm of DTPA is administered at each treatment. This sequence will be administered 3 times, approximately 1 month apart.
Duration of administration	3 months
Reference therapy	No treatments established for this condition
Statistical Methodology	R, a language and environment for statistical computing (R Core Team, Vienna, Austria), will be used for all statistical computing. Statistical significance will be defined as p value less than 0.05.

1 Introduction

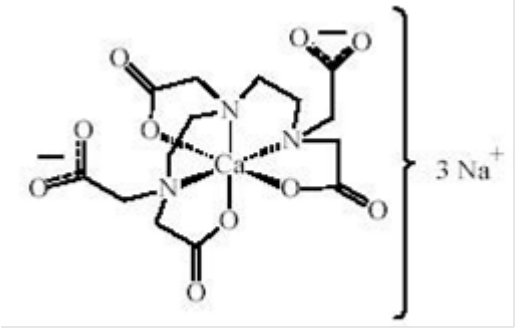
This document is a clinical research protocol and the described study will be conducted in compliance with the protocol, Good Clinical Practices standards and associated Federal regulations, and all applicable University research requirements. This study involves the treatment of patients with Gadolinium Deposition Disease (GDD) by intravenous administration of the chelating agents Ca-DTPA and Zn-DTPA. These chelating agents are approved for the treatment of individuals who have been contaminated by the actinide elements Plutonium (Pu), Americium (Am) and Curium (Cm). Based on the similarity of Gadolinium (Gd) to these elements and the use of DTPA as a chelate in Gd-based contrast agents (GBCAs) used in Magnetic Resonance Imaging (MRI), it is expected that Ca-DTPA and Zn-DTPA will be effective in reducing the total body burden of Gd in patients suffering from GDD.

1.1 Background

Gadolinium (Gd) is a heavy metal that is a principal component of many MRI contrast agents. Gd is always administered as a chelate in which the chelating agent (ligand) is expected to tightly bind the Gd ions. The Gd-chelate prevent the toxic Gd ions from directly interacting with cells and tissues. They also help to promote rapid elimination of the contrast agent from the body after the imaging procedure is complete. There are several commercially available Gd-based contrast agents (GBCAs). There have been growing concerns about the in vivo stability of some of these chelators. Nephrogenic Systemic Fibrosis (NSF) was first described in 2007 and was directly attributed to the release of Gd ions from MRI contrast agents, OptiMARK and Omniscan in particular, in patients with renal failure. More recently, patients with normal renal function have been reporting serious illnesses after administration of all classes of GBCAs.

1.2 Investigational Agent

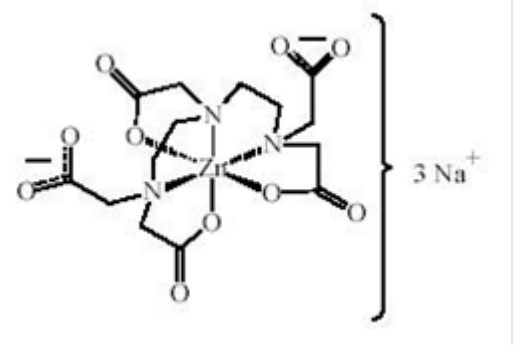
Ca-DTPA (Pentetate calcium trisodium injection) and Zn-DTPA (Pentetate zinc trisodium injection) are FDA-approved products for removal of the radioactive actinides americium (Am), curium (Cm) and plutonium (Pu) for use in a radiological disaster or following nuclear terrorism event. Their mechanism of action is to chelate (bind) heavy metals in the circulation and enhance their renal elimination.



Pentetate calcium trisodium injection contains the sodium salt of calcium diethylenetriaminepentaacetate. Pentetate calcium trisodium is also known as trisodium calcium diethylenetriaminepentaacetate and is commonly referred to as Ca-DTPA. It has a molecular formula of $Na_3CaC_{14}H_{18}N_3O_{10}$ and a molecular weight of 497.4 Daltons. Ca-DTPA is supplied as a clear, colorless, hyperosmolar (1260 mOsmol/kg) solution in a colorless ampoule containing 5 mL. The ampoule contents are sterile, non-pyrogenic and suitable for intravenous administration. Each mL of solution contains the equivalent of 200 mg pentetate

calcium trisodium (obtained from 158.17 mg pentetic acid, 40.24 mg calcium carbonate and NaOH) in Water for Injection, USP. The pH of the solution is adjusted with NaOH and is between 7.3 - 8.3.

Pentetate zinc trisodium injection contains the sodium salt of zinc diethylenetriaminepentaacetate. Pentetate zinc trisodium is also known as trisodium zinc diethylenetriaminepentaacetate and is commonly referred to as Zn-DTPA. It has a molecular formula of $Na_3ZnC_{14}H_{18}N_3O_{10}$ and a molecular weight of 522.7 Daltons. Zn-DTPA is supplied as a clear, colorless, hyperosmolar (1260 mOsmol/kg) solution in a colorless ampoule containing 5 mL. The ampoule contents are sterile, non-pyrogenic and suitable for intravenous administration. Each mL of solution contains the equivalent of 200 mg pentetate zinc trisodium (obtained from 150.51 mg pentetic acid, 31.14 mg zinc oxide and NaOH) and water for injection, USP. The pH of the solution is adjusted with NaOH and is between 6.5 – 7.5.



