CLINICAL RESEARCH PROTOCOL

NATIONAL INSTITUTE OF ENVIRONMENTAL HEALTH SCIENCES (NIEHS)

DATE:	July 07, 2023
PROTOCOL NUMBER:	17-E-0161
PROTOCOL VERSION:	6.8
IND NUMBER:	135039
IND NAME:	sodium thiosulfate injection
IND HOLDER:	NIEHS
IND MFG:	Hope Pharmaceuticals

TITLE: An Open-label Study of Sodium Thiosulfate for Treatment of Calcinosis Associated with Juvenile and Adult Dermatomyositis

SHORT TITLE: Sodium Thiosulfate (STIC)

IDENTIFYING WORDS: Calcinosis, dystrophic calcification, dermatomyositis, juvenile dermatomyositis, sodium thiosulfate, biomarkers, imaging, adult and pediatric autoimmune disease, idiopathic inflammatory myopathy, treatment trial

PRINCIPAL INVESTIGATOR: Adam Schiffenbauer, M.D., Investigator, Environmental Autoimmunity Group (EAG), NIEHS, National Institutes of Health (NIH), Bethesda, MD*

ESTIMATED DURATION OF STUDY: 6 Years (5 years for enrollment, 1 year for study completion and analysis)

START DATE: July 24, 2017 **END DATE:** December 31, 2022

NUMBER AND TYPE OF PATIENTS:

Accrual ceiling: 250 for screening protocol, 18 enrolled in full protocol (to obtain 13 who complete the study)

	Number	Sex	Age Range
Patients:	250	M/F	≥7 years
Volunteers:	N/A		

PROJECT USES IONIZING RADIATION:

__X___Research and medically indicated:

RSC Approval Number: 2640 **Expiration Date:** 03/23/2026

PROJECT USES "DURABLE POWER OF ATTORNEY": No

Drug Name:	**sodium thiosulfate injection**
IND Number:	**135039**
Sponsor:	**NIEHS**
Manufacturer:	**Hope Pharmaceuticals**

OFF-SITE PROJECT: No

[If yes, please list the location/s:]

MULTI-SITE STUDY: No

Précis

Calcinosis, a serious complication of dermatomyositis, involves deposition of calcium (carbonate apatite) in soft tissue, and can result in negative impacts on quality of life and physical function. To date, there are no known effective therapies that are approved for the treatment of dermatomyositis-associated calcinosis, and there is no consensus within the medical community on the optimum treatment strategy for this often-debilitating condition.

A few reports in the literature describe treatment successes with a variety of therapeutics; however, these data are from anecdotal reports or case series and thus provide limited scientific evidence of effectiveness. Recently published reports as well as personal observations within our group have suggested that intravenous sodium thiosulfate treatment may benefit calcinosis patients. In order to gather more robust data on the utility of this medication in the treatment of calcinosis associated with adult and juvenile dermatomyositis, we propose to evaluate its effects in the context of a prospective clinical trial.

We plan to enroll participants at a single center into a single-arm, open-label study, with the overall objective of evaluating the efficacy and safety of intravenous sodium thiosulfate use in patients with moderate to severe extensive calcinosis associated with juvenile and adult dermatomyositis.

The study will enroll a maximum of 18 participants over 4 years into the full study, but up to 250 patients may screen for study entry. Eligible patients will be age 7 or older, and will have extensive calcinosis (defined as calcinosis involving the torso or 2 extremities) and moderate to severe calcinosis (indicated by a calcinosis activity visual analogue scale score of greater than or equal to 3.5 cm out of 10 cm).

Two separate evaluations performed at the NIH prior to initiation of therapy will be used as baseline data to compare in a pairwise manner to the change in assessments following treatment with sodium thiosulfate, with all other medications remaining stable. Study treatment will be 16 g/m^2 sodium thiosulfate administered 3 times weekly over a period of 10 weeks at the NIH. Subjects who complete 10 weeks of treatment or reach the primary end point by week 6 will be considered completers. Following the treatment period, all participants will return to the NIH for evaluations at weeks 24 and 62.

The primary outcome will be change in calcinosis activity visual analogue scale score from week 0 to week 10 on therapy, compared to the baseline change in calcinosis activity visual analogue scale score from week -10 to week 0 pre-treatment. Secondary measures will evaluate safety and changes in components of the Calcinosis Assessment Tool, clinical assessments of calcinosis, Mawdsley Calcinosis Questionnaire, quality of life, functional disability, muscle testing (manual and quantitative), laboratory parameters (muscle enzymes, inflammatory markers, and endothelial activation markers), gene expression, calcification pathogenesis, time to improvement, and imaging. Myositis disease activity and damage will also be assessed by validated measures.

A number of research studies will be incorporated into this clinical trial in an attempt to understand the immunologic markers associated with calcification in dermatomyositis as well as the immunologic effects of sodium thiosulfate treatment.

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Abbreviations

AE	Adverse event
ACR	American College of Rheumatology
AHRP	Association of Rheumatology Health Professionals
ALT	Alanine aminotransferase
AMAT	Adult Myopathy Assessment Tool
AST	Aspartate aminotransferase
BMD	Bone mineral density
CAT	Calcinosis Assessment Tool
CCD	Charge-coupled device
CD	Clinical director
CFR	Code of Federal Regulations
CHAQ	Childhood Health Assessment Questionnaire
CHQ	Childhood Health Questionnaire
CHQ-PF50	50-Item Child Health Questionnaire – Parent Form
CK	Creatine kinase
CMAS	Childhood Myositis Assessment Score
CT	Computed tomography
CTDB	Clinical Trials Database
dbGaP	Database of Genotypes and Phenotypes
DEXA	Dual-energy x-ray absorptiometry
DLQI	Dermatology Life Quality Index
DM	Dermatomyositis (adult)
DNA	Deoxyribonucleic acid
DSMB	Data and Safety Monitoring Board
EAG	Environmental Autoimmunity Group
EMP	Endothelial microparticles
ESR	Erythrocyte sedimentation rate
FDG	Fluorodeoxyglucose
FI3	Functional Index 3
GCP	Good Clinical Practice

GWAS	Genome-wide association study
HAQ	Health Assessment Questionnaire
HED	Human equivalent dose
ICH	International Conference on Harmonization
IMACS	International Myositis Assessment and Clinical Studies (Group);
IR	Infrared
IRB	Institutional Review Board
IV	Intravenous
JDM	Juvenile dermatomyositis
LD50	Lethal dose, 50%
LDH	Lactate dehydrogenase
LSCI	Laser speckle contrast imaging
MFM	Motor Function Measure in neuromuscular diseases
MMT	Manual muscle testing
MP	Microparticles
MRI	Magnetic resonance imaging
NET	Neutrophil extracellular traps
NIBIB	National Institute of Biomedical Imaging and Bioengineering
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NIEHS	National Institute of Environmental Health Sciences
NIH	National Institutes of Health
NIST	National Institute of Standards and Technology
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PD	Protocol deviation
PDMP	Platelet-derived microparticles
PET	Positron emission tomography
PI	Principle investigator
PROMIS	Patient Reported Outcomes Measurement Information System
PT	Prothrombin Time
PTT	Partial Thromboplastin Time
QOL	Quality of life

rem	Unit of radiation (derived from Roentgen equivalent man)
RNA	Ribonucleic acid
ROM	Range of motion
SAE	Serious adverse event
SF36	36-Item Short Form Health Survey
STS	Sodium Thiosulfate
UADE	Unanticipated adverse device effect
ULN	Upper limit of normal
UP	Unanticipated problem
US	United States
VAS	Visual analogue scale
WHO	World Health Organization

1. Background

Juvenile and adult dermatomyositis (JDM, DM respectively) are inflammatory muscle diseases that can also have protean non-muscular manifestations. One such non-muscle complication is calcinosis, which is the development of dystrophic soft tissue calcification. This complication is more common in pediatric patients with JDM than adults, with calcinosis occurring in up to 35% of JDM patients and up to 20% of adult DM patients. Spontaneous resolution of cutaneous calcification is rare, treatment remains a challenge, and calcinosis has been associated with decreased physical function and poor outcomes (1). Thus, it is critical to identify and develop effective treatment options for patients experiencing this comorbid condition.

1.1. Treatment of Dermatomyositis-Associated Calcinosis

Calcinosis as a complication of JDM and DM has remained elusive to treat. Reports in the literature have described the administration of a number of different medications in JDM or DM patients—including alendronate, etidronate, pamidronate, probenecid, colchicine, diltiazem, thalidomide, aluminum hydroxide, warfarin, and sodium thiosulfate, as well as immunosuppressive therapies (2–4)—and in some cases, there has been documented improvement in calcinosis following treatment. However, these data come from single reports or case series, and often, such improvements have not been successfully replicated. There are also no validated outcome measures to assess improvement in calcinosis, so improvement is often made by clinical opinion of the treating physician supported by plain radiographs showing decrease in a calcinosis lesion's size. There have been several recently published reports on the use of sodium thiosulfate (STS) in JDM/DM and other autoimmune disease patients with calcinosis, that describe significant improvement in calcinosis after treatment with this drug. These reports have included patients receiving the medication topically, intradermally, and intravenously (5–8).

Our group has collected a number of unpublished reports (from within our clinic and from outside physicians) of significant responses to therapy after administration of intravenous (IV) sodium thiosulfate (STS) for the treatment of severe calcinosis:

- One boy with JDM was dosed with 12.5 mg IV sodium thiosulfate twice weekly. After 3 doses, there was a noticeable improvement in the calcinosis in his hamstring and elbow. After 15 doses, the calcinosis had softened and continued to improve, and both his strength and range of motion improved considerably. The patient, who was previously unable to perform many of his usual activities due to calcinosis, is now able to ride his bicycle, his skin is almost back to normal per physician report, and he has experienced no side effects attributable to the sodium thiosulfate treatment.
- A second child with JDM was encased in calcinosis and wheelchair bound. Within 1 month of initiating IV sodium thiosulfate, her calcinosis noticeably improved by physician assessment. She remained on therapy for 1.5 years, along with an increase in her cyclosporine dose and the addition of abatacept. Since then she has continued to improve in the resorption and excretion of the calcinosis (some calcinosis was excreted in the stool). She is much stronger now and is able to climb stairs. Her grip strength has also improved. Her dual-energy X-ray absorptiometry (DXA) scan showed a drop in her bone density to -3.5.

- A third child with JDM developed calcinosis in the gluteal area and posterior thighs with methicillin-resistant *Staphylococcus aureus* infection, including an acute presentation with septic shock. She received 7 g/m^2 IV sodium thiosulfate (10 g per infusion) 3 times a week for 4 weeks, then 2 times a week for 2 weeks. Within the first week, she began experiencing softening in the lesions, less pain, reduction of erythema, and draining of liquefied calcium (after each infusion, liquefied calcium was drained). Following treatment she experienced approximately an 80% improved in her calcinosis based on physician and radiographic assessment.
- In another case a 11 year old girl from Chicago was treated with IV sodium thiosulfate twice weekly for 6 weeks, and then once weekly for the following 4 months. The initial dose was 12.5 grams for 3 weeks and then increased to 15 grams. The patient weighed 37 kg at the time. She tolerated the treatment well. Her only other medication during this time was methotrexate. She had improvement in finger, wrist and knee range of motion and questionable improvement in ankle range of motion. She also had improvement in the fluidity of her gait. Her x-rays post treatment showed mild improvement compared to x-rays taken 8 months prior in terms of showing less ankle and forearm soft tissue calcifications. Her hand x-rays showed an increase in finger calcifications with a decrease in palmar calcification. X-rays revealed no change in knee calcinosis.

- Within our group, Dr. Schiffenbauer treated a 51 year old woman with DM with 37.5 g of IV sodium thiosulfate 3 times a week for 1.5 months and then switched to 2 times a week infusions for 1.5 months. She had significant improvement in pain after 2 weeks of infusions. At 6 weeks, she had increased functional ability demonstrated by not needing her rolling walker. She also had noticeable softening of her calcinosis. During this period, her other medications were stable except for one intramuscular administration of corticosteroid for a disease flare. The sodium thiosulfate was stopped due to compliance issues. The patient felt significantly better to the point where she stopped coming for scheduled infusions.

Three additional cases of improvement in calcinosis following IV sodium thiosulfate treatment were reported at the 2014 American College of Rheumatology (ACR)/Association of Rheumatology Health Professionals (ARHP) Annual Meeting in Boston, MA:

- Thibodaux *et al*. (9) reported on two cases: A 63 year old Caucasian female with limited scleroderma (Patient #1) and a 46 year old Black female with polymyositis (Patient #2). Both had recurrent calcinosis deposits of the skin and soft tissue causing pain and functional loss. Patient #1 reported severe pain and decreased mobility of her hands, and Patient #2 reported deposits in her posterior thigh causing severe pain on sitting down and standing up. There was little improvement in pain or function after aggressive therapy that included corticosteroids, colchicine, calcium channel blockers, and surgical interventions. Each patient regularly reported pain VAS scores of 8–9/10. IV sodium thiosulfate was administered starting at 12.5 grams over one hour weekly and then was advanced as tolerated. The maximum doses achieved in Patient #1 and Patient #2 were 15 g/week and 25 g/week, respectively. Infusions were continued weekly for approximately 7 months. The infusions were tolerated well, and the most

common patient reported side effects were nausea and blurry vision; the most common laboratory abnormality was a non-anion gap metabolic acidosis, which we would consider an AE. As early as two weeks after starting the infusions, improvements in pain scores and softening of calcinosis deposits were observed. Both patients reported improved pain VAS scores of 3–4/10 at 2 weeks and 0–1/10 at 4 weeks, and this persisted throughout therapy. At 4 weeks, functional status improved; specifically, Patient #1 was able to grip a drinking glass without difficulty, and Patient #2 was able to sit and stand with ease.

- Florestano and Alamo (10) described a case involving a 38 year old male, who presented with classic DM in 2007. He was treated with corticosteroids and methotrexate, with recovery of muscle strength and normalization of creatine kinase. In May 2008, without evidence of DM disease activity, he presented with progressive painful subcutaneous calcifications in the axillary, gluteal and popliteal regions, and the hands and back, which were confirmed by plain x-ray. Metabolic studies were normal. Infliximab was initiated (200 mg three times a day, every 8 weeks, totaling 5 doses), with poor response and progression of calcinosis. In August 2009, therapy with IV sodium thiosulfate was initiated: 50 ml at 25% (12.5 g) in prolonged infusion over 60 minutes two times a day, with 10 doses per session. He received 17 monthly sessions, with a slow but significant regression of pain and calcinosis. Adverse effects were nausea, headache, and infusion site pain, all mild. The patient has remained asymptomatic, without any new calcinosis lesions.