Ca-DTPA and Zn-DTPA form stable chelates with metal ions by exchanging calcium or zinc for a metal of greater binding capacity. The radioactive chelates are then excreted by glomerular filtration into the urine

1.3 Preclinical Data

The log of thermodynamic binding constant (log K) of DTPA for Gd is comparable to those of the three heavy metals for which Ca-DTPA and Zn-DTPA are approved and far greater than the two most relevant physiological metals (Ca and Zn):

	Log K
Am	22.92
Cm	22.99
Pu3+	20.58
Gd	22.46
Ca	10.74
Zn	18.75

Note that these are log binding constants; thus, Gd binds to DTPA as tightly as the approved metals (Am, Cm and Pu) and 4-12 orders of magnitude greater than Zn and Ca.

1.4 Clinical Data to Date

Prior to formal approval by the FDA in 2005, Ca-DTPA and Zn-DTPA had been used to treat contaminated workers in the nuclear energy industry. In the period from 1958 to 1987, 485 patients received a total of 3077 doses of DTPA, about two-thirds as the calcium salt. Minor transient effects were observed in 12 patients, but no serious or long-term effects were reported. One worker was administered a total of 583 g of DTPA, primarily as the zinc salt, over a 4-year period without any observed toxicological effects (the usual recommended human dosage for a single administration is 0.5 to 1 gram).

In France, over 500 workers have been given a single dose of these agents by slow intravenous infusion and over 200 workers have received multiple dosages without adverse effects.

One of the most widely used GBCA, Magnevist, is actually a chelate of Gd and DTPA. It is estimated that 80 million doses Magnevist have been administered to patients undergoing an MRI procedure, and it has exhibited a very high safety profile. Of note, 50 million doses of Omniscan, which employs a di-amide chelator, have been administered to patients. This agent incorporates 5% free ligand in order to improve safety as it serves to scavenge Gd ions released from its chelated form. A similar di-amide Gd chelate, Optimark, contains 10% free ligand for the same reason.

DTPA is also used as a chelator for radiopharmaceuticals employing other heavy metals, e.g., 99mTc-DTPA (TechneScan®DTPA) and 111In-DTPA (Pentetate Indium Disodium In 111®) used as diagnostic agents in nuclear medicine. These agents have been safely used in millions of patients over the past four decades.

1.5 Dose Rationale and Risk/Benefits

The doses selected (1 gram each of Ca-DTPA and Zn-DTPA) are based on information in the package insert of these products for use as radionuclide decorporation agents.

It is worth emphasizing that DTPA (and other chelators in different GBCA products) represents the component that makes MRI contrast agents 'safe', and we will administer it in approximately 1/4 the standard dose of the MR contrast agents. As a side note, it should be observed that the total amount of DTPA ligand is approximately the same amount of free ligand that is delivered when a magnetic resonance angiogram is performed using Optimark as the contrast agent. Thus, the precedent exists for this in clinical practice, with the comparable intention of scavenging free Gd to minimize the potential for deposition.

2 Study Objectives

Primary Objective:

- To observe an increase in the elimination of Gd as measured by analysis of 24-hour urine collections.

Secondary Objective:

- To observe a measurable reduction or elimination of symptoms caused by administration of a GBCA.
- Evaluate chemokine mediators and function of the mononuclear phagocyte system prior to and after treatment with DTPA.

3 Study Design

3.1 General Design

This is an open-label study. Patients will be evaluated at a baseline visit to determine eligibility and obtain informed consent before proceeding on to study-related procedures. Most patients coming for treatment will have had prior gadolinium testing greater than 30 days post MRI/contrast agent that shows an elevated result, thus eligibility for chelation therapy.

In addition to previous gadolinium testing, subjects will be instructed to perform a 24-hour urine collection (at visit 0) to determine a baseline for evaluating the elimination of gadolinium before initiating the first treatment.

At the first and subsequent chelation therapy visits, a baseline blood pressure will be obtained, an IV will be placed by Vascular Interventional Radiology (VIR) nursing staff, and 1L of normal saline will be connected. Initially, 2.5 mL of Ca-DTPA (1 g/5 mL) will be administered over a period of 1 minute. Five (5)

minutes post DTPA blood pressure will be collected again. The saline will then be administered at a rate of 300 mL/hr over 30 minutes with the patient seated and with hands lowered by their sides to allow the chelator to dwell slightly in the soft tissues of the hands and feet. The patient will then be positioned in a supine position on a hospital bed/gurney and the infusion rate will then be increased to 750 mL/hr over the next 60 minutes. With 10 minutes remaining (80 minutes after the start of infusion), the remaining 2.5 mL of Ca-DTPA will be administered I.V. over a period of 1 minute. The infusion of saline will continue another 9 minutes, and the I.V. line will then be removed. The patient will be instructed to drink plenty of fluids that evening. The following day, the treatment will be repeated using Zn-DTPA.

The patient will be instructed to collect 24-hour total urine samples beginning once chelation therapy starts, and bring back to the lab the following days.

The process will be repeated monthly, for a total of 3 chelation treatment time-points.

Patients will come to clinic for follow up to be assessed for adverse events one week following each treatment. Patients will be asked to fill out pain/QOL questionnaires and given a brief physical exam. Patients who live greater than 3 hours away will be assessed via phone call and instructed to get labs drawn locally. Patients may be advised to seek medical attention if the investigator feels any adverse reaction is not expected or is severe.

The number and function of monocytes and macrophages will be evaluated using 6 mL of blood. A 6 mL blood sample will be obtained in a heparin (green-top) tube and used to determine of the number and function of monocytes and macrophages via flow cytometry (phagocytosis and respiratory burst). This sample will be kept at ambient temperature, and must be processed within 39 hours. These assays will be performed in accordance with the standard operating procedures in Dr. William Zamboni's laboratory and the UNC Flow Cytometry Core Facility.

Mediators of the MPS, including cytokines/chemokines, will be measured with a 6 mL blood sample obtained in a heparin (green-top) tube. Blood will be processed to plasma by centrifugation at 1500 x g for 5 minutes at 4°C. Plasma should be removed using a pipet and distributed evenly between two screw-top tubes. Plasma should be processed within 2 hours and stored at -80C until evaluation in accordance with standard operating procedures within Dr. William Zamboni's laboratory.

3.2 Primary Study Endpoints

To observe a reduction or elimination of symptoms caused by gadolinium deposition after administration of a GBCA.

3.3 Secondary Study Endpoints

To observe increased elimination of gadolinium in 24-hour urine collections post infusion therapy, estimated at greater than two-fold increase.
The pre-chelation Gd amount in urine should decrease from the first to third treatment.

3.4 Primary Safety Endpoints

Clinically significant hypocalcemia will be monitored.

4 Subject Selection and Withdrawal

4.1 Inclusion Criteria

1. Have been administered a GBCA ≥ 30 days, but less than 10 years, prior with a confirmation lab result of elevated levels by 24 hour urine
2. Exhibiting ≥ 3 of the following symptoms which must be present after having been administered a GBCA:
    a. Cognitive disturbance

b. Extremity pain
c. Headache
d. Chest wall pain
e. Skin induration
f. Skin hyperpigmentation
g. Skin pain
h. Arthralgia

4.2 Exclusion Criteria

1. Pregnant/lactating
2. Less than 18 years old
3. If there is no evidence of gadolinium (has to have shown previous demonstration of Gd by bone biopsy or urine analysis)
4. Known connective tissue disease such as Systemic Lupus Erythematosus and scleroderma
5. Severe hemochromatosis or Wilson's disease
6. Glomerular filtration rate (GFR) ≤60
7. Have not had an investigational drug within the past 30 days
8. Unable to give written consent
9. Multiple sclerosis
10. Chronic heart failure
11. Cirrosis of the liver

4.3 Subject Recruitment and Screening

Study subjects will be recruited as they seek us out from patient activist groups of gadolinium toxicity. In most cases it will be a first come first serve basis, but patients will also be prioritized based on levels of incapacitation by the gadolinium deposition disease.

Screening will include:

24-hour gadolinium urine
Pain/QOL assessments
Blood chemistries: calcium, zinc, manganese, magnesium, sodium, potassium, chloride, Creatinine, BUN, CO2, Phosphorus, CBC/Diff, urinalysis, creatinine clearance.

4.4 Early Withdrawal of Subjects

Patients may remove themselves from the study at any time for any reason. The investigators may remove patients from the study for any reason, such as non-compliance, or patient safety concerns.

4.4.1 When and How to Withdraw Subjects

Study subjects will be withdrawn from the study if they experience any known or unknown severe adverse event following administration of either Ca-DTPA or Zn-DTPA. Treatment will be discontinued immediately following any severe adverse event.

4.4.2 Data Collection and Follow-up for Withdrawn Subjects

The same labs done at screening will be collected, and a follow up phone call will be made one week after the event.

5 Study Drug

5.1 Description

Ca-DTPA (Pentetate calcium trisodium injection 1g/5 mL) and

Zn-DTPA (Pentetate zinc trisodium injection 1 g/5 mL).

5.2 Treatment Regimen

Initially, 2.5 mL of Ca-DTPA (1 g/5 ml) will be administered over a period of 1 minute. A solution of normal saline will then be administered at a rate of 300 mL/hr over 30 minutes. The patient will be seated with hands lowered to their sides to allow the chelator to dwell slightly in the soft tissues of the hands and feet. The patient will then be moved to a supine position and the infusion rate will then be increased to 750 mL/hr over the next 60 minutes. At 90 minutes the remaining 2.5 mL of Ca-DTPA will administered I.V. over a period of 1 minute, and the normal saline will continue for the remaining 9 minutes. The I.V. line will then be removed. The patient will be instructed to drink plenty of fluids that evening. The following day, the treatment will be repeated using Zn-DTPA. The same treatment therapy will consist of 3 time-points, approximately 1 month apart.

5.3 Method for Assigning Subjects to Treatment Groups

No randomization will be used. All patient subjects will receive the same treatment therapy.

5.4 Preparation and Administration of Study Drug

The appropriate doses will be drawn into a syringe from the Ca-DTPA and Zn-DTPA ampoules by the trained nursing staff and administered intravenously by a physician through an IV infusion line with the agents administered through a T-valve connection as a bolus. No placebo will be used.

5.5 Subject Compliance Monitoring

Subjects are treated and discharged from the clinic. They will be instructed to drink plenty of fluids, and collect urine for the following 24 hours to be returned the following day. Subjects will also have blood chemistries drawn the day following the second infusion for monitoring. Subject treatment will discontinue if they do not collect the 24 hour urine sample for testing following the infusion therapy, or refuse blood chemistry monitoring.

5.6 Prior and Concomitant Therapy

We will be excluding patients who have had an investigational product administered within the previous 30 days.