Interestingly, sodium thiosulfate has also been reported to have efficacy in treating patients with other disorders involving ectopic calcium deposition, including calciphylaxis (11,12), calcific nephrolithiasis (13), and tumoral calcinosis (14). Sodium thiosulfate is a calcium chelator, and thus its mechanism of action in these disorders, as well as in calcinosis associated with DM, may be to bind excess calcium that would have otherwise been ectopically deposited. Another possible mechanism of action is for sodium thiosulfate to increase the solubility of calcium deposits by making highly soluble thiosulfate salts of calcium, which are then eliminated through the urinary tract.

1.2. Non-Clinical Toxicology and Pharmacology of Sodium Thiosulfate

Non-clinical toxicology and pharmacology data has been provided to NIEHS by Hope Pharmaceuticals (the supplier of investigational product), including information that has been submitted to FDA as part of the initial IND on file (NDA #201,444). This data is summarized below.

Single Dose Toxicity

The sodium thiosulfate material safety data sheet (MSDS) provided by Hope Pharmaceuticals (supplier of the investigational product) provides information about the intravenous dose required to kill 50% of a test animal population (LD_{50}) for several species (15). This data is summarized in Table 1 in relation to its human equivalent dose (HED), which was estimated in accordance with Center for Drug Evaluation and Research (CDER) guidance (16).

Table 1 LD_{50} Values for STS Administered Intravenously in Relation to HED

Species	LD_{50} (mg/kg)	HED[a] (mg/kg)
Rat	>2,500	>403
Mouse	1190	97
Dog	3000	1667

[a]HED assumes a 60 kg adult and was derived by dividing the rat dose by 6.2, mouse dose by 12.3, and dog dose by 1.8

As dog is the species most comparable in size to a human and has been used in animal experiments of calcinosis, it is considered the most relevant species for a human safety comparison in this instance (17). The proposed sodium thiosulfate dose in this study is 16 g/m^2 3 times weekly for 10 weeks. Assuming a 60 kg adult, as used for the HED in table 1, would have a body surface area of 1.13 m^2 they would be receiving in our study a dose of 18.08 grams at each infusion, which would come to 301.3 mg/kg body weight. This is well below the HED of the LD_{50} for rats and dogs as noted in Table 1. It is important to note that, as described in Section 1.3, sodium thiosulfate has been administered to humans in clinical trial settings in combination with chemotherapeutic agents to decrease toxicity and for the treatment of vascular calcification in end stage renal disease (ESRD) using intravenous doses as high as 25 g/m^2 3x/week for up to 5 months in adults and up to 20 g/m^2 in children in frequencies up to 3 times weekly.

Repeated Dose Toxicity

Rats were administered 125 mg/kg intramuscular for 4 weeks or 3 months. Changes in the capillary walls of the thyroid and adrenal cortex were visible at 4 weeks. The vessels were dilated and exhibited a lowered alkaline phosphatase activity. After 3 months, the vessels of the kidneys displayed clear changes, including atrophy of the glomeruli and dilation of the glomerular capillaries, which were permeable to plasma. An increased permeability of liver capillary walls and an increase in Kupffer cells were also noted (18).

Genotoxicity

A study published by the U.S. Food and Drug Administration reports that sodium thiosulfate tested negative for mutagenic potential in the bacterial reverse mutation assay (Ames test) using S. typhimurium strains TA98, TA100, TA1535, TA1537, TA1538, and E. coli strain WP2 (± S9 metabolic activation) (19).

Carcinogenicity

Long-term studies in animals have not been performed to evaluate the potential carcinogenicity of sodium thiosulfate.

Reproductive Toxicity

Reproductive toxicity data is from unpublished data from original IND submission for sodium thiosulfate. Pregnant mice (N=20-22/group) were administered sodium thiosulfate via oral gavage at doses of 0, 5.5, 25.5, 118 or 550 mg/kg from day 6 through day 15 of gestations. On day 17 all dams were subjected to caesarean section and the number of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were recorded. All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations. The remaining two-thirds were stained with

alizarin red S dye and examined for skeletal defects. Sodium thiosulfate was not found to be embryotoxic or teratogenic in this study.

Pregnant rats (N=20-22/group) were administered sodium thiosulfate via oral gavage at doses of 0, 4, 19, 86 or 400 mg/kg from day 6 through day 15 of gestations. On day 20 all dams were subjected to caesarean section and the number of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were recorded. All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations. The remaining two-thirds were stained with alizarin red S dye and examined for skeletal defects. Sodium thiosulfate was not found to be embryotoxic or teratogenic in this study.

Pregnant hamsters (N=20-22/group) were administered sodium thiosulfate via oral gavage 0, 4, 19, 86 or 400 mg/kg from day 6 through day 10 of gestation. On day 14, all dams were subjected to caesarean section and the number of implantation sites, resorption sites, and live and dead fetuses were recorded. The body weights of the live pups were recorded. All fetuses were examined grossly for the presence of external congenital abnormalities. One-third of the fetuses of each litter underwent detailed visceral examinations. The remaining two-thirds were stained with alizarin red S dye and examined for skeletal defects. Sodium thiosulfate was not found to be embryotoxic or teratogenic in this study.

Pharmacology

Sodium thiosulfate has been shown both *in vitro* and *in vivo* studies to reduce calcification of a variety of tissues in a number of animal models.

In an *in vitro* model, 3T3-L1 preadipocytes were induced into mature adipocytes (20). The mature adipocytes underwent calcification after being treated with high phosphorus media leading to increased expression of osteopontin and the osteoblast transcription factor Runx2 and decreased expression of adipocyte transcription factors peroxisome proliferator-activated receptor γ (PPARγ) and CCAAT-enhancer-binding protein α (CEBPα). When the mature adipocytes were exposed to sodium thiosulfate, calcification was inhibited in a dose dependent manner and the secretion of leptin and vascular endothelial growth factor (VEGF) from adipocytes was also decreased.

The effectiveness of sodium thiosulfate was investigated in a Wistar albino rat model that were bilaterally myringotomized, thus inducing myringosclerosis (MS, calcification of the tissues of the middle ear) around the handle of the malleus and near the annular region (21). Thirty rats were administered topical sodium thiosulfate or saline daily in the right ear. The left ear of all rats was left untreated to serve as the control. It was observed that rats who received sodium thiosulfate showed reduced calcium deposition and less formation of MS ($p < .05$).

Sodium thiosulfate was administered to uremic rats to determine if it could prevent the development of vascular calcifications in chronic kidney disease (22). The uremic rats treated with sodium thiosulfate had no histological evidence of calcification in the aortic wall in comparison to the control group (untreated rats) in which three-fourths showed aortic calcification. In those rats receiving sodium thiosulfate, urine calcium excretion was elevated and the calcium content of aorta, heart, and renal tissues was significantly reduced in comparison to the control animals. Sodium thiosulfate also lowered plasma

ionized calcium, induced metabolic acidosis and lowered bone strength in relation to the control group.

Sodium thiosulfate orally has also been shown to reduce the formation of calcium kidney stones in an uncontrolled clinical trial (23). To examine this further, genetic hypercalciuric rats, an animal model of calcium phosphate stone formation, were fed normal food either with or without sodium thiosulfate for 18 weeks. Urine chemistries, supersaturation, and the upper limit of metastability of urine were assessed. Eleven of the 12 untreated rats formed stones compared to three of the 12 sodium thiosulfate-treated rats ($P < 0.002$). Urine calcium and phosphorus levels were higher in rats receiving sodium thiosulfate in the feed. Urine citrate, urine volume and urine pH were lower in the sodium thiosulfate-treated rats. Overall, the administration of sodium thiosulfate reduced the calcium phosphate stone formation in this rat model.

The above data show the promise of sodium thiosulfate administration to reduce calcification. Based upon these results, a clinical trial is needed to test the potential efficacy and safety of sodium thiosulfate administration for the treatment of juvenile and adult dermatomyositis.

1.3. Sodium Thiosulfate Use in Humans

Sodium thiosulfate (used in conjunction with sodium nitrite) is currently approved by the FDA for the treatment of cyanide poisoning at a dose of 12.5 g in adults and 0.25 g/kg in children (not to exceed 12.5 g) (24). This dose can be repeated at half of the original dose if needed, leading to a total dose of 18.75 g. It is administered via intravenous infusion of a sodium thiosulfate injection solution containing 12.5 g/50 mL, at a rate of 0.625 to 1.25 g/min (approximately 10-20 minutes).

There are no clinical trials to date that have evaluated the safety of intravenous administration of sodium thiosulfate in humans for the treatment of juvenile and adult dermatomyositis. George Washington University is currently evaluating the topical use of sodium thiosulfate in combination with fractional carbon dioxide laser in the treatment of dermatomyositis associated calcinosis (ClinicalTrials.gov Identifier: NCT01572844). Additional human experience with this drug across other indications is extensive. The numerous reports available in the literature span several decades, with several describing longer-term and repeat-dose use of sodium thiosulfate in humans. For example, a 1985 publication by Yatzidis (13), who was evaluating it as a treatment for recurrent calcium urolithiasis, reported the administration of 20 mmol sodium thiosulfate daily (~5 g/day, or ~3 g/m^2/day for an average adult) over the course of 4 years in a cohort of 34 patients (overall, the group had a decline in new stone development during the sodium thiosulfate treatment period).

Table 2 summarizes clinical studies identified as of 08 June 2015 (through a PubMed and clinicaltrials.gov search) that have been conducted with sodium thiosulfate via the intravenous (IV) route and associated dose levels as available.

Table 2. Clinical Trials Conducted with Sodium Thiosulfate

Sodium thiosulfate Dose	**Indication**	**Subject Ages**	**Clinicaltrials.gov Identifier**	**Current Status & Findings**
20 g/m^2 IV at 4 hours post carboplatin 16 g/m^2 IV at 8 hours post carboplatin	Chemoprotectant	1-30 years	NCT00983398	Currently recruiting
16 g/m^2 IV at 4 hours & 8 hours post carboplatin	Chemoprotectant	1-18 years	NCT00238173	Suspended participant recruitment
12.5-25 g/m^2 IV after each thrice weekly hemodialysis treatments for 5 months	Treatment of Vascular Calcification in ESRD	18+ years	NCT00568399	Completed - One patient with prolonged QT interval due to hypocalcemia. Event resolved by use of a higher dialysate calcium concentration. Sodium thiosulfate has shown to be well tolerated in both children and young adults with calcific uremic arteriopathy and has mild adverse effects (25).
10–16 g/m^2 IV 2-4 hours post carboplatin, second dose 4 hours post first dose if impaired baseline hearing.	Safety in children with malignant brain tumors treated with intra-arterial (i.a.) carboplatin in conjunction with blood-brain-barrier disruption	17 months – 12 years	Started prior to clinical trials.gov	High dose sodium thiosulfate is well tolerated in children under 12 years of age (26).
16 g/m^2 or 533 mg/kg IV over 15 minutes 6 hours after cisplatin	Preventing Hearing Loss in Young Patients Receiving Cisplatin	1-18 years	NCT00716976	Ongoing, but not recruiting

Sodium thiosulfate Dose	**Indication**	**Subject Ages**	**Clinicaltrials.gov Identifier**	**Current Status & Findings**
IV over 15 minutes 6 hours after cisplatin on day 1 Repeats every 2 weeks for 4 courses 3 weeks after surgery, IV over 15 minutes 6 hours after cisplatin, repeats every 2 weeks for 2 courses (dose not provided)	Chemoprotectant	0-18 years	NCT00652132	Unknown
6 g IV over 5 minutes	Pharmacologic Profile in renal failure and healthy volunteers	18-90 years	NCT01008631	Completed No adverse events reported.
IV immediately post chemotherapy (dose not provided)	Chemoprotectant	18+ years	NCT00571298	Active, but not recruiting
20 g/m^2 IV at 4 hours post carboplatin 16 g/m^2 IV at 8 hours post carboplatin	Chemoprotectant	18-75 years	NCT00075387	Currently recruiting

Sodium thiosulfate Dose	**Indication**	**Subject Ages**	**Clinicaltrials.gov Identifier**	**Current Status & Findings**
50 ml IV drip twice/week post hemodialysis	Coronary Calcification in Patients on Hemodialysis	18+ years	NCT00720772	Completed Clinical adverse events reported included anorexia in 75%, sneezing 19%, transient hypotension 10%, vertigo 5% of patients. Laboratory results showed increased sodium, chloride, anion gap, and decreased bicarbonate and calcium.
20 g/m^2 IV at 4 hours post carboplatin 16 g/m^2 IV at 8 hours post carboplatin	Chemoprotectant	18-75 years	NCT00303849	Currently recruiting
IV after one hour lavage of cisplatin (dose not provided)	Chemoprotectant	18+ years	NCT00165516	Completed No adverse events reported due to sodium thiosulfate, but was done in the setting of experimental setting of cisplatin use.
20 g/m^2 IV at 4 hours post carboplatin 16 g/m^2 IV at 8 hours post carboplatin	Chemoprotectant	18-75 years	NCT00074165	Terminated

Sodium thiosulfate Dose	**Indication**	**Subject Ages**	**Clinicaltrials.gov Identifier**	**Current Status & Findings**
Loading dose of 7.5 g/m^2 infused prior to cisplatin, then maintenance infusion 25.56 g/m^2 delivered by continuous infusion pump over 12 hours	Chemoprotectant	18+	NCT02092298	Currently recruiting
20 g/m^2 IV at 4 hours post carboplatin 16 g/m^2 IV at 8 hours post carboplatin	Chemoprotectant	18-75	NCT00293475	Currently recruiting
IV over 6 hours after lavage of cisplatin (dose not provided)	Chemoprotectant	18+	NCT00165555	Completed No adverse events reported due to sodium thiosulfate, but was done in the setting of experimental setting of cisplatin use.
sodium thiosulfate infusion given on days 1, 8, 15, for a total of 4 cycles, each cycle totaling 7 days (dose not provided)	Chemoprotectant	18+	NCT01587820	Terminated
IV during cisplatin treatment (dose not provided)	Chemoprotectant	18+	NCT00004547	Completed

There are generally no serious adverse reactions associated with sodium thiosulfate use, even when used long-term and at doses well above the FDA-approved dose for its one approved indication of cyanide poisoning treatment. Reported side effects of sodium thiosulfate include hypotension, metabolic acidosis, nausea, and osteoporosis. Section 10.1.2.1 further discusses sodium thiosulfate use in humans.

We also point out an additional study from Bolliger and Earlam from 1933 in which 17 pregnant women were given 4 grams of IV sodium thiosulfate from two to twelve times

during their pregnancies and all of the births are reported as normal, with healthy children born (27).

1.4. Proposed Mechanisms of Action for Sodium Thiosulfate in Myositis Patients

The role of sodium thiosulfate in treating ectopic soft tissue calcification has been historically attributed to many of its properties, including its ability to act as a calcium chelator, an antioxidant, and a vasodilator (11). We believe its main action is through the ability of sodium thiosulfate to make calcium more soluble. We have also clinically seen improvements in patient parameters beyond those impacted directly by calcinosis, including improvement in muscle strength, physical function, endurance, and decreased pain. Based on newer evidence we propose some of those changes are due to an anti-inflammatory property of sodium thiosulfate, potentially through reduction in IL-6.

Sodium thiosulfate acts as a calcium chelator by reacting to form calcium thiosulfate, which is an extremely soluble product and is easily excreted in the urine and feces. This is the believed mechanism of action for reducing ectopic calcification in calciphylaxis and what we believe to be the main mechanisms for reduction in calcinosis in myositis patients. Our study will evaluate this mechanistic interaction by evaluating the amount of calcium excreted in urine after treatment with sodium thiosulfate to demonstrate an increased calcium excretion and to evaluate whether this correlates with decreasing calcinosis burden by exam and imaging studies.

The patients we have evaluated and have received reports from who have received this treatment also have noted a very rapid decrease in the pain from their calcinosis sites, which has happened too rapidly to be attributed to reduction in calcinosis burden. This finding has been reported in other disorders of calcification treated with sodium thiosulfate (11,28). This mechanism is thought to be due to its vasodilator properties and/or its ability to affect the endothelium. The vasodilator properties of this medication and their association with decreased pain was documented in one case of critical limb ischemia in a dialysis patient with arteriolar calcification (29). To better understand the role these mechanisms may play in the decrease in pain we have seen in myositis patients treated with sodium thiosulfate we plan several evaluations focused on endothelial function and perfusion. We will look at changes in markers of endothelial activation with treatment. We will use laser speckle contrast imaging, nailfold capillaroscopy, and infrared photography to evaluate for changes in perfusion.

We have also seen clinically and had reported to us improvements in patient strength with treatment with sodium thiosulfate. Although part of this may be due to the medications effect on vasodilation and endothelial function, we also believe this may be due to anti-inflammatory properties of sodium thiosulfate. Inhaled H_2S has been shown to decrease IL-6, IL-1β, TNF-α, NOS2, and ICAM-1 expression, cytokines that are likely important in myositis disease activity (30) and increase IL-10 expression in mice (31). One of the main effects of inhaled H_2S is also an increased in serum thiosulfate levels. This lead to an experiment in which mice were given a LPS challenge with varying amounts of sodium thiosulfate and survival was observed to be improved in a dose-dependent manner with the addition of the sodium thiosulfate. Further studies in mice have shown that sodium thiosulfate can decrease polymorphonuclear neutrophils migrating into lung in response to LPS (32). In addition in the same lung model, sodium thiosulfate decreased IL-6 and TNF-α protein levels and RNA expression. Experiments

showed that sodium thiosulfate decreased IL-6 and IL-8 production in a dose-dependent manner in endothelial cell culture systems. Based on further experiments it is hypothesized that sodium thiosulfate inhibits the activation of TAK1 and TRAF6 leading to inhibition of NFκB signaling. To evaluate these possible mechanisms of action we will be performing cytokine analysis at multiple points in the study to evaluate for any change in cytokines with therapy and also examine the proteomics of aspirated liquefied calcinosis fluid.

1.5. Assessment of Calcinosis

There is currently no agreed upon criteria for assessing calcinosis by means of validated physician forms, patient response forms, laboratory measures, or imaging studies. In the literature, the current most common assessments are physician opinion where the physician says the calcinosis improved or x-ray images showing the area of calcinosis has decreased in size. There are significant limitations to both of these. Physician evaluation is non-quantitative and is very unclear what goes into selecting whether an effected area has improved or not. X-ray is limited by its use of radiation, inability to tell if a lesion is active or quiet, difficulty in identifying lesions that overlay bone, inability to detect liquefied lesions, inability to assess 3-dimensional structure of an area, small field of view, and difficulty in quantification.

To this end we have developed and been testing many new outcome parameters. The first is a visual analogue scale for physician assessment that specifically comments on the areas to be considered in assessing calcinosis activity, damage, and severity. This provides the improvement of quantification and standardization over the current physician qualitative statement outcome.

We have also assessed several new imaging modalities to replace the use of x-ray. We have performed 16 CT scans, 19 DEXA scans, and 38 MRI/PETs in adult and juvenile myositis patients. From CT scan we have been able to overcome many of the limitations of x-ray. We can identify small lesions even when near bone with good spatial resolution we can detect many liquefied lesions, we can image the entire body at once, and we can produce quantitative volumetric analysis of the amount of calcinosis. From our experience, we have seen patients who have had extra procedures performed due to x-rays where a calcinosis appeared to be inside one tissue and when a CT scan was finally performed it was clear the calcinosis lesion was elsewhere and the procedure was not indicated. Thus, CT scan has overcome almost all of the issues with conventional x-ray except that it still uses radiation, cannot assess the activity level of a lesion, and still misses some liquefied lesions. From DEXA scan images we have been able to significantly reduce the amount of radiation use, image the entire body, and easily quantitate lesions in terms of the amount of calcium present. We have seen a patient who had DEXA scans on the outside and at NIH and they showed changes with time in their calcinosis burden. Thus, DEXA scan has a very low radiation dose making it useful for following patients as it can safely be performed more frequently then CT and x-ray, but it lacks the volumetric imaging abilities of CT, has difficulties in detecting lesions that overlay bone, has difficulty detecting liquefied lesions, and still cannot determine lesion activity. MRI/PET offers the ability to use low dose radiation, can detect if a lesion is metabolically active, where that activity is (soft tissues or calcified area), can image lesions that are near or overlaying bone, is very good at detecting liquefied lesions, can produce 3-dimensional spatial images, can image the entire body at once, and can be quantitated both in terms of activity and volume involved. MRI/PET has the weaknesses

that it uses more radiation then a DEXA scan, is significantly longer and more involved than a DEXA scan or CT, and can miss small calcified areas. Thus, from our experience in the patients we have imaged to date, we have found all three imaging modalities are superior to x-ray and all three offer different advantages and are complimentary to each other. It is with this experience as well as a strong desire to promote safety and minimize radiation dosing that we have developed the imaging regiment used in this protocol.

1.6. Summary

We currently lack proven efficacious treatments for dermatomyositis-associated calcinosis. Preliminary reports in the literature and our personal clinical observations of positive outcomes following administration of sodium thiosulfate indicate the potential utility of sodium thiosulfate in dermatomyositis-associated calcinosis. Our clinical experience is supported by the plausible mechanisms of actions of this agent in the treatment of calcinosis. In addition, extensive off-label use and chronic use in studies evaluating sodium thiosulfate as a chemotherapy adjuvant have raised side effects that have been treatable and non-life threatening. More robust scientific evidence of the safety and efficacy of sodium thiosulfate as a treatment for calcinosis associated with JDM or DM is needed.

We therefore propose to conduct a clinical trial that will evaluate the efficacy and safety of sodium thiosulfate use in the treatment of calcinosis associated with dermatomyositis for juvenile and adult patients. There are several unique aspects of the proposed trial:

- This would be the first adequately-powered trial of any agent evaluating its efficacy in the treatment of calcinosis in JDM/DM.
- It would be one of few studies to evaluate a JDM/DM treatment in both adults and children.
- It will be designed to validate an assessment tool for calcinosis which is needed to promote research in this area.
- It will integrate a series of patient reported outcome measures allowing their utility in myositis and calcinosis to be assessed.
- It will allow the evaluation of novel imaging modalities in calcinosis to better understand not just the distribution of calcinosis, but also its activity.
- It will capture information about the natural history of calcinosis during the pretreatment phase, of which there is a dearth in the literature.
- It will assess a variety of proposed mechanisms of action of the drug in a multidimensional manner.

2. Study Objectives/Hypothesis

The *primary objective* of this study is to evaluate the efficacy of intravenous sodium thiosulfate in the treatment of moderate to severe, extensive calcinosis associated with juvenile and adult dermatomyositis. Since the treatment of calcinosis is currently a challenge for practitioners, and descriptions of therapeutic successes are anecdotal and thus provide limited scientific evidence, the findings of this study are intended to provide information to the medical community of high scientific value regarding a potential therapy for calcinosis.

Our *primary hypothesis* is that participants treated with sodium thiosulfate will experience greater improvement in calcinosis activity as assessed by a change in the calcinosis visual analogue scale (VAS) score after treatment, compared with any change in calcinosis activity VAS score during the observation period prior to treatment with sodium thiosulfate.

Secondary objectives include assessing the safety of longer-term use (10 weeks) of sodium thiosulfate in children and adults, and evaluating the impacts of treatment on quality of life, functional disability, muscle strength, laboratory values (including biomarkers of inflammation and endothelial activation), and gene expression, as well as myositis activity and damage. Some of these will also be used as safety measures. In addition, we will evaluate the effect of treatment on calcinosis lesions by clinical, patient-reported, and imaging assessments. We will also evaluate the mechanisms of action of sodium thiosulfate by evaluating for changes in endothelial markers and vascular flow with treatment.

Our *secondary hypotheses* are that participants treated with sodium thiosulfate will experience improvements in the secondary clinical endpoints and immunologic biomarkers of calcification compared to those measured during the observation period prior to treatment with sodium thiosulfate.

There are currently no agreed upon or validated methods for evaluating changes in calcinosis. For important research to occur in treating this disease complication there is a need for validated outcome measures. To this end, we plan to evaluate several different methods for assessing calcinosis, including an array of imaging modalities, patient-reported outcomes, and physician assessments to capture meaningful changes in calcinosis. Our group has developed a patient-reported Calcinosis Assessment Tool (CAT), and we will seek to validate and improve the tool as part of this objective. We will also examine the validity of the Mawdsley Calcinosis Questionnaire, which was developed to assess calcinosis associated with systemic sclerosis.

2.1. Outcome Measures

- The primary outcome measure of the study will be greater improvement in calcinosis activity VAS rating from Week 0 to Week 10, compared with any change in calcinosis activity VAS rating observed during the pre-treatment period (from Week -10 to Week 0).

Secondary outcome measures include:

- Greater improvement in quality of life score (CHQ-PF50 for children, SF-36 for adults) from Week 0 to Week 10, compared with any change in quality of life score observed during the pre-treatment period (from Week -10 to Week 0).
- Changes in components of quality of life over time, as measured by quality of life questionnaires (e.g., SF-36, CHQ-PF50, PROMIS, Skindex-29)
- Changes in functional disability over time, measured by CHAQ/HAQ, CMAS/AMAT/Myositis Functional Index, and physical therapy-related assessments (e.g., range of motion, 6-minute walk, timed up and go, sit to stand, Motor Functional Measure (MFM))
- Change in muscle strength over time, measured by manual and quantitative muscle testing

- Improvement of calcinosis lesions, assessed by Calcinosis Assessment Tool, Sentinel Calcinosis Lesion form, Mawdsley Calcinosis Questionnaire, durometry measurements, photography, and imaging studies
- Changes in gene expression, measured by RNA and protein analyses (e.g., to evaluate changes due to therapy, or differences between responders and non-responders to therapy)
- Incidence and severity of adverse events, including laboratory abnormalities, over time
- Improvement in or stabilization of myositis activity and damage, as assessed by the IMACS core set measures

Secondary outcomes will evaluate for differences in change from week -10 to 0 compared to week 0 to 10 as well as changes at week 24 and 62.

An additional outcome will be validation of the Calcinosis Assessment Tool and the Mawdsley Calcinosis Questionnaire and new imaging modalities for calcinosis, which are under development by our group and associate investigators of the protocol.

3. Study Design

3.1. Overview of the Study Design

This is a single-arm, open-label study to evaluate the efficacy and safety of IV sodium thiosulfate use in patients with extensive and moderate to severe calcinosis associated with juvenile and adult dermatomyositis. Participants will be enrolled at a single center. Children (age 7 years or older) and adults with moderate to severe calcinosis will be recruited to participate. We will attempt to enroll equal proportions of patients with juvenile and adult DM. Refer to Section 3.3 for full inclusion/exclusion criteria.

We plan to screen up to 250 patients and to enroll 18 fully eligible participants into the trial, with an expected minimum of 13 participants completing the trial over up to 4 years due to the involved schedule for the study treatment and assessments. Assessments will all be conducted at the NIH Clinical Center in Bethesda, Maryland, as well as, potentially over the phone, by e-mail, and/or through internet or mailed questionnaires.

The first two patients enrolled will be adults. In addition to all labs and procedures discussed for other patients, these patients will also have additional laboratory studies with the first 3 doses of sodium thiosulfate, including serum calcium and sodium levels checked prior to each infusion of sodium thiosulfate and hourly after the start of the infusion until 6 hours post infusion. Although there is extensive evidence about the transient nature of any hypocalcemia or hypernatremia in patients receiving this drug, as a precaution we will perform this monitoring, as this is a different disease population. In the first pediatric patient enrolled in which blood draw limits will allow we will draw a serum calcium and sodium prior to their first infusion and at the time point we observed the lowest calcium and highest sodium respectively level in the adult patients. If any lab abnormalities are noted, the data will be brought to the DSMB for evaluation to determine if further safety monitoring with infusion beyond what is already described in the protocol should occur.

The specific procedures performed at each study week are listed in the time and events table found in Appendix B, and details of the study procedures and study treatment are provided in Section 4.1 and Section 12, respectively.

Week 0 assessments may occur +/- 32 days and all assessments following it will be adjusted so that they start the appropriate number of weeks after week 0. **All assessments and contacts during the infusion period of Week 1 to Week 10, as described below, may occur in a window of +/- 4 days of the time point stated** (e.g., Week 6 may take place 6 weeks +/- 4 days after Week 0). **The Week 24 and Week 62 assessments may occur in a window +/- 14 days. The Week -4 assessments may occur in a window of +/- 10 days.**

Timing of study assessments and contacts from the study staff are as follows (see also Figure 1 for a study schematic):

- **Screening**: Participants will be screened over the phone prior to coming to the NIH Clinical Center for any study visits; section 9.1 outlines the procedures that will be followed to screen participants over phone and conduct the consenting process. Participants will sign a screening consent before any procedures are performed. Participants may come to NIH for an in-person screening evaluation. Interested individuals will provide access to their medical records for review prior to enrollment, and qualified study staff will discuss the potential participants' medical history with them to assess eligibility for the study. A Screening Evaluation Form will be completed for each potential participant; a copy of the form is attached as Appendix F, along with history and physical, laboratory studies and imaging studies as needed (Tables 4-8, found in Appendix B). Some of the blood and imaging testing may be performed at home prior to NIH screen. The main study consent will also be provided and reviewed with subjects at the time of screening evaluation.