5.7 Packaging

The study drug will be used as supplied by the manufacturer. Vials will be received from the manufacturer packaged as 10 ampoules (1g/5 mL) Ca-DTPA and 10 ampoules (1gm/5 mL) of Zn-DTPA. A total of four packages (40 ampoules) of each product will be received. This will allow enough doses for each subject to be administered up to three doses of Ca-DTPA and three doses of Zn-DTPA as well as allowing extra vials for breakage, spillage, etc.

5.8 Blinding of Study Drug (if applicable)

No blinding of drug will be incorporated. All patient subjects will be treated with the same therapy regimen.

5.9 Receiving, Storage, Dispensing and Return

5.9.1 Receipt of Drug Supplies

Study drug receipt, storage, and dispensing and accountability will be managed by our Investigational Drug Services pharmacy. Upon receipt of the of the study treatment supplies, an inventory is performed and a drug receipt log filled out and signed by the person accepting the shipment. The investigator must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site.

5.9.2 Storage

The manufacturer recommends that the products be stored between 15 - 30°C (59 - 86°F), and will be stored in the Investigation Drug pharmacy.

5.9.3 Dispensing of Study Drug

Study drug receipt, storage, and dispensing and accountability will be managed by our Investigational Drug Services pharmacy. The pharmacy must notify study sponsor of any damaged or unusable study treatments that were supplied to the investigator's site. Regular study drug reconciliation will be performed to document drug assigned and when.

5.9.4 Return or Destruction of Study Drug

At the completion of the study, there will be a final reconciliation of drug shipped, drug consumed, and drug remaining. This reconciliation will be logged on the drug reconciliation form, signed and dated. Any discrepancies noted will be investigated, resolved, and documented prior to return or destruction of unused study drug. Drug destroyed on site will be documented in the study files.

6 Study Procedures

6.1 **Visit 0**- The patient will be seen and evaluated by a UNC clinician regarding symptoms, assessments, a physical exam, a medication history and lab values up to one week prior to anticipated therapy start date. After agreeing to participate in the study, a coordinator will obtain informed consent signatures and the patient will be instructed to collect a 24-hour urine so that gadolinium can be measured for pre-treatment values.

6.2 **Visit 1**- The patient will come for infusion chelation therapy with Ca-DTPA to be done in the Clinical and Translational Research Center. Patient will void and have a baseline blood pressure taken before start of treatment. A urine pregnancy test will be performed if the subject is a woman of child-bearing potential. Blood samples will be collected by phlebotomy trained staff at the Clinical & Translational Research Center (CTRC) at UNC Memorial hospital. Patient blood samples will be obtained just prior to intravenous administration of Ca-DTPA or Zn-DTPA. The IV will be placed by nursing staff, and the infusion of normal saline will begin. The first injection of Ca-DTPA is performed by the investigator, or one of the co-investigators. Five (5) minutes post injection of Ca-DTPA another blood

pressure will be taken. Saline will continue for another 80 minutes, and the second injection of Ca-DTPA will be given by an investigator, followed by the last 10 minutes of saline infusion. IV will be discontinued, vitals checked, and patient will be discharged home. Patient will begin 24-hour urine collection once the infusion is started. They will be instructed to drink plenty of fluids. Analog pain scale sheets will be given to the subject to fill out starting with day 1 through day 6 post treatment, which will be returned on the day 7 visit. Urine is to be brought in on 2nd day of therapy.

6.3 **Visit 2**- The patient subject will come for the second infusion therapy with Zn-DTPA. Patient will void and have a baseline blood pressure taken prior to initiation of therapy. Blood samples will be collected by phlebotomy trained staff at the Clinical & Translational Research Center (CTRC) at UNC Memorial hospital. Patient blood samples will be obtained just prior to intravenous administration of Ca-DTPA or Zn-DTPA. IV placement will be done by nursing staff, normal saline infusion started, and Zn-DTPA injections will be done by investigator or one of the co-investigators exactly as the day prior. Five (5) minutes post injection of Zn-DTPA another blood pressure will be taken. Another 24-hour urine sample will be collected beginning after chelation therapy is initiated, to be returned then next day.

6.4 **Visit 3**- The patient will return to the hospital to drop off 24 hour urine for gadolinium testing, as well as a creatinine clearance. Blood chemistries calcium, zinc, magnesium and manganese will be evaluated, along with BUN, creatinine, sodium, potassium, chloride, phosphorus and CO2.

6.5 **Visit 4**- The patient subject will come for a follow up assessment by a clinician 1 week (+5 days) following IV therapy, where pain and overall health assessments will be performed. If the patient lives more than 3 hours away, a phone-call follow up will be done and questionnaires will be supplied to be filled out and emailed prior to leaving the infusion therapy. The second therapy session will be scheduled for the following month.

6.6. **Visit 5**- Patient will return for 2nd round of infusion therapy to be done exactly as first, with day one of Ca-DTPA including pregnancy testing on women of child-bearing potential and pain scale assessment sheets for the week. Blood samples will be collected by phlebotomy trained staff at the Clinical & Translational Research Center (CTRC) at UNC Memorial hospital. Patient blood samples will be obtained just prior to intravenous administration of Ca-DTPA or Zn-DTPA.