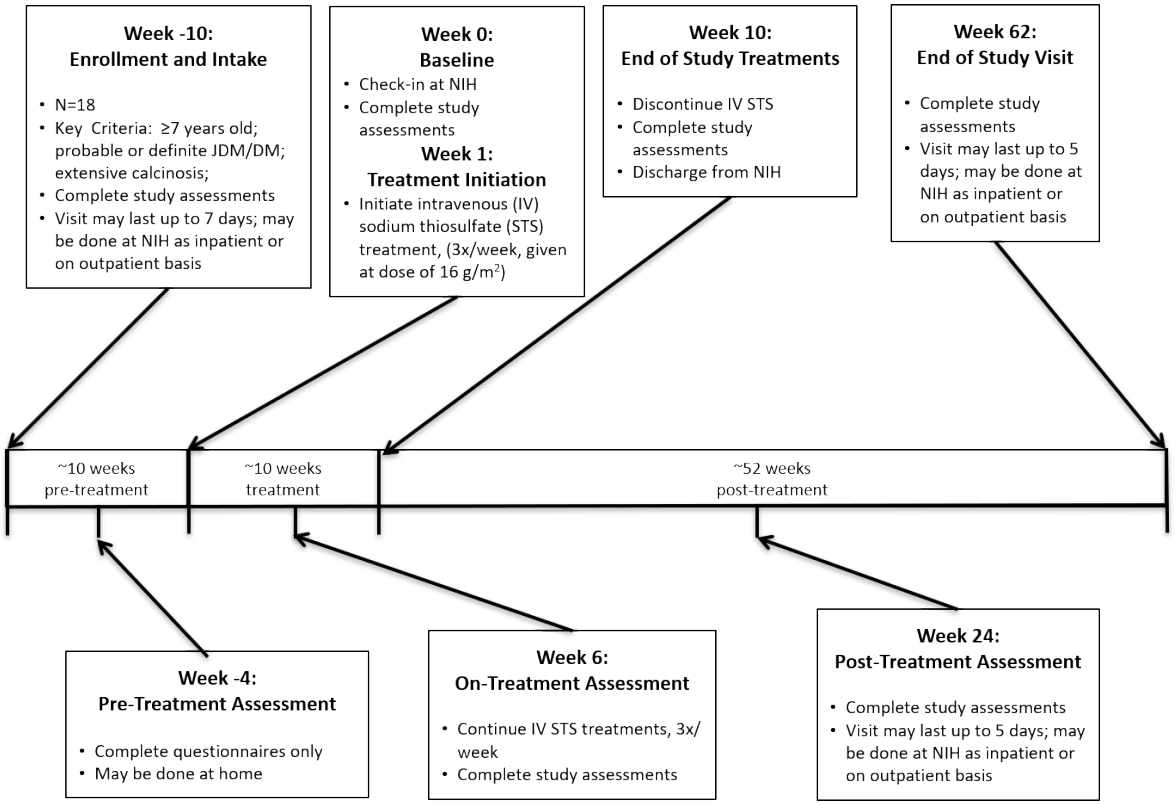
- **Week -10 (Enrollment/Intake)**: Participants will come to the NIH Clinical Center for an initial evaluation to confirm qualification for the study and to conduct baseline evaluations. Study participants (or their parent(s)/guardian(s)) must sign a consent form, and minors must provide assent before they can be enrolled in the study and before any study assessments are performed. Any participants who are diagnosed with a medical condition or have laboratory abnormalities that were previously unknown will be notified and referred for medical care. For example, if a participant is found to be vitamin D deficient their physician will be notified and they can replace based on Institute of Medicine or National Osteoporosis Foundation guidelines. The Week -10 visit is expected to last up to approximately 7 days and may be done on either an inpatient or outpatient basis depending on the needs and mobility of the participant. Participants who have equivalent laboratory evaluations, imaging studies, or physical assessments within 1 week of their -10 visit may have those results used for this protocol instead of repeating those tests under this protocol.
- **Week -4**: Participants will complete questionnaires only (Calcinosis Assessment Tool, Mawdsley Calcinosis Questionnaire, Quality of Life, and patient-reported assessment measures); this may be done at the participant's home or at NIH.

- **Week 0 (Baseline)**: Eligible participants will return to the NIH approximately 10 weeks following the Week -10 visit for a second evaluation, during which time they will be admitted to the NIH Clinical Center as an inpatient and will initiate IV sodium thiosulfate (STS) therapy, dosed at 16 g/m^2 three times weekly for 10 weeks. Female individuals of reproductive potential must have a negative urine or serum pregnancy test prior to administration of sodium thiosulfate. Any participants experiencing intolerable side effects may discontinue therapy earlier. Participants who tolerate the therapy without an adverse event for 2 infusions and are deemed by the medical staff to be medically stable will be given the choice of becoming local outpatients to the NIH Clinical Center and returning 3 times weekly for infusions and evaluations during the 10-week sodium thiosulfate treatment period. Safety monitoring will be performed throughout the treatment period (see Table 8).
- **Week 6**: The Week 6 visit takes place approximately 6 weeks after the Week 0 visit. Participants continue with three times weekly IV sodium thiosulfate treatments and complete study assessments. Safety monitoring will continue. Study assessments for this time point are expected to take up to 3-5 days and may be done as an inpatient or outpatient, depending on the needs and mobility of the participant.
- **Week 10**: The Week 10 visit takes place approximately 10 weeks after the Week 0 visit, and occurs on the last day of the inpatient (or local outpatient) period wherein participants are receiving three times weekly IV sodium thiosulfate treatment. Study assessments for this time point are expected to take up to 3-5 days. Once all Week 10 evaluations are complete, the participant will be discharged from the active treatment portion of the study.
- **Week 10-24**: Patients or their parents will receive two contacts to assess adverse events. These will involve a short set of questions of medical issues that may have arisen during this time. If a patient or patient's parent responds that they have had any of these issues they will be called and additional information will be collected. These contacts will be by e-mail, mail, or phone based on what devices the patient is able to use. The first contact will be between week 11 and week 13 and the second contact will be between week 17 and week 19. They will also be advised during this period to contact the study staff should any new medical issues or questions arise.
- **Week 24**: At approximately 24 weeks following the Week 0 visit, participants will return to the NIH for an evaluation. The study visit is expected to take up to 3-5 days and may be done either as an inpatient or outpatient, depending on the needs and mobility of the participant. For participants who are unable to physically return to the NIH, the study team will attempt to complete as many of the assessments as possible by using modalities such as telehealth, phone, e-mail, and mail.
- **Week 62**: At approximately 62 weeks following the Week 0 visit, participants will return to the NIH for a final evaluation and study completion. The study visit is expected to take up to 3-5 days and may be done as an inpatient or outpatient, depending on the needs and mobility of the participant. For participants who are unable to physically return to the NIH, the study team will attempt to complete as many of the assessments as possible by telehealth, phone, e-mail, and mail.

- **Reminder Contacts**: Approximately 1 week and 6 weeks (as relevant; e.g., for Week 24 and Week 62 visits) prior to each study visit, participants may receive reminder contacts (e.g., phone, text, or email) with information about the date and time of future scheduled visits and, if applicable, any instructions necessary to prepare the participant for the visit. Additional reminders may be made as needed.
- **Early Withdrawal Due to Rapid Response**: Patients may be deemed completers at week 6 if they have scored a moderate improvement on the physician assessment of change and are believed to have reached maximal benefit as evidenced by a calcinosis VAS score of less than or equal to 10 mm. They will receive the week 6 assessments and then will not complete any of the infusions or assessments for the study for week 7-9. They then will return at week 10 for those evaluations, without receiving any sodium thiosulfate infusions that week, and will go on to receive the normal week 24 and 62 assessments.
- **Early Withdrawal Due to SAE**: Patients who experience a SAE where it is felt it is unsafe for them to continue to receive study drug will continue to be assessed according to the protocol schedule. Those assessments thought to be of increased risk due to their SAE will not be performed. For patients who are limited in their ability to follow up, the focus will be on assessments at week 6, 10, 24, and 62.
- **Early Withdrawal Due to Patient Desire/ Non-medical Inability to Continue**: Efforts will be made to continue to have the patient complete all assessments as indicated in the study. A focus will be placed if there are limited opportunities on performing the week 6, 10, 24, and 62 assessments. If it is known ahead of time a patient will be withdrawing from treatment and unable to return for the week 6 or 10 assessments, they will be given the assessments for those visits at the last time they are able to complete them prior to those dates.

3.2. Schematic of Study Design

Figure 1 Study Schematic



* Patients at week 6 who reach the rapid response criteria may choose to stop active treatment with STS.

** Not pictured in the schematic is a screening visit prior to study initiation at which time study eligibility will be determined. Some patients may complete some screening tests at home and provide copies of the results.

Follow-up/Termination Procedures

At the final visit (i.e., visit occurring at ~week 62), the participant will be told to contact the study staff with any safety issues or complications that arise after completion of the study. The participant's contact information will be confirmed.

3.3. Discussion of Outcome Selection

There is currently no gold standard for evaluating calcinosis either clinically, via laboratory testing or radiographically. In the majority of reports on calcinosis any change is assessed by physician exam and/or by x-ray. X-ray exams are limited as an assessment tool for changes in calcinosis, as they will fail to detect changes in erythema, pain, and consistency of lesions which are important aspects of improvement. Based on this we have chosen a physician-based outcome of calcinosis using a global visual analog scale in order to better quantify the overall physician assessment of calcinosis activity. To help alleviate differences based on personal experience, we will

also include directions explaining what variables to consider for the VAS and provide examples of where certain calcinosis activity levels should fall on the scale.

To this end, this study also seeks to define better ways of evaluating calcinosis changes over time to promote research in this area. As secondary outcomes, we have included several tools that have been proposed as ways to assess calcinosis or to assess dermatomyositis patients in general. The goal is to be able to evaluate these tools to determine which measures are useful in evaluating calcinosis, and if there are particular combinations of tools that are useful together. This will allow future studies to minimize the assessments they perform on patients.

3.4. Discussion on Timing of Imaging Time Points

Wholebody MRI data collected as either a dedicated wholebody MRI or as part of a MRI/PET is collected at week -10, 0, 6, 10, 24, and 62. Time points -10 and 0 are needed to assess baseline muscle, pericalcinosis, and fascial edema at baseline. The baseline fluctuation in this disease is unknown and a baseline variance is needed as a comparator to the change over the treatment period to assess for drug benefit in these parameters which are all thought to be signs of active disease. Week 0 and 6 are needed to help in assessing if the patient is able to stop medication at the 6 week point. MRI is an important parameter for the physicians to assess for disease activity as well as to make sure all liquefied areas of calcinosis are visualized that may still benefit from treatment. This will also allow us to better understand the trajectory of response when used with the 0 and 10 week assessments. Week 10 is our primary end point assessment and will be used to assess if the study medication has improved either muscle or pericalcinosis inflammation/edema. As one of the main factors we are analyzing is the change in amount of calcinosis, it is also important to make sure we capture any liquefied areas of calcinosis that may be missed by CT scan. The week 24 and 62 week MRIs are for evaluation of long term effect. It is unknown how long the effect of this medication will last and these will allow us to determine if inflammation returns and the time line of a return in inflammation. This is important in thinking about developing re-dosing plans for future patients and for better elucidating the mechanism of action by determining if the changes in calcinosis burden and inflammation/edema are linked together or whether they appear to be independent.

CT scan data is collected at week 10 for research purposes and baseline data will be available for all patients. Among a number of methods to assess calcinosis burden and extent, CT scan is the best. The patient population we are targeting can have well over 100 calcinosis lesions and many may not be identifiable by any other method of detection. The change between weeks 0 and 10 serves as an objective measure of medication response. It also will help demonstrate that parameters that change on the physician and patient global assessments are due to a decrease in calcinosis burden, not solely other effects. The week 62 assessment we believe would occur outside of the study by outside physicians if not completed here to assess for sustained effect of medication and evaluate if patients should be on additional therapy. By offering this inside of the study we are able to offer patients reduced radiation burden compared to an outside scan and we can collect uniform data to determine the sustainability of benefits received from the sodium thiosulfate. For scientific discussion, one of the weaknesses raised about the study is the lack of week -10 and week 24 CT scans to assess for baseline variation in CT and for the rate of ongoing improvement or worsening after the cessation of therapy. In order to accommodate concerns over the

radiation dose, we have not included CT scans at these time points with the understanding that it does create some scientific uncertainty. At the times the CT scans occur they will also be used to evaluate for kidney stones as part of the safety evaluation.

PET as part of a MRI/PET is performed at weeks 0, 10 in adults, and potentially week 62. PET imaging offers a way to look at metabolic activity of the calcinosis lesions. We have seen that calcinosis lesions can be PET active or not and can have different patterns of activity. Those lesions that have been PET active have tended to correspond to lesions the patients describe as new, active, or changing in some aspect. Based on some of the cases we have been part of it does not appear all calcinosis lesions respond equally to sodium thiosulfate and part of this may be due to their activity. Thus, we are obtaining PET imaging to assess for lesion activity to see if there is a correlation between PET activity and which lesions respond to therapy. To accomplish this, we need to know PET activity prior to study drug initiation (week 0). This will allow us to determine if specific types of calcinosis lesions based on PET activity respond better or worse to sodium thiosulfate. It will also allow us to see if the sodium thiosulfate affects the energy usage of calcinosis lesions in followup. The week 62 time point allows us to determine the metabolic changes from treatment. Also, if a differential effect is seen, it would allow a clinical opinion to be given of what lesions are most likely to respond in that patient to additional sodium thiosulfate, or even to generalize this knowledge to the treatment of other patients in the future. The 10-week evaluation in adults will allow us to evaluate the direct drug effect on lesion activity. A decrease in the energy consumption of lesions would help indicate that the medication is working through pathways besides purely causing chemical reabsorption which would not necessarily decrease energy consumption.

DEXA scan is performed at weeks -10, 0, 6, 10, 24, and 62. As discussed in detail in the risks and benefits section of this protocol, DEXA scan imaging allows us to determine bone density for safety monitoring, evaluate for calcinosis in a way that is significantly lower in radiation than CT imaging at the cost of missing some areas of involvement, and to quantify the amount of calcium inside specific lesions. The week -10 assessment allows for the determination of the patients' baseline bone density for the study to ensure they are eligible and will confirm the extent of patients' calcinosis burden. Due to the limitation of being unable to perform CT scans at weeks -10 and 0, by performing a DEXA scan at these times we can understand some of the baseline variation in calcinosis burden in these patients, which is important in trying to determine if any changes seen during the treatment phase represent true effect versus normal temporal variation. The week 6 assessment allows again for bone density determination. It also allows for assessment of calcinosis lesions that cannot be identified on physical exam in deciding if patients will be offered the option of stopping treatment at week 6 or not. It also allows evaluation along with the week 10 time point on the rate of change in the calcinosis burden. The week 10 time point allows again for safety monitoring and evaluation of drug effect. If CT scans are obtained at weeks 0 and 10, the change in burden is determinable from those studies, but the baseline variation will be unknown, in contrast to DEXA scans, where the baseline variation will be determined, so the importance of a change can be better understood. One of our goals is also to assess the use of DEXA scan as a surrogate for CT imaging in monitoring these patients which requires obtaining DEXA scan and CT scan data at the same time points to determine if change in the two tests correlates tightly enough for future studies to focus on DEXA instead of CT for assessing imaging outcome measures. The week 24 and 62 time

points serve to evaluate for any continued worsening in bone density or whether patients have improvement post study drug cessation. It also allows for timing to be determined for when any worsening post treatment of calcinosis might occur or calcinosis lesions continue to improve. The time 62 assessment when it is paired with a CT scan will also be used for validating DEXA as an alternative to CT scan for clinical monitoring of calcinosis and for assessments in future clinical studies.

4. Inclusion and Exclusion Criteria

Participants will be enrolled without regard to gender, race, or ethnicity. Each potential participant must meet all of the following inclusion criteria in order to be eligible to enroll in the study:

INCL 1. At least 7 years of age

INCL 2. Meets Bohan and Peter criteria, as modified by the International Myositis Assessment and Clinical Studies Group (IMACS), (13,14, oddis) for probable or definite DM or JDM

INCL 3. Has extensive calcinosis, defined as calcinosis involving at least 2 extremities or the torso

INCL 4. Has moderate to severe calcinosis, defined as having a calcinosis activity visual analogue scale score of ≥3.5 cm out of 10 cm

INCL 5. Is willing and able to comply with the requirements of the protocol and to undergo all testing

INCL 6. Can have IV access established to receive study infusions

INCL 7. Myositis disease activity is stable*

INCL 8. Medications for myositis are stable for at least 6 weeks prior to study entry**

INCL 9. Men and women of reproductive potential must agree to use a reliable form of birth control during the 62-week duration of the study

INCL 10. Subjects or their legal guardian must sign a written informed consent

*Stable myositis disease activity will be defined by physician global and patient/parent global VAS that are <4 cm (35), as well as creatine kinase (CK), lactate dehydrogenase (LDH), aldolase, aspartate aminotransferase (AST), and alanine aminotransferase (ALT) that are less than or equal to 2X upper limit of normal (ULN).

**If a patient has a medication for myositis changed in this window for reasons besides their myositis activity and has returned to their baseline medication use prior to enrollment they will still be eligible.

A potential participant meeting any of the following exclusion criteria is not eligible to enroll in the study:

EXCL 1. Is pregnant or breastfeeding

EXCL 2. Has known allergies to sodium thiosulfate, any of its components, or dextrose

EXCL 3. Has severe myositis disease activity as defined by patient/parent or physician global activity visual analogue scale score >4 cm out of 10 cm

EXCL 4. Has had an escalation of immunosuppressive therapy in the 2 months prior to enrollment for the purpose of treating active myositis disease activity, including the addition of a new agent to treat the patients underlying disease or an increase in dose of an existing medication used to treat the patient's disease (other than an adjustment for weight or body surface area in children)

EXCL 5. Has a malignancy or had a malignancy within 5 years of diagnosis of their DM (except for benign skin lesions or basal cell carcinoma)

EXCL 6. Known or suspected history of alcohol or drug abuse in the 6 months prior to study enrollment

EXCL 7. Has systemic lupus erythematosus, scleroderma, or a condition other than DM that is associated with calcinosis as a complication

EXCL 8. Has had a change in medications used specifically for calcinosis in the 2 months prior to enrollment, including but not limited to alendronate, etidronate, pamidronate, probenecid, colchicine, diltiazem, thalidomide, and aluminum hydroxide

EXCL 9. Has used probenecid, diltiazem, aluminum hydroxide, or hydrochlorothiazide in the 2 months prior to enrollment

EXCL 10. Has currently or has a history of any of the following: heart failure, renal impairment (GFR less than 30 representing severe renal disease), liver disease (Child-Pugh class C), arrhythmias (that are symptomatic or are concerning for progression to symptomatic arrhythmias), or recurrent kidney stones (more than one episode of symptomatic kidney stones separated by at least 1 month), or QT prolongation, or hypocalcemia, or metabolic acidosis, or hypotension

EXCL 11. Has severe osteoporosis or has had a bone fracture within a year prior to enrollment. For adults, severe osteoporosis as defined by the World Health Organization (WHO) as bone mineral density (BMD) 2.5 standard deviations below that of a young, normal adult (T-score at or below -2.5 and one or more fractures). For individuals, less than age 18, severe osteoporosis as defined by the First Pediatric Consensus Development Conference as a Z-score below -2 and one or more fractures.

EXCL 12. Has a psychiatric illness or medical non-compliance that the study team feels will make the patient unlikely to complete the study

EXCL 13. Has dysphagia where non-oral feeding alternatives are needed.

EXCL 14. Requires supplemental oxygen therapy

EXCL 15. Has >3 episodes of cellulitis requiring IV antibiotics related to calcinosis within a year prior to enrollment or cellulitis within 1 month of enrollment

EXCL 16. Previously received or currently receiving sodium thiosulfate by any route

EXCL 17. Is on an oral prednisone dose of more than 1mg/kg/day or other oral corticosteroid equivalent.

EXCL 18. Is taking any concomitant medications that are thought to alter sodium thiosulfate's effects or pharmacokinetics. Once patients have met all other inclusion criteria and no other exclusion criteria this criteria will be checked. A PharmD will evaluate the patient's current medication list for medications with the potential for interaction with sodium thiosulfate. Methodology is as follows: He or she will perform a search in two individual validated medication interaction software programs. He or she will also perform a literature search via PubMed for case reports of interactions with sodium thiosulfate. As an additional safeguard, the PharmD will evaluate the medication list utilizing principles of pharmacology and pharmacokinetics to attempt to identify any potential interactions not yet documented in the literature.

EXCL 19. Has any health conditions that, in the opinion of the investigator, significantly increase the risk of taking sodium thiosulfate or participating in any of the study procedures

EXCL 20. Weighs less than 26 kilograms.**

EXCL 21. Has a regimen of pulse steroids or IVIG that is at an interval besides every 1, 2, or 5 weeks.

EXCL 22. Has a chronic infection that makes assessment of muscle disease difficult including, but not limited to, hepatitis, HIV, HTLV 1, and HTLV 2.

EXCL 23. Has had a severe complication of diabetes in the past year prior to enrollment.

EXCL 24. Anemia with a HgB less than 10 at time of screening or deemed to be too low to safely complete study by hematology consult team.

**We will attempt to enroll patients at a weight greater than 28 kg as these patients will be able to obtain all lab work for the study. Patients weighing 26 to 28 kg will only be able to obtain some of the research blood work. Patients less than 26 kg of body weight will be unable to obtain all safety labs, so will not be able to enroll.

4.1. Concomitant Medications

Every effort will be made to keep patient medications stable throughout the course of the study. Medications can be increased as clinically indicated for a flare of DM during the study. Criteria for disease worsening in a clinical trial, by which patients could be termed treatment failures and offered alternative therapies, were preliminarily defined by IMACS (36). They will be incorporated into this trial and include: (1) physician global worsening of ≥ 2 cm on the 10 cm visual analog scale (VAS) and a worsening of the manual muscle testing by ≥ 20%, or (2) global extra muscular organ disease activity (a composite of constitutional, cutaneous, skeletal,

gastrointestinal, pulmonary and cardiac activity) worsening by ≥ 2 cm on a 10 cm VAS, or (3) any 3 of 6 IMACS core set activity measures worse by ≥ 30%. In order to be classified as “flaring” in this trial, subjects will have to meet the above criteria on 2 consecutive assessments and have the physician rate the patient as at least moderately worse. Patients who are considered flaring can have their prednisone increased up to the study maximum of 2mg/kg/day and can have an increase in any DMARD they are currently taking at the discretion of a physician from the study team or their home physician. At the discretion of the treating physician, if the prednisone dose is increased, the patient may be started on vitamin D and calcium supplementation for bone health. If a flare is uncontrolled with this change or the treating physician feels the patient needs additional therapy it can be provided. To maintain safety if a study physician or home physician feels a patient needs additional therapy for the management of their myositis they may institute that therapy even if the patient has not met the official criteria for a flare. If a patient exceeds the 2 mg/kg/day dose of prednisone or needs additional agents started they will not be withdrawn from the study. If the patient exceeds these limits prior to week 10, an effort will be made to enroll an additional patient (up to the study maximum of 18) and if such an additional patient is enrolled and completes treatment the patient who did not exceed the medication limits will be used for the primary end point analysis instead of the initial patient. A secondary analysis would be performed examining all patients regardless of additional medication used. Secondary end points will be assessed both for the set of patients staying within the flare treatment guidelines and for all patients enrolled for which we have data. We feel flares are more likely to be noticed during the treatment phase when patients are monitored more closely than during the lead in phase. Due to this we also think patients are more likely to receive prednisone for a flare during the treatment phase and we do not want a false positive effect of the treatment phase due to patients in it receiving more prednisone.

Prednisone can be tapered if at visit 10 and 24 the patient has inactive disease as defined by having a 30% or greater improvement in the calcinosis activity VAS from baseline and a stable or improved value for MMT8, CK, and physician global disease activity VAS, as well as the CMAS for pediatric patients. These variables were selected based off of the Centres of the Paediatric Rheumatology International Trials organization (PRINTO) criteria for inactive disease (37). We have modified these criteria due to the high amount of damage in the patient population that would enroll in this trial, such that even without active disease it may be difficult for patients to have a MMT8 ≥ 78 out of 80, CMAS ≥ 48 out of 52. Prednisone during the tapering period may only be tapered by ≤ 20% every two months. Medications may be tapered or stopped at any time if a serious adverse event occurs that is thought to be possibly related to that medication.

Pulse steroids and IVIG will be allowed in patients during the study. The patient must be on a stable regimen prior to initiation of the study for at least 2 months. The frequency of the doses must be every 1, 2, or 5 weeks, and study assessments will be performed prior to administration of these infusions whenever possible. The combined dose for IVIG should be 1 to 2 grams/kg/month. No single dose of IV steroid should exceed the equivalent of 30 mg/kg of solumedrol.

4.2. Reproductive Health

All participants will be required to use birth control during study participation. All participants will be told about the importance of using birth control. Approved birth control methods will be based on the mycophenolate REMS contraception methods for females listing with the modifications being altering some language to make it applicable to both males and females. This was chosen as a model as it is an accepted system for birth control and both myositis and lupus have shared concerns about estrogen therapy potentially causing disease flares and thus pursuing low estrogen or no estrogen birth control options. The modified recommendations would be:

For female participants who become pregnant after week 0 they will still be in the study for their entire pregnancy if they became pregnant on study drug (drug up to week 10 and last follow up is week 62) and we would assess how the pregnancy is advancing at the times of adverse event monitoring by asking them if they are having any problems with the pregnancy and if there were any complications with the birth process or the child at birth. More details on the management of females who become pregnant can be found in other sections of this protocol. For males, whose partners become pregnant at those points of adverse event monitoring, we will ask the male participant if there are any complications of their partner's pregnancy and after the birth if there were any complications of the birth process or with the baby. If a female participant or a male participant's partner becomes pregnant during the study, we will offer the opportunity for additional meetings with the study team to discuss reproductive risk based on the situation to the participant and inform them that they may invite their partner to any such discussions. In accordance with NIH HRPP Policy 400 - *Research Involving Pregnant Women, Fetuses, and Neonates*, no member of the study team will have any part in any decisions pertaining to the timing, method, or procedures used to terminate a pregnancy if a patient becomes pregnant during the study. In addition, no individuals engaged in the study will have any part in determining the viability of a neonate.

If a female participant or the partner of a male participant in the study becomes pregnant during the study, but has not given birth we will ask them to contact us after the end of the pregnancy to let us know if there were any problems with the pregnancy, birth, or child. If we do not hear back from participants who fall into this category in the two months after they were expected to have the child born, we will reach out to the participant to try and determine the outcome of the pregnancy and if there were any complications associated with it.

5. Clinical and Laboratory Methods

Timing of each study procedure is provided in Table 4 found in Appendix B. No equipment for these tests is being used to diagnose, cure, mitigate, treat, or prevent a disease or condition in this protocol.

During the study, no exercise interventions will be offered by Department of Rehabilitation Medicine unless felt to be important for patient safety. Patients may continue an exercise regimen that they have been on prior to the trial initiation, although certain activities may be restricted at the time of specific assessments.

5.1. Medical History

A full, thorough medical history will be obtained from all participants and/or their parents at the screening visit and at week -10 visit. No medical history will be taken at Week -4 (although some medical information may be given by the participant and/or their parents as part of the questionnaires completed during this at-home assessment). At the Week 0 visit and all subsequent visits, the medical history will be reviewed and updated if any changes have occurred.

Self-administered questionnaires will be completed by the participant/parents and the examining physicians. Requests will be made to obtain the participant's relevant medical records, review all previous biopsy materials and radiographic studies, and speak to their primary physician regarding their illness. All clinically meaningful conditions (e.g., inflammatory/autoimmune diseases, metabolic diseases, cardiovascular disease, and renal disease) will be recorded along with onset and resolution dates if applicable. Participants and/or their parents will be asked to identify any exclusionary conditions, such as heart failure, renal impairment, or other renal disease. Participants of reproductive potential will be asked if they are pregnant or breastfeeding.

A registered nurse or physician may take the medical history. A board-certified medical doctor who is licensed to practice medicine will review the medical history.

5.2. Medication History and Concomitant Medication

Participants and/or their parents will be asked about recent and current medication use at the screening visit and at weeks -10, -4, 0, 24, and 62. Between Week 0 and Week 10 most participants will have been inpatients and medication use will already be documented; for those participants who choose to receive treatment on a local outpatient basis during the 10-week treatment period, their medication use will be reviewed weekly. Medication history will include a review of antibiotic use, probiotics, supplements, vitamins, and herbs or non-traditional medications.

After the Week 0 visit, an extensive medication history is not required; rather, any changes that have occurred since the prior study visit will be recorded.

5.3. Physical Exam

Participants will receive a physical examination at all study visits (excluding the Week -4 at-home assessment). The initial physical exam will confirm the participants' dermatomyositis and calcinosis and will help rule out any exclusionary conditions. Exams at all visits will minimally include heart auscultation, lung auscultation, abdominal palpation, skin and joint examination, and muscle strength assessment.

Details of the physical examination procedures are provided in the study's Manual of Procedures (MOP).