6.7 **Visit 6**- Patient returns for day two of infusion therapy with Zn-DTPA. Blood samples will be collected by phlebotomy trained staff at the Clinical & Translational Research Center (CTRC) at UNC Memorial hospital. Patient blood samples will be obtained just prior to intravenous administration of Ca-DTPA or Zn-DTPA

6.8 **Visit 7**- The patient will return to the hospital to drop off 24-hour urine for gadolinium testing, as well as a creatinine clearance. Blood chemistries calcium, zinc, magnesium and manganese will be evaluated, along with BUN, creatinine, sodium, potassium, chloride, phosphorus and CO2.

6.9 **Visit 8**- One week post treatment follow up visit for health assessments as before. Third therapy session will be scheduled for one month out.

6.10 **Visit 9**- Patient returns for final round of infusion therapy to be done exactly as first two treatments with day one of Ca-DTPA, including pregnancy testing on women of child-bearing potential and pain assessment sheets for the week. Blood samples will be collected by phlebotomy trained staff at the Clinical & Translational Research Center (CTRC) at UNC Memorial hospital. Patient blood samples will be obtained just prior to intravenous administration of Ca-DTPA or Zn-DTPA

6.11 **Visit 10**- Patient returns for day two of infusion therapy with Zn-DTPA. Blood samples will be collected by phlebotomy trained staff at the Clinical & Translational Research Center (CTRC) at UNC Memorial hospital. Patient blood samples will be obtained just prior to intravenous administration of Ca-DTPA or Zn-DTPA

6.12 **Visit 11**- The patient will return to the hospital to drop off 24-hour urine for gadolinium testing, as well as a creatinine clearance. Blood chemistries calcium, zinc, magnesium and manganese will be evaluated, along with BUN, creatinine, sodium, potassium, chloride, phosphorus and CO2.

6.13 **Visit 12**- Subject returns for final follow up assessments 1 week (+5 days) following last infusion therapy. If patient lives more than 3 hours away, a phone-call will be done by the treating physician or clinician.

6.14 **Visits 13-14**- Subjects will receive a phone call from the coordinator to go over pain the pain assessment scale at months 6 and 12 following treatment.

At any time in between treatments if the clinician or patient feels they need to be seen, they will be brought in to the clinic (or to their local healthcare provider) for evaluation and possible discontinuation of therapy.

7 Statistical Plan

7.1 *Sample Size Determination*

Twenty subjects will be evaluated to determine the safety profile of chelation in this setting and to provide information on potential endpoints for future studies in this area. The distributions of urinary measures of Gd excretion as well as for pain and quality of life will be evaluated at baseline and after each treatment, as will the differences in these measures between baseline and each treatment.

7.2 *Statistical Methods*

Changes between baseline and each treatment will be evaluated for direction and magnitude. Although the goal is estimation of effect size and variation, statistical tests for changes pre/post treatment will be evaluated to understand the pre/post changes toward development of a future trial. These tests will evaluate the null hypothesis of no change in urine measures or symptoms (via questionnaire). If the changes in measures are normally distributed, then paired t-tests will be used. However, it is more likely that the data will not be normally distributed and that Wilcoxon signed-rank tests will be need. For any nominal (yes/no) questionnaire data, a McNemar's or sign test will be used for the paired contingency table. Since these tests will likely be used in future studies, direction of effect measures (differences) and ranges of estimates (confidence intervals) will provide information on what to expect in testing and analyzing more individuals

Subject Population(s) for Analysis

All-treated population: All subjects consenting to study will be treated with the same therapy, receiving at least one dose of study drug.

8 Safety and Adverse Events

8.1 Definitions

Unanticipated Problems Involving Risk to Subjects or Others
Any incident, experience, or outcome that meets all of the following criteria:

- Unexpected in nature, severity, or frequency (i.e. not described in study-related documents such as the IRB-approved protocol or consent form, the investigators brochure, etc)
- Related or possibly related to participation in the research (i.e. possibly related means there is a reasonable possibility that the incident experience, or outcome may have been caused by the procedures involved in the research,

- Serious (as defined below) "***Serious" is different than "severe" as reported in the CTC criteria that applies a grade to the AE.***

Adverse Event
An ***adverse event*** (AE) is any symptom, sign, illness or experience that develops or worsens in severity during the course of the study. Intercurrent illnesses or injuries should be regarded as adverse events. Abnormal results of diagnostic procedures are considered to be adverse events if the abnormality:

- results in study withdrawal
- is associated with a serious adverse event
- is associated with clinical signs or symptoms
- leads to additional treatment or to further diagnostic tests
- is considered by the investigator to be of clinical significance

Serious Adverse Event
Adverse events are classified as serious or non-serious. A ***serious adverse event*** is any AE that is:

- fatal
- life-threatening
- requires or prolongs hospital stay
- results in persistent or significant disability or incapacity
- a congenital anomaly or birth defect
- an important medical event

Important medical events are those that may not be immediately life threatening, but are clearly of major clinical significance. They may jeopardize the subject, and may require intervention to prevent one of the other serious outcomes noted above. For example, drug overdose or abuse, a seizure that did not result in in-patient hospitalization or intensive treatment of bronchospasm in an emergency department would typically be considered serious.

All adverse events that do not meet any of the criteria for serious should be regarded as ***non-serious adverse events***.

For the purpose of this trial, the following will be considered expected adverse events:

> **Ca-DTPA—headache, lightheadedness, chest pain, allergic reaction, dermatitis, metallic taste, nausea and diarrhea, and injection site reactions.**
>
> **Zn-DTPA—headache, lightheadedness, and pelvic pain**.
>
> Possible "flare effect", resulting in exacerbation of current symptoms.