5.4. Physician Assessment Forms

As part of their evaluation assessing physicians will complete forms documenting the state of the patient's disease at the time of the visit. One of these forms will be the Physician Calcinosis Activity Visual Analogue Scale. This form will contain directions for the physician on how to score patient calcinosis activity and several ways for them to record this information. This will include a VAS where physicians will mark with a vertical

line the activity of calcinosis taking into account amount of calcinosis, presence of erythema, consistency of calcinosis, calcinosis warmth, ulceration associated with the calcinosis, texture of the calcinosis, the location of the calcinosis, and any pain associated with the calcinosis. Physicians will also complete the Calcinosis Type and Extent Form. This form records information about the location of a patient's calcinosis and the subtype of calcinosis based on the distribution pattern of the calcinosis. We will also use a Calcinosis Sentinel Lesion form to follow up to 5 calcinosis lesions clinically throughout the study based on assessment by examination, durometry, and photography.

5.5. Pregnancy and Breastfeeding Status

Female participants of reproductive potential will receive a urine or serum pregnancy test at all study visits and during the infusion period of 0 to 10 weeks on a weekly basis, they will also be asked if they are breastfeeding at each of these time points; both are assessed as part of clinical care to eliminate any risks to an unborn fetus or nursing child. The pregnancy tests will be performed at the NIH Clinical Center laboratory.

Any participant found to be pregnant at the screening visit will not be eligible for enrollment until they are no longer pregnant at which time they would undergo a second abbreviated screening visit. Any participant with a positive pregnancy result or who is breastfeeding at the Week -10 visit through Week 10 (during periods of baseline assessments or study treatment administration) will be excluded from enrolling or treatment. Patients found to be pregnant at week 0 will not receive study drug and will complete week 0 assessments that are not contraindicated in the setting of pregnancy. Patients found to be pregnant between weeks 0 and 10 if they have received any study drug will also be asked to return for the week 10, 24 and 62 assessments, again excluding any tests that are contraindicated while pregnant. Patients who are found to be pregnant after week 0 will not receive any additional study drug as long as they remain pregnant. Any patient found to be pregnant prior to week 10 will not be considered a completer for evaluation of the primary outcome. A positive pregnancy result at Weeks 24 or 62 will exclude the participant from undergoing assessments involving ionizing radiation [i.e., dual-energy x-ray absorptiometry (DEXA) and computerized tomography (CT) scans], but will not result in changing their status from being a completer for evaluation.

5.6. Muscle Strength and Functional Testing

Muscle strength and function testing will be administered in this study for research purposes. Details of testing by visit are provided in Table 7 found in Appendix B.

The functional assessment tools including the Health Assessment Questionnaire or Childhood Health Assessment Questionnaire will be given to participants and/or their parents.

Muscle strength and functional assessment will be performed by a physiatrist, as well as physical therapists in the Department of Rehabilitation Medicine, who will administer muscle strength assessment tools. Some additional MMT evaluations may also occur as clinical assessments or as part of other tools that may be performed by a NIEHS physician or practitioner.

Testing will include the Adult Myopathy Assessment Tool (AMAT) and Childhood Myositis Assessment Score (CMAS), passive range of motion (ROM), sit to stand, 6-minute walk, timed up and go, quantitative muscle testing, Motor Function Measure in neuromuscular disease (MFM), Grip strength, key pinch strength, targeted ROM, Myositis Functional Index version 3 (FI3) and manual muscle testing (MMT).

The AMAT, CMAS and FI3 rate physical function and muscle endurance using timed functional maneuvers and endurance tasks, with higher scores indicating better performance.

For the 6-minute walk assessment, participants will be asked to walk as briskly as is comfortable for them for a 6-minute period on a flat gym surface. Vital signs, including blood pressure, are monitored before, immediately after, and 10 minutes after the test. Distance traveled at specific timed intervals will also be included.

Passive range of motion testing will assess increases in joint range of motion on therapy while minimizing the impact of muscle strength. Full passive ROM will be performed initially, and then on subsequent time points targeted ROM will be performed on hip extension, knee extension, elbow extension, shoulder flexion, shoulder abduction, and the three other joint motions that had the greatest limitation on the initial assessment.

5.7. Magnetic Resonance Imaging (MRI)

MRI will be administered for research purposes to assess changes in muscle inflammation over the course of the treatment.

Participants without a contraindication will undergo MRI examination of the whole body, which will be conducted in collaboration with the NIH Department of Radiology.

For the MRI scans, a 1.5 Tesla magnet will be used with standardized scanning parameters, including T1, fat-suppressed T2, diffusion weighted images, T2-Map, and STIR images. Scanning time will be 1 hour and will occur as a separate scan then the whole body MRI or MRI/PET. Quantitation of the MRI scans, including T2 and T1 maps, will be developed. For those patients with contractures or other physical limitations stopping them from having MRI performed in the typical position we will make efforts to perform the MRI in a position they can tolerate and that would be reproducible between scans. Research MRI will be performed without gadolinium and without sedation. All efforts possible will be made to make the experience as tolerable as possible, which may include such activities as providing supportive padding, providing blankets during the test, playing the patient music, and any other action that can increase the tolerability for that patient, with the understanding that all options may not be available for all patients.

5.8. Computed Tomography (CT) Scan

CT scans will be performed for research purposes to evaluate calcinosis burden/extent of calcinosis disease. One CT scan will be done for research purposes and patients may have up to two clinical CT scans during the protocol. The specific unique advantage of a CT scan and what it offers that other imaging modalities cannot accomplish is discussed in Section 10.1.2.5.

Participants will undergo non-contrast CT imaging of the trunk (thorax, abdomen, and pelvis) and extremities as a sensitive method to examine the morphology of the calcinosis (superficial plaques, deeper tumoral deposits, or fascial planar deposits), as

well as the distribution of the lesions and their mass. 3D images will be reconstructed to obtain the volume of the lesions, which will be assessed over the course of the protocol.

After the CT data, have been acquired and stored, research software may be used to reconstruct images from the acquired data. This reconstruction could be performed on the scanner itself or off-line on a separate computer system. Data and images may be stored for future evaluation and additional processing.

The week 10 research CT scan is performed to analyze for response to therapy. This is the end of the treatment phase and when we expect to see the maximal effect of the study medication. Due to the extent and depth of disease often seen in this patient population, it is impossible to properly assess the amount of calcinosis by clinical exam. This CT scan will serve to quantify the change in the amount of calcinosis. Based on our prior experience, patients have had responses of a change in calcinosis as quickly as a few weeks, with some requiring longer therapy to show improvement. This time point we feel strikes a balance between giving patients enough time to have a response and not overly burdening them with additional time at or near the clinical center for additional infusions of study medication. We have performed CT scans of calcinosis in DM and JDM and have reviewed CT scans of other calcific disorders that have accurately reflected the areas of calcification, showing not only what has been determined from a thorough physical exam, but in addition, reveals additional areas of calcinosis that are not identifiable by examination. We feel that the quantitative assessment of calcinosis burden by CT imaging is critical in determining whether any improvement noted by patients or physicians is due to actual changes in calcinosis burden. CT scan changes have also correlated with clinical improvement in one published case (38). Understanding whether the areas of calcinosis improve uniformly or not also is important information in designing future studies and for monitoring future patients on this therapy.

Study involvement can involve up to two clinical scans. The first CT scan would occur at week 0. If the patient had a CT scan in the year prior to this time they would not receive a CT scan at week 0 for clinical purposes as part of this protocol. This scan will serve to determine if the patient's symptoms are due to calcinosis and to ensure that they would not benefit more from surgery or local therapy than from treatment with the investigational drug in this protocol. The time point of week 0 is chosen since it is the closest time period when the patient is available to when study drug would be initiated. This will allow us to ensure patients would not better be managed with surgery at the time of deciding to implement medical therapy. This is important in determining that if a patient has specific acute problems due to their calcinosis whether or not it would be superior for them to receive surgical management or potentially other treatments compared to study medication. Also, if areas thought to have calcinosis from exam were found not to have calcinosis by CT imaging, then the patient could potentially fall into a less severe category and we would need to reassess the risk/benefit ratio of study participation for them.

The second potential clinical scan would occur at week 62. If the patient has signs of continued calcinosis severity by physical exam, symptoms, or DEXA scan, they will receive a CT scan to evaluate for re-accumulation of calcinosis. If the patient has had a clinically relevant CT scan in a time frame that makes the week 62 CT scan redundant, this CT scan will not be performed. This information will be used to decide if the patient should be tried on additional off-label therapy outside of the protocol. Change in calcinosis is decided in clinical practice primarily based on imaging studies, utilizing ionizing radiation, and physical exam. Patients with the severity of calcinosis we are

enrolling are likely to still want additional therapy for their calcinosis if they did not have complete resolution as part of this study. Whether this therapy should be more sodium thiosulfate administered off label, surgery, another experimental agent, or another therapy would be guided by the clinical exam and imaging results. Important aspects of this decision will be whether the patient improved on sodium thiosulfate as part of the study and the severity of their current calcinosis. In discussing with other physicians this would entail imaging studies prior to initiating a new therapy for the same purpose of monitoring for improvement and knowing the patient's true calcinosis burden so the appropriate therapy can be chosen. We plan to do this clinical scan as part of this protocol so that it can be performed on the same scanner as prior scans to allow for more accurate comparison and assessment of the change in the lesions. The patient can also benefit from the decreased radiation offered by this scanner's research parameters versus a standard CT scan, and also, the data from the scan can be used for research purposes, in addition to its clinical purposes.

All efforts possible will be made to make the experience as tolerable as possible, which may include such activities as providing supportive padding, providing warm blankets during the test, supportive pads, cartoon animations of the CT gantry display for children to watch, and any other action that can increase the tolerability for that patient, with the understanding that all options may not be available for all patients.

5.9. Positron Emission Tomography - Magnetic Resonance Imaging (MRI/PET)

MRI has proven useful for evaluating patients with myositis for disease activity. Preliminary work from a pilot study we have performed shows that PET imaging can add additional information about areas of disease activity and particularly about calcinosis activity. We plan to further pursue these initial findings by performing MRI/PET in study participants. We will perform a MRI/PET from the base of the skull to the toes. All participants will receive a brief medical history to determine if they have any condition that would preclude a MRI/PET. These contraindications include, but are not limited to, pregnancy, implanted metal that is deemed to be dangerous, or age older than 95 years. A history of radiation exposure will be taken prior to enrollment and prior to performing any research study involving ionizing radiation. If the patient has received more than 0.5 rem for research purposes in the past 12 months prior to enrollment, we will delay enrollment until their time 0 would be 12 months or more from their last research radiation exposure. If the patient is already enrolled in the study and they have received more than 0.5 rem for research purposes outside of this protocol, we will review their situation with the DSMB and the IRB to decide whether to continue with currently planned research assessments involving radiation or to alter such assessments. All female subjects will receive a pregnancy test prior to this study and will be excluded from this testing if they are pregnant. All patients will have their blood sugar checked immediately prior to the study to ensure it is below 200 mg/dl, because hyperglycemia interferes with the uptake of radioactive glucose required for the PET scan. If blood glucose is found to be elevated, the medical team will work to safely lower the blood sugar and reassess if the blood sugar is at a level where the scan results would be interpretable. Prior to undergoing the scan, all subjects or their parents/guardians will complete a standard radiology questionnaire that determines other potential risks of the procedure that may have previously been missed. Prior to the scan patients will receive a detailed instruction sheet explaining that they need to minimize exercise for 48 hours prior to the scan,

consume a high fat diet for 24 hours prior to the scan, and fast for 6 hours prior to the scan.

The MRI/PET will consist of having an IV line placed and receiving 10 mCi. of FDG. The patient will then sit in a quiet room for an uptake period of approximately one hour. The patient will then be placed on the scanner for the MRI/PET scan. These images will then be analyzed by a nuclear medicine physician and/or radiologist. If any abnormality the subject did not know about is detected by MRI/PET, the subject and the subject's physician will be informed so that further care can be provided. In pediatric patients, there is the potential to exceed the radiation safety limit of pediatric patients of 0.5 rem/year from each of these scans.

MRI/PET is scheduled to occur at week 0, 10, and 62 in adults and 0 and 62 in pediatric patients. If a patient has become pregnant, at week 62 they will receive a full body MRI instead of the MRI/PET. If a patient has no PET abnormalities on MRI/PET at week 0 they will not receive any further MRI/PET scans in the study.

All efforts possible will be made to make the experience as tolerable as possible, which may include such activities as providing supportive padding, providing blankets during the test, playing the patient music, providing videos or movies for the patient to watch during the study, and any other action that can increase the tolerability for that patient, with the understanding that all options may not be available for all patients.

For an in-depth review of the potential direct clinical benefits to the patient and the reasoning for what MRI/PET provides that the other imaging modalities being used do not, please refer to Section 10.1.2.6.

5.10. Thermography and Vascular Imaging

Thermography and vascular imaging procedures will be performed for research purposes to study the impact of calcinosis on local inflammation and the peripheral vascular bed, and will include infrared (IR) photography, nailfold capillaroscopy, and laser speckle contrast imaging.

Infrared Photography: A non-invasive, real-time, in vivo imaging system will be used to determine the spatial distribution of blood flow, by measuring the infrared emission from the skin. The procedure does not require direct contact between the instrument and the participant. The imaging system utilizes an infrared imaging modality, which combines two-dimensional, digital camera and computer to store and analyze the data for calculating power spectra low frequency oscillations of the temperature. For ease of use, the entire instrument is mounted on a standard movable tripod. No illumination is necessary. An IR measurement reflects a local reaction depending mainly on changed blood flow in subcutaneous and cutaneous tissues. This will be performed as a standalone assessment on a schedule as described in Appendix B. It will also be performed during up to 5 infusions of sodium thiosulfate to determine if there is an immediate effect from drug infusion.

The participant will be adapted to room temperature for ~20 minutes and, after that, the extremity being imaged will be kept immobile for one minute to obtain a blood flow distribution image and 20 minutes during data acquisition for assessment of low frequency vasomotion. Data analyses will be performed later using computer software. Standard digital photographs also will be taken for comparison and anatomical orientation of the infrared photographs.

Nailfold Capillaroscopy: Advanced computer capillaroscopy using a portable video capillaroscope operating at low (90X) magnification (designed by NIBIB device based on an iPhone 7 plus) or high (200-500X) magnification (adapted by NIBIB hand held microscope made by Braedius LLC.) will be utilized to capture nailfold capillary density, tortuosity, dimensions of the individual capillary loops, and quantification of capillary blood flow and velocity. The time required for adaptation to the room and to set up the participant's hand with a controllable heating pads is 20 minutes Snapshot assessment for nailfold capillaries will last about one hour. The second, third, fourth, and fifth fingers of the non-dominant hand will be studied. To monitor individual capillary blood flow fluctuations during 4-5 hours will be done with one of the portable capillaroscope as patient will be laying on the bed or sitting in reclining chair. This will be performed as a standalone assessment on the time table as described in Appendix B. It will also be performed during up to 5 infusions of sodium thiosulfate to determine if there is an immediate effect from drug infusion. Continuous capillaroscopic monitoring of the patient with portable hand-held microscope may be performed overnight to observe long term capillaroscopic changes.

To prepare for the capillary examination, the participant will be instructed to keep their hand free from rings, bracelets, tight clothing, and take care of their nail bed for 60 minutes prior to the study start, and this includes the 20 minutes for adapting to the rooms temperature. On the day before the test, they should not expose the skin of the finger to petrol, laundry detergent, acetone, or nail polish. One day prior to the study the patient should not consume any caffeinated drinks. The participant will be asked to refrain from physical activity in the 5 hours preceding the procedure. The test will generally be conducted several hours after a meal, rather than in the fasting state. The participant will be asked to refrain from a high fat content in the preceding meal, as well as coffee, nicotine, or excess liquid intake within 1 hour of the examination, and avoid consuming alcohol within 24 hours of the test.

During the examination, the hand will be positioned at heart level under the objective on an adjustable microscope stage that has been preheated to ~28°C. The pulse rate and blood pressure will be measured in the contralateral arm. To minimize motion artifact, the finger and palm will be held by a medical-grade double-side adhesive paper for the duration of the exam. A drop of viscous liquid used for imaging will be applied to those fingers visualized In order to assess the capillary density and concordance of capillary perfusion, the whole nail fold margin will be first scanned at a low magnification. When perfusion has become concordant and continuous, the images obtained from one selected finger will be recorded continuously on video memory for in 10 minute intervals over several hours at a low and high magnification for later analysis. The time-averaged erythrocyte velocities will be calculated from these images. To ensure that all the capillaries are in good focus, an area with the smallest curvature at the center part of the nailfold will be selected where preferably all capillaries are in the same depth of focus of the microscope objective and are sharply imaged. If 4 capillaries cannot be assessed (e.g. when capillary density is low, imaging quality is poor, or neighboring capillaries are not in the same depth of focus, etc.), a second area will be imaged for another 3 minutes.

Laser Speckle Contrast Imaging (LSCI): LSCI provides a semi-quantitative assessment of microvascular blood perfusion, which is expressed in arbitrary units. LSCI measurements from the skin reflect blood flow in capillaries, arterioles, venules, and dermal vascular plexuses.

The LSCI technique exploits the random speckle pattern, which is generated when tissue is illuminated by coherent laser light. Using a predefined integration time on a charge-coupled device (CCD), the changing pattern is affected by motion of the underlying particles. If there is flow in the region of interest, the speckle pattern is decorrelated (or “blurred”) and the contrast in that area is reduced. Thus, the amount of correlation is dependent on the speed and volume of the RBCs in the tissue area. In this manner, low contrast is related to a high amount of blood flow (since the light is scattered by moving RBCs only), and conversely, high contrast represents an area of low blood flow. The speckle size is determined by the aperture size of the imaging device alone, and is usually chosen to be the size of a single CCD pixel (~15 μm). The detector integration time is sufficiently small in comparison to the correlation time of the intensity fluctuations so that the signal is not averaged out. In typical systems for skin perfusion imaging, the integration time is set to between 5 and 20 ms. The 3- to 30-mW laser beam used by LSCI is expanded over a larger area, and there is no rastering of the beam, as all measurements are acquired in parallel.

Moor Instruments LSCI system will be used for this study. The 780-nm laser source is expanded over an area, rather than a line as in other approaches. There are two modes available: high resolution/low speed or high speed/low resolution. For low resolution/high speed, acquisition is 25 frames per s at a size of 152×113 pixels. High resolution/low speed allows an adjustable integration time, constant for temporal averaging (1 s per frame - 25 frames, 4 s per frame - 100 frames, 10 s per frame - 250 frames, and 60 s per frame - 1500 frames) at 568×760 pixels for each image. The imager allows a repeat image function, video mode (25 frames per s), and single image acquisitions, all simultaneously if required. The camera gain is adjustable to increase the area of image acquisition, although care should be taken to not saturate the CCD. This will be performed as a standalone assessment on a schedule as described in Appendix B of affected areas. It will also be performed during up to 5 infusions of sodium thiosulfate to determine if there is an immediate effect from drug infusion.

5.11. DEXA

DEXA scans will be used for adverse event monitoring and for research purposes to evaluate calcinosis burden.

Participants will undergo a full-body DEXA scan as a way to monitor for osteoporosis in response to treatment. Due to the underlying disease, it can be difficult to obtain a bone density value for patients in a focused area so a full-body DEXA will be performed and an evaluation for osteoporosis will be performed in the area most easily read. In addition, areas of calcinosis will be evaluated for the amount of calcium present. Bone density and calcinosis burden will be tracked over the time of the study. Bone density will be followed to monitor for severe worsening of bone density as a possible side effect of the study treatment. Changes in calcinosis burden will be tracked as a secondary end point. Each DEXA scan will consist of two-five images: one with the patient lying on their back and if the patient is able to, a second image with the patient lying at an angle to the first image, in order to allow a better determination of where the calcinosis lesions are in 3D space. Additional images of targeted areas may also be undertaken if a separate view is needed to visualize an area of calcinosis or to more accurately determine bone density. Evaluation of the images will be performed on the scanner itself or on a separate off-line computer system. Data and images will be stored for future evaluation and additional processing. There is clinical utility in this test in that it will provide a bone density

measurement for these patients, who frequently have osteoporosis, as well as a body fat measurement.

DEXA scans will be performed as described in Appendix B. Assessments at week -10 will allow for baseline assessment for osteoporosis and calcinosis burden. Week 0 assessments will allow for assessment of baseline variation in calcinosis burden and bone density. Since CT scan frequency is more limited by radiation, DEXA is our best imaging test for baseline variation in calcinosis burden and extent. Patients are allowed to stop treatment at week 6 due to good response and a DEXA scan will help in making this assessment. Also, it will be used to monitor for any worrisome changes in bone density from a safety standpoint and will allow us to also evaluate for any potential new fractures. Week 10 serves as the primary end-point time and DEXA will allow for safety assessment of bone density as well as evaluation of change in calcinosis burden. It will be compared to CT scan changes over the same 10 weeks when possible to also determine if it can serve in the place of CT as a lower radiation alternative in future studies. The week 24 and 62 week DEXA scans will allow us to determine long-term bone safety of the study medication overall, as well as in the individual patients. It will also serve to evaluate for changes in calcinosis over this time by checking for continued improvement, stability, or worsening. Due to the radiation limitations of CT imaging, it cannot be performed at week 24 however; the DEXA scan affords the opportunity to more accurately assess the trajectory of any changes in calcinosis burden. At week 62 we will also compare DEXA to CT scans for evaluating if DEXA scans may be a suitable monitoring technique for future research studies in calcinosis.

All efforts possible will be made to make the experience as tolerable as possible, which may include such activities as providing supportive padding, providing blankets during the test, providing short breaks between views, and any other action that can increase the tolerability for that patient, with the understanding that all options may not be available for all patients.

5.12. Renal Ultrasound

Renal ultrasound used in this study is indicated for medical/safety monitoring. This will be used to monitor for the development of renal stones, which is a theoretical risk with use of this medication. Renal ultrasound will be performed according to the NIH Clinical Center's standard clinical protocol, including determining kidney size and echogenicity to evaluate for kidney disease. The frequency of testing is based on consultation with nephrologists about when they might expect to see new stone formation, and to ensure the patient does not have stones prior to initiation of therapy. On those visits where a CT scan is performed, a renal ultrasound was felt to be redundant unless the kidneys were missed for some reason; thus, we have scheduled renal ultrasounds at those times only if the kidneys cannot be assessed by the CT scans at those visits, or if a CT scan does not occur at week 62.

5.13. Blood Draw Evaluations/Collections

Blood samples will be collected and assessed both as part of clinical care and for research purposes. The laboratory assessments performed and the samples being collected at each visit, as well as the volumes required, are detailed in Table 5 found in Appendix B. Additional blood tests may be ordered if they are felt needed by the clinical team for safe medical management of a participant. As hospital supply and testing

procedures change, the exact tubes and volumes for blood draws may differ from that listed in the protocol, and we will draw the needed volume and containers as these change, assuring we remain inside the total limits of blood draw listed in the protocol.

A nurse, physician, or qualified phlebotomist will draw blood at all visits. Phlebotomy will be performed according to NIH Clinical Center established Standard Operating Procedures (SOPs), and details of the procedure are provided in the MOP.

The amount of blood drawn will strictly adhere to NIH guidelines and policies. For adult patients, blood for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period. For pediatric patients, no more than 5 mL/kg shall be drawn for research purposes per day, and no more than 9.5 mL/kg shall be drawn over any eight week period.

The blood drawn from the participant will be labeled “human blood” and will be labeled and tracked using an NIH Clinical Research Information System (CRIS) barcode. Blood samples will be processed, transferred/shipped, and stored according to the NIH Clinical Center laboratory test procedures and SOPs.

The following tests will be performed to evaluate study efficacy and/or safety and the timing of the tests is found in Appendix B:

- Acute care chemistry panel, cystatin C, uric acid, mineral panel, hepatic panel, CK, LD, total protein, Quantitative Immunoglobulins, C-Reactive Protein-High sensitivity, Pro Brain Natriuretic Peptide, CK-MB Immunoassay
- Aldolase
- ANA, ENA, JO-1
- Thyroid screen (TSH, FT4)
- Complete blood count and differential
- Erythrocyte sedimentation rate (ESR)
- Hepatitis serologies
- PT and PTT
- HLA typing
- Myositis autoantibody testing
- CHI RNA gene expression analysis
- Inflammatory markers (acute phase reactants that promote or inhibit calcification, cytokines)
- Endothelial activation markers
- CHI proteomics
- CHI and NIH Clinical Center lab Flow cytometry
- CHI Cytokine Analysis
- von Willebrand factor related antigen panel, thrombomodulin, ionized calcium, Vitamin D levels (1,25 and 25-OH), PTH, blood FGF23, alkaline phosphatase, bone-specific alkaline phosphatase

5.14. Urine Collection and Urinalysis

Spot, first morning void, and 24-hour urine samples will be collected from participants. The laboratory assessments performed and the samples being collected at each visit, as well as the volumes required, are detailed in Table 5 found in Appendix B.

For spot-urine assessments (e.g., for pregnancy testing), the volume of one void will be collected. If an inadequate volume is obtained, a second spot urine may also be collected on the same day. These will be analyzed for electrolytes and proteins. Sodium thiosulfate levels will also be checked.

First morning void urine will be collected weekly during the periods when participants are receiving sodium thiosulfate infusions to evaluate renal function and urinary excretion of calcium. These may be repeated up to 7 times during a given week based on patient availability and tolerability of the collection. Participants will be instructed to collect all urine from their first void of the day, and those who are outpatients will be instructed to keep the urine refrigerated until it is brought to the clinic.

For 24-hour collections, participants will be instructed to collect all urine, using a stream splitter, into an acidified and non-acidified container, for a period of 24 hours around study visits at Week -10, 0, 6, 10, 24, and 62. Participants and/or their parents who are outpatients will be instructed to keep the urine refrigerated until it is brought to the clinic. The volume of the total urine sample will be measured and recorded.

The following urine assessments will be performed to monitor safety during sodium thiosulfate administration:

- Sodium
- Phosphorus
- Calcium
- Creatinine
- Ca/Cr ratio
- Citrate
- Urate

5.15. Iothalamate Clearance

The iothalamate clearance assessments used in this study are indicated for research purposes to evaluate renal clearance and for safety monitoring to better evaluate kidney function in this population.

Study participants will undergo administration of 5 ml of 600mg/ml iothalamate intravenously as a bolus. A syringe containing the iothalamate will be weighed, the contents injected, and the syringe weighed again to allow for calculation of the administered dose. 2-3 mL of blood will be collected in heparinized tubes at 150, 180, 200, 220, and 240 minutes after infusion. In subjects with an eGFR < 40ml/min/1.73m^2, a sixth plasma sample will be obtained at 24 hours. Samples will be placed on wet ice and promptly centrifuged at 1500 g, with the plasma frozen at -20°C or -80°C. Plasma iothalmate concentrations will be determined and GFR will be calculated using the one compartment model, using the slope of the final elimination phase, denoted Cl_1; this value will be corrected by the Brochner-Mortensen formula: $Cl = 0.9907783*Cl_1 \pm 0.0012183*Cl_1$. Due to the amount of blood drawn for this testing, not all individuals may be able to undergo it (particularly children). Individuals with an iodine allergy or a history of bronchial asthma also will not receive this test, but will be able to enroll in the protocol and complete all other assessments. Patients who are pregnant or breastfeeding will be unable to complete this assessment.

All patients will have this evaluated at week 0, 6, and 10. Adults will have an additional assessment at week -10. Any patient with abnormal clearance will have additional

testing at weeks 24 and 62. Given that these patients often have active inflammation and low body muscle mass, other traditional assessments for renal function may be incorrect in them. There are other techniques for clearance, but given that many of these patients have mobility problems and can have calcinosis in the pelvic region, an assessment technique that did not involve timed urine collections or significantly increasing the number of times the patient has to urinate we felt would minimize the patients' discomfort and problems with the procedure. We are assessing at week -10 in adults to understand if they have renal clearance issues as early as possible to make sure we can more closely monitor issues that may occur in patients with renal dysfunction. It is particularly important to assess their volume status given the volume of fluid given in the study and to potentially reduce the normal saline given post-infusion per the details given in the sodium thiosulfate administration section of the protocol. All patients will be assessed at weeks 0,6, and 10 to ensure they are maintaining renal function and are appropriately able to handle the volume and to excrete study medication. This is also needed to ensure all patients are seeing the same effective dose of sodium thiosulfate, since given its short half-life a decrease in renal function could greatly increase the circulating time of the medication and impact efficacy. There is also the potential, through its vasodilator properties, that the medication may impact renal function and this is an important impact to know for further drug use. The follow-up assessments in those who have abnormal function are to determine a trend of improvement, worsening, or stabilization after sodium thiosulfate is discontinued, which is important for their general health management and for understanding the risks and benefits of sodium thiosulfate.

5.16. Calcinosis Aspiration

For calcinosis lesions believed by the investigator to have liquefied, a needle will be inserted into the calcinosis lesion using aseptic technique and the volume of fluid will be aspirated. The evaluation for liquefaction of the calcinosis will be based on physical exam and imaging studies. In addition, ultrasound of the calcinosis may be performed to guide the aspiration. Major aspirations (if any liquefied calcinosis lesions are identified) will be allowed at Weeks 10, 24, and 62 and will involve aspirating the maximum amount of fluid that can be removed. Minor aspirations may be performed between Week 0 and Week 10 for small volumes if they are detected (<15 cc). Refer to the MOP for additional details. These samples will be used for the following testing:

Inductively coupled plasma mass spectrometry (ICP-MS): This method allows us to identify elements and their relative concentrations in solutions. Elements such as: Ca, S, P, C, Na, Mg and others can be identified and quantified. This allows is to perform two analyses simultaneously, both elemental identification and quantification. This analysis will be performed first in order to learn more about material composition. That knowledge will be useful in determining if there are any elements that are more prevalent and maybe associated with the hydroxyapatite dissolution. There is the potential to learn if treatments are more effective in some patient populations because of specific element prevalence. In addition, this information can be used later to interpret crystal structures, potentially identify chemical end products of the thiosulfate reaction and maybe elucidate reaction chemistry and kinetics.