Adverse Event Reporting Period
The study period during which adverse events must be reported is normally defined as the period from the initiation of any study procedures to the end of the study treatment follow-up. For this study, the study treatment follow-up is defined as 30 days following the last administration of study treatment.

Preexisting Condition
A preexisting condition is one that is present at the start of the study. A preexisting condition should be recorded as an adverse event if the frequency, intensity, or the character of the condition worsens during the study period.

General Physical Examination Findings

At screening, any clinically significant abnormality should be recorded as a preexisting condition. At the end of the study, any new clinically significant findings/abnormalities that meet the definition of an adverse event must also be recorded and documented as an adverse event.

Post-study Adverse Event
All unresolved adverse events should be followed by the investigator until the events are resolved, the subject is lost to follow-up, or the adverse event is otherwise explained. At the last scheduled visit, the investigator should instruct each subject to report any subsequent event(s) that the subject, or the subject's personal physician, believes might reasonably be related to participation in this study. The investigator should notify the study sponsor of any death or adverse event occurring at any time after a subject has discontinued or terminated study participation that may reasonably be related to this study. The sponsor should also be notified if the investigator should become aware of the development of cancer or of a congenital anomaly in a subsequently conceived offspring of a subject that has participated in this study.

Abnormal Laboratory Values
A clinical laboratory abnormality should be documented as an adverse event if any one of the following conditions is met:

- The laboratory abnormality is not otherwise refuted by a repeat test to confirm the abnormality
- The abnormality suggests a disease and/or organ toxicity
- The abnormality is of a degree that requires active management; e.g. change of dose, discontinuation of the drug, more frequent follow-up assessments, further diagnostic investigation, etc.

Hospitalization, Prolonged Hospitalization or Surgery
Any adverse event that results in hospitalization or prolonged hospitalization should be documented and reported as a serious adverse event unless specifically instructed otherwise in this protocol. Any condition responsible for surgery should be documented as an adverse event if the condition meets the criteria for and adverse event.

Neither the condition, hospitalization, prolonged hospitalization, nor surgery are reported as an adverse event in the following circumstances:

- Hospitalization or prolonged hospitalization for diagnostic or elective surgical procedures for a preexisting condition. Surgery should ***not*** be reported as an outcome of an adverse event if the purpose of the surgery was elective or diagnostic and the outcome was uneventful.
- Hospitalization or prolonged hospitalization required to allow efficacy measurement for the study.
- Hospitalization or prolonged hospitalization for therapy of the target disease of the study, unless it is a worsening or increase in frequency of hospital admissions as judged by the clinical investigator.

8.2 Recording of Adverse Events

At each contact with the subject, the investigator must seek information on adverse events by specific questioning and, as appropriate, by examination. Information on all adverse events should be recorded immediately in the source document, and also in the appropriate adverse event module of the case report form (CRF). All clearly related signs, symptoms, and abnormal diagnostic procedures results should recorded in the source document, though should be grouped under one diagnosis.

All adverse events occurring during the study period must be recorded. The clinical course of each event should be followed until resolution, stabilization, or until it has been determined that the study treatment or participation is not the cause. Serious adverse events that are still ongoing at the end of the study period must be followed up to determine the final outcome. Any serious adverse event that occurs after the study period and is considered to be possibly related to the study treatment or study participation should be recorded and reported immediately.

8.3 Reporting of Serious Adverse Events and Unanticipated Problems

Investigators and the protocol sponsor must conform to the adverse event reporting timelines, formats and requirements of the various entities to which they are responsible, but at a minimum those events that must be reported are those that are:

- related to study participation,
- unexpected, and
- serious or involve risks to subjects or others
  (see definitions, section 8.1).

If the report is supplied as a narrative, the minimum necessary information to be provided at the time of the initial report includes:

- Study identifier
- Study Center
- Subject number
- A description of the event
- Date of onset
- Current status
- Whether study treatment was discontinued
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study treatment

8.3.1 Investigator reporting: notifying the study sponsor

Any study-related unanticipated problem posing risk of harm to subjects or others, and any type of serious adverse event, must be reported to the study sponsor by telephone within 24 hours of the event. To report such events, a Serious Adverse Event (SAE) form must be completed by the investigator and faxed to the study sponsor within 24 hours. The investigator will keep a copy of this SAE form on file at the study site. Report serious adverse events by phone and facsimile to:

Within the following 48 hours, the investigator must provide further information on the serious adverse event or the unanticipated problem in the form of a written narrative. This should include a copy of the completed Serious Adverse Event form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing serious adverse events should be provided promptly to the study sponsor

8.3.2 Investigator reporting:

For reportable deaths, the initial submission to the UNC IRB may be made by contacting the IRB Director or Associate Director. The AE/Unanticipated Problem Form is required as a follow up to the initial submission.

Other Reportable events:
For clinical drug trials, the following events are also reportable to the UNC IRB:

- Any adverse experience that, even without detailed analysis, represents a serious unexpected adverse event that is rare in the absence of drug exposure (such as agranulocytosis, hepatic necrosis, Stevens-Johnson syndrome).
- Any adverse event that would cause the sponsor to modify the investigators brochure, protocol or informed consent form, or would prompt other action by the IRB to assure protection of human subjects.
- Information that indicates a change to the risks or potential benefits of the research, in terms of severity or frequency. For example:
  - An interim analysis indicates that participants have a lower rate of response to treatment than initially expected.
  - Safety monitoring indicates that a particular side effect is more severe, or more frequent than initially expected.

  - A paper is published from another study that shows that an arm of your research study is of no therapeutic value.
- Change in FDA safety labeling or withdrawal from marketing of a drug, device, or biologic used in a research protocol.
- Breach of confidentiality
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research participant.
- Incarceration of a participant when the research was not previously approved under Subpart C and the investigator believes it is in the best interest of the subject to remain on the study.
- Complaint of a participant when the complaint indicates unexpected risks or the complaint cannot be resolved by the research team.
- Protocol violation (meaning an accidental or unintentional deviation from the IRB approved protocol) that in the opinion of the investigator placed one or more participants at increased risk, or affects the rights or welfare of subjects.

8.3.3 Sponsor reporting: Notifying the FDA

The study sponsor is required to report certain study events in an expedited fashion to the FDA. These written notifications of adverse events are referred to as IND safety reports. The following describes the safety reporting requirements by timeline for reporting and associated type of event:

- ***Within 7 calendar days***
  Any study event that is:
  - associated with the use of the study drug
  - unexpected,
  - fatal or life-threatening, and

- ***Within 15 calendar days***
  Any study event that is:
  - associated with the use of the study drug,
  - unexpected, and
  - serious, but not fatal or life-threatening

    -or-
  - a previous adverse event that was not initially deemed reportable but is later found to fit the criteria for reporting (reporting within 15 calendar days from when event was deemed reportable).

  Any finding from tests in laboratory animals that:
  - suggest a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.

Additional reporting requirements
Sponsors are also required to identify in IND safety reports all previous reports concerning similar adverse events and to analyze the significance of the current event in light of the previous reports.

Reporting Process
Adverse events will be submitted on FDA Form 3500A or in a narrative format, along with FDA form 1571. If supplied as in a narrative format, the minimum information to be supplied is noted above at the beginning of section 8.3.

8.4 Stopping Rules

The study will be stopped for review if there is greater than 2 of the first 3 patients, or 5 of the first 10 patients experience an unanticipated severe adverse event, or more than one grade IV expected adverse events as defined by CTCAE v.4.0.

Study subjects will be withdrawn from the study if they experience any unknown severe adverse event (grade 3 or 4) following administration of either Ca-DTPA or Zn-DTPA. We will be using the CTCAE v.4.0 scale to measure severity of adverse events.

Any patient who shows a Grade 3 hypocalcemia (<7.0-6.0 mg/dl) post infusion therapy will be discontinued from the study.

The study will be stopped for any study-related death.

8.5 Medical Monitoring

It is the responsibility of the Principal Investigator to oversee the safety of the study at his/her site. This safety monitoring will include careful assessment and appropriate reporting of adverse events as noted above, as well as the construction and implementation of a site data and safety-monitoring plan (see section 9 Auditing, Monitoring and Inspecting). Medical monitoring will include a regular assessment of the number and type of serious adverse events.

Safety Monitoring will be performed by Jeff Neitlich, MD, who is not a study investigator. The CRFs and any relevant source documents will be sent to the medical safety monitor (as above) who will review them after treatment is complete for subject 1, 3, 10 and 20.

The research coordinator will complete the appropriate report form and logs; assist the PI to prepare reports and notify the IRB, FDA, and/or safety monitor of all Unanticipated Problems/SAE's.

The research coordinator and co-principal investigators will confirm that all Adverse effects (AE) are correctly entered into the AE log by the coordinator; be available to answer any questions that the coordinators may have concerning AEs; notify the IRB and FDA of all Unanticipated Problems/SAEs and AEs as appropriate. All assessments of AEs will be made by a licensed medical professional who is an investigator on the research.

Complications will be assessed by the co-PIs, categorized into major and minor categories and recorded on the CRF. CRFs and appropriate source documents will be made available to this individual for bi-monthly (every 2 months) review to ensure completeness of data collection. Any discrepancies will be immediately addressed by the co-PIs. All adverse events will be recorded and then summarized for inclusion in the final manuscript.

Data monitoring will be performed by an individual who is not a study investigator. CRFs and appropriate source documents will be made available to this individual for review to ensure completeness of data collection. Any discrepancies will be immediately addressed by the co-PIs.

- Verify receipt of all documents and supplies needed to conduct study
- Informed consent obtained for each participant
- Source document verification 100%
- CRF completion
- Investigational product accountability
- Check and review of the regulatory binder and all essential documents
- Clinical supply inventory
- SAE reporting
- Protocol amendment and their approval by the IRB
- Significant protocol deviations

• Personnel changes
• Updated regulatory documentation
• Any other issue as deemed important to the conduct of the study

9 Data Handling and Record Keeping

9.1 Confidentiality

Information about study subjects will be kept confidential and managed according to the requirements of the Health Insurance Portability and Accountability Act of 1996 (HIPAA). Those regulations require a signed subject authorization informing the subject of the following:

- What protected health information (PHI) will be collected from subjects in this study
- Who will have access to that information and why
- Who will use or disclose that information
- The rights of a research subject to revoke their authorization for use of their PHI.

In the event that a subject revokes authorization to collect or use PHI, the investigator, by regulation, retains the ability to use all information collected prior to the revocation of subject authorization. For subjects that have revoked authorization to collect or use PHI, attempts should be made to obtain permission to collect at least vital status (i.e. that the subject is alive) at the end of their scheduled study period.

9.2 Source Documents

Source data is all information, original records of clinical findings, observations, or other activities in a clinical trial necessary for the reconstruction and evaluation of the trial. Source data are contained in source documents. Examples of these original documents, and data records include: hospital records, clinical and office charts, laboratory notes, memoranda, subjects' diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies or transcriptions certified after verification as being accurate and complete, microfiches, photographic negatives, microfilm or magnetic media, x-rays, subject files, and records kept at the pharmacy, at the laboratories, and at medico-technical departments involved in the clinical trial.

9.3 Case Report Forms

The study case report form (CRF) is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the procedure was not done or the question was not asked, write "N/D". If the item is not applicable to the individual case, write "N/A". All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, draw a single straight line through the incorrect entry and enter the correct data above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, print the clarification above the item, then initial and date it.

9.4 Records Retention

It is the investigator's responsibility to retain study essential documents for at least 2 years after the last approval of a marketing application in their country and until there are no pending or contemplated marketing applications in their country or at least 2 years have elapsed since the formal discontinuation of clinical development of the investigational product. These documents should be retained for a longer period if required by an agreement with the sponsor. In such an instance, it is the responsibility of the sponsor to inform the investigator/institution as to when these documents no longer need to be retained.

10 Study Monitoring, Auditing, and Inspecting

10.1 Study Monitoring Plan

This study will be monitored by an independent monitor from outside of UNC. The investigator will allocate adequate time for such monitoring activities. The Investigator will also ensure that the monitor or other compliance or quality assurance reviewer is given access to all the above noted study-related documents and study related facilities (e.g. pharmacy, diagnostic laboratory, etc.), and has adequate space to conduct the monitoring visit.

10.2 Auditing and Inspecting

The investigator will permit study-related monitoring, audits, and inspections by the IRB, the sponsor, government regulatory bodies, and University compliance and quality assurance groups of all study related documents (e.g. source documents, regulatory documents, data collection instruments, study data etc.). The investigator will ensure the capability for inspections of applicable study-related facilities (e.g. pharmacy, diagnostic laboratory, etc.).

Participation as an investigator in this study implies acceptance of potential inspection by government regulatory authorities and applicable University compliance and quality assurance offices.

11 Ethical Considerations

This study is to be conducted according to US and international standards of Good Clinical Practice (FDA Title 21 part 312 and International Conference on Harmonization guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent Ethics Committee (EC) or Institutional Review Board (IRB), in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the EC/IRB concerning the conduct of the study will be made in writing to the investigator and a copy of this decision will be provided to the sponsor before commencement of this study. The investigator should provide a list of EC/IRB members and their affiliate to the sponsor.

All subjects for this study will be provided a consent form describing this study and providing sufficient information for subjects to make an informed decision about their participation in this study. This consent form will be submitted with the protocol for review and approval by the EC/IRB for the study. The formal consent of a subject, using the EC/IRB-approved consent form, must be obtained before that subject undergoes any study procedure. The consent form must be signed by the subject or legally acceptable surrogate, and the investigator-designated research professional obtaining the consent.

12 Study Finances

12.1 Funding Source

The radiology department will be using internal funds. Hameln Pharma GmbH will be providing study drug.

12.2 Conflict of Interest

Any investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must have the conflict reviewed by a properly constituted Conflict of Interest Committee with a Committee-sanctioned conflict management plan that has been reviewed and approved by the study sponsor prior to participation in this study. All University of UNC investigators will follow the University conflict of interest policy.

13 Publication Plan

Neither the complete nor any part of the results of the study carried out under this protocol, nor any of the information provided by the sponsor for the purposes of performing the study, will be published or passed on to any third party without the consent of the study sponsor. Any investigator involved with this study is obligated to provide the sponsor with complete test results and all data derived from the study.

14 References

1. Sieber MA1, Lengsfeld P, Walter J, Schirmer H, Frenzel T, Siegmund F, Weinmann HJ, Pietsch H., Gadolinium-based contrast agents and their potential role in the pathogenesis of nephrogenic systemic fibrosis: the role of excess ligand. J Magn Reson Imaging. 2008 May;27(5):955-62 doi: 10.1002/jmri.21368

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15 Appendices

A. Table of Events
B. Visual Analog Pain Scale
C. Quality of Life scale
D. Copy of Consent Form

Time and Events Table:

	Pre-treatment Visit 0	V 1 Day 1	V 2 Day 2	V 3 Day 3	V 4 Day 7	V 5 Day 1 Mo 2	V 6 Day 2 Mo 2	V 7 Day 3 Mo 2	V 8 Day 7 Mo 2	V 9 Day 1 Mo 3	V10 Day 2 Mo 3	V 11 Day 3 Mo 3	V 12 Day 7 Mo 3	V 13-14 Mo 6 & 12
Physical Assessments	X				X				X				X	
Labs	X	X[c]		X		X[c]		X		X[c]		X		
24 hr urine gd	X	X[a]	X[a]			X[a]	X[a]			X[a]	X[a]			
Pain/QOL questionnaire	X				X[e]				X[e]				X[e]	X[d]
IV Ca-DTPA therapy		X				X				X				
IV Zn-DTPA therapy			X				X				X			

a. Patients will begin 24hr urine collection immediately following therapy
b. Assessments may be done by phone when patient lives greater than 3 hours away
c. Urine pregnancy testing will be done on day one of therapy for WCBP

d. Patients will be called to collect pain and QOL data at months 6 and 12
e. Pain/QOL questionnaires will be filled out days 1-6 and returned day 7