Mapping of mineral, thiosulfate and protein content in small nodules (or their agglomerates) by using FTIR and RAMAN spectroscopy. Nondestructive analysis is one of the advantages of these two complementary techniques. This will be done with a Nicolet Continuμm FT-IR microscope and Nicolet 6700 FT-IR spectrophotometer

(Thermo Scientific, Madison, Wisconsin). When using FTIR-RM analyses in reflectance mode, we can obtain maps of calcified deposits and identify their density (area under the v3 PO4 peak). In addition, protein maps can be generated (area under the amides peaks). Density distributions of mineral and protein content can be generated and displayed as color contour maps. The spectra from experimental samples will be compared against amorphous and hydroxyapatite standards. Peaks related to S will be identified and mapped if possible. Similar studies but complementary to FTIR can be run using RAMAN analysis.

Identification and characterization of material crystallinity and type of impurities incorporated in the hydroxyapatite lattice. (Transmission Electron Microscopy (TEM) and Electron Energy Loss Spectroscopy (EELS): TEM & EELS will allow us to determine crystal structure, impurities and chemical bond type present in small nodules. Since we have small volumes of liquid samples, this is likely the only technique allowing for structural and more comprehensive chemical analysis. Since the sodium thiosulfate can either directly or indirectly interact with CaP nodules (initial calcinosis nodule structure is more similar to enamel than bone) it would be important to see if the small nodules (collected after treatment) differ in structure over time. It is possible that thiosulfate acts as a calcium chelator and substitutes (or allows for other ions to substitute) for Ca in the crystal lattice and by doing so weakens the structure. It is also possible that other elements (impurities) substitute for Ca as a result of thiosulfate treatment, for example: Na, Mg, K, C or others.

Evaluation of proteins found in calcinosis lesions. We will evaluate acute phase reactants and protein mineralization matrix promoters and inhibitors over time in the liquefied calcinosis samples, and how these change with therapy. Calcium resins isolated from each patient at up to four different time points are extracted and divided into two samples, liquid and precipitate. Both samples are immersed in 2 mM EDTA containing protease inhibitors to elute the proteins. Protein concentrations are determined by Bradford assay using immunoglobulin as a standard.

The various proteins are separated by two-dimensional gel electrophoresis with a focusing range between pH 3 and 7 as the first dimension and 10% polyacrylamide SDS gel as the second dimension. The gel is stained and scanned by PDQuest Basic 2-D analysis software to analyze and annotate differentially expressed spots (proteins). The relevant spots are cut out of the gel into smaller pieces and the proteins are digested into tryptic peptides by Trypsin for MALDI-TOF MS analysis. Alternatively, some samples may be evaluated by using calcium-dependent affinity chromatography where the proteins are bound to a Sepharose matrix and eluted when the matrix is washed with increasing concentrations of Ca+2 ions ($CaCl_2$). Fractions with absorption at 280 nm are monitored and collected. The proteins in each fraction are separated through SDS-PAGE gel electrophoresis, and the purified bands are excised from the gel and digested in the same manner for MALDI-TOF MS analysis.

The peptides are analyzed by MALDI-TOF MS. The mass spectra data of the peptides is identified as a fingerprint after calibration with trypsin autolysis products and the protein is classified according to the Swiss-Prot and NCBI databases search.

The determined proteins may then be verified by western blot or immunocytochemical staining with a specific antibody.

5.17. Neutrophil Extracellular Traps (NETs) Analysis

Recent evidence implicates neutrophil extracellular traps (NETs; a microbiocidal strategy characterized by externalization of nuclear and neutrophil granular material) in the pathogenesis of various autoimmune disease including lupus, rheumatoid arthritis and vasculitis. It has been proposed that NETs may serve as source of modified autoantigens and also promote targeted tissue damage. It is unknown if patients with inflammatory myopathies have enhanced NETosis and whether this phenomenon may plan important role in the pathogenesis of these diseases. We will work in collaboration with Dr. Mariana Kaplan's laboratory to evaluate this process in dermatomyositis patients and to evaluate the effect that sodium thiosulfate administration has on NETosis. Blood samples will be collected for evaluation to quantify neutrophil extracellular trap formation in the presence or absence of stimulation using fluorescent microscopy and/or sytox green.

5.18. Microparticle and Other Endothelial Biomarker Evaluation

Endothelial microparticles (EMP) and platelet-derived microparticles (PDMP), which have been observed by others to be increased in small numbers of patients with lupus and dermatomyositis, will be assessed by flow cytometry in a 10-mL plasma citrate blood sample from patients using an LSR II digital flow cytometer, which provides higher sensitivity, resolution and faster rate of data acquisition. The features of LSR II are especially beneficial for rare event analysis, because EMP may represent less than 10% of total circulating MPs in the blood, and the machine may detect microparticles of smaller dimensions (100-200 nm). These will be correlated with established endothelial markers: Intercellular Adhesion Molecule – 1 (ICAM-1), vascular cell adhesion molecule (VCAM-1), E-selectin (CD62E), VE-cadherin (CD144). PECAM-1 (CD31), and others performed in the NIH Clinical Center laboratory, such as von Willebrand factor VIII related antigen and C-reactive protein levels. Monocyte-derived microparticles (CD14+), T lymphocyte-derived (CD3+) and B-lymphocyte-derived (CD19+), and platelet-derived microparticles (CD41), (CD61) will also be monitored. These studies may be performed on some of the patients enrolled in the protocol.

5.19. Center for Human Immunology (CHI) Assessments

Samples will be collected for exploratory evaluations by the CHI for drug effects on the immune system using a series of techniques to attempt to clarify which immune parameters are perturbed in DM patients with calcification and how they change with treatment. Based on our experience with sodium thiosulfate we believe it is having a beneficial impact on patients' underlying myositis disease processes in addition to their calcinosis, and these studies will aid in better understanding the biologic causes for this observed clinical improvement. These assessments will include proteomics evaluation to assess changes in serum protein expression with drug treatment to evaluate which pathways may be altered by medication treatment. We will also evaluate this question at the RNA level. In addition, we will assess cytokines to determine if there is a decrease in signs of inflammation and what may be leading to that change. Cell surface marker and cell population changes may also provide insight into additional mechanisms of this medication so we will also evaluate changes in these areas. Blood samples will be collected at -10 and 0 weeks to establish a baseline value and variation in this population then again at weeks 2,6, and 10 to evaluate for drug effect, and finally at weeks 24 and 62 to evaluate the longevity of any affect that was observed. Calcinosis

samples that are aspirated will also be sent for cytokine analysis and flow cytometry to better understand the composition of liquefied calcinosis and its response to therapy.

5.20. Questionnaire Assessments

Quality of life questionnaires will be administered in this study as part of the participants' clinical care and for research purposes. Generally, parents, children and adult patients will complete these assessment tools. Details of tool administration by visit, including the individual(s) completing the assessment, are provided in Table 7 found in Appendix B.

We plan to administer the following quality of life assessments in this study:

- Child Health Questionnaire - Parent Form 50 (CHQ-PF50 for pediatric participants) *or* Medical Outcomes Study Short Form Health Survey (SF-36; for adult participants), which include a pain score as one of their components
- Childhood Health Assessment Questionnaire (CHAQ; for pediatric participants) or Health Assessment Questionnaire (HAQ; for adult participants), to capture activities of daily living functional assessments and a pain visual analog scale score (these are also part of the IMACS activity core set measures as noted in Section 5.21)
- Calcinosis Assessment Tool (CAT), which will assess calcinosis severity and change with time (as noted in Section 5.22)
- Mawdsley Calcinosis Questionnaire, which is an experimental tool for assessing calcinosis in scleroderma patients will also be used to try and capture information on calcinosis severity.
- Patient Reported Outcomes Measurement Information System (PROMIS®) questionnaire, which will be used to capture daily life functions and health-related quality of life
- Skindex-29, which will assess dermatology-related quality of life impairment

5.21. International Myositis Assessment and Clinical Studies (IMACS) Group Activity and Damage Core Set Measure Assessments

The IMACS forms will be completed by the patients, parents of the patients, and a physician in order to assess patients' disease throughout the study. Details of the timing of form administration are provided in Table 7 found in Appendix B.

The IMACS Disease Activity Core Set Measures and Damage Core Set Measures have been described by Rider *et al*., (35), and are summarized on the IMACS website: http://www.niehs.nih.gov/research/resources/imacs/.

IMACS Disease Activity Core Set Measures include the following:

- Physician Global Activity - Visual Analogue Scale/Likert
- Patient/Parent Global Activity - Visual Analogue Scale/Likert
- Muscle Strength Testing - Manual Muscle Testing (MMT)

- Functional Assessment Tools - Childhood Health Assessment Questionnaire (CHAQ; for pediatric participants), Health Assessment Questionnaire (HAQ; for adult participants), Childhood Myositis Assessment Scale (CMAS)
- Laboratory - Muscle Enzymes
- Extramuscular Assessment - Myositis Disease Activity Assessment Tool

The IMACS Damage Core Set Measures include the following:

- Myositis Damage Index (MDI)
- Physician Global Assessment of Disease Damage
- Patient/Parent Global Assessment of Disease Damage
- Muscle Strength Testing – MMT (also part of the Disease Activity Core Set Measures, as listed above)
- Functional Assessment Tools - HAQ, CHAQ (also part of the Disease Activity Core Set Measures, as listed above)

5.22. Calcinosis Assessment Tool (CAT)

The CAT is a new tool our group has developed that we will be using to assess calcinosis severity and change with time, with the goal of validating this tool as part of this study. These questionnaires will involve a short form completed by the participant and the parent of a juvenile patient. The CAT also includes a pain VAS. Details of the timing of form administration are provided in Table 7 found in Appendix B.

5.23. Cutaneous Dermatomyositis Disease Area and Severity Index (CDASI)

The CDASI is a physician completed assessment of cutaneous disease in dermatomyositis. We will administer the CDASI version 2 to patients at specific time points as described in Appendix B Table 5. Whenever possible, these measurements will be performed by the same clinician throughout the trial.

5.24. Durometry

Durometry will be performed on patients. At least three separate areas of calcinosis will be evaluated during each assessment and the same area will be evaluated across different points in the study. The durometer small cylinder with a tiny mobile bump at the measuring end called the footpad. During an assessment, the evaluator will place the footpad at an angle to the calcinosis lesion and then roll it into a perpendicular orientation to the calcinosis lesion and press it firmly for several seconds. This will be performed 3 times at each site evaluated and the results averaged to provide a reading for that site. The schedule for durometry measurements is as described in Appendix B Table 4. Whenever possible, these measurements will be performed by the same clinician throughout the trial. They will be done using an OO digital DD-4 rex gauge digital durometer model number digital DD-4-OO. These will be recorded on the Calcinosis Sentinel Lesion Form along with additional physical exam comments on these areas of calcinosis.

5.25. CT/PET

Due to technical issues the MRI/PET machine may not be available for obtaining protocol described MRI/PET studies. CT/PET is an additional method of collecting this PET data although it involves additional radiation over that of MRI/PET. We will perform a CT/PET from the base of the skull to the toes. All participants receiving a CT/PET will receive a brief medical history to determine if they have any condition that would preclude a CT/PET. These contraindications include, but are not limited to, pregnancy, uncontrolled diabetes or age older than 95 years. A history of radiation exposure will be taken prior to enrollment and prior to performing any research study involving ionizing radiation. If the patient has received more than 0.5 rem for research purposes in the past 12 months prior to enrollment, we will delay enrollment until their time 0 would be 12 months or more from their last research radiation exposure. If the patient is already enrolled in the study and they have received more than 0.5 rem for research purposes outside of this protocol, we will review their situation with the DSMB and the IRB to decide whether to continue with currently planned research assessments involving radiation or to alter such assessments. All female subjects will receive a pregnancy test prior to this study and will be excluded from this testing if they are pregnant. All patients will have their blood sugar checked immediately prior to the study to ensure it is below 200 mg/dl, because hyperglycemia interferes with the uptake of radioactive glucose required for the PET scan. If blood glucose is found to be elevated, the medical team will work to safely lower the blood sugar and reassess if the blood sugar is at a level where the scan results would be interpretable. Prior to undergoing the scan, all subjects or their parents/guardians will complete a standard radiology questionnaire that determines other potential risks of the procedure that may have previously been missed. Prior to the scan patients will receive a detailed instruction sheet explaining that they need to minimize exercise for 48 hours prior to the scan, consume a high fat diet for 24 hours prior to the scan, and fast for 6 hours prior to the scan.

The CT/PET will consist of having an IV line placed and receiving 10 mCi. of FDG. The patient will then sit in a quiet room for an uptake period of approximately one hour. The patient will then be placed on the scanner for the CT/PET scan. These images will then be analyzed by a nuclear medicine physician and/or radiologist. If any abnormality the subject did not know about is detected by CT/PET, the subject and the subject's physician will be informed so that further care can be provided.

CT/PET has occurred at week 0 for the second patient enrolled in the study instead of a MRI/PET. This patient received a whole body MRI as described in section 5.7 during the week 0 visit as well for colocalization with this CT/PET.

CT/PET will be used for up to two additional adult patients instead of MRI/PET if MRI/PET is unavailable. These patients would receive a whole body MRI as described in section 5.7 during the visit as well for colocalization with this CT/PET.

All efforts possible will be made to make the experience as tolerable as possible.

CT/PET combined with the whole body MRI provides the same potential for direct clinical benefit as the MRI/PET and offers comparable, and likely superior, research data. To this end please refer to section 10.1.2.6 for what MRI/PET provides that the other imaging modalities do not with the understanding that all of the described uses of MRI/PET also

apply to CT/PET colocalized with a whole body MRI. We have also added additional information to section 10.1.2.20 about CT/PET.

6. Collection and Storage of Human Specimens or Data

We will collect and store clinical and laboratory data and biologic specimens on all participants. Long-term storage of all biological specimens in repositories will be coordinated by Dr. Lisa Rider's laboratory by dividing specimens between her laboratory facilities and NIEHS storage facilities for redundancy.

Privacy/confidentiality will be protected by: labeling all data and samples with a code; limiting distribution only to IRB-approved collaborators; limiting all data sharing procedures to only those conducted through encrypted electronic files or secure fax machines; and by keeping all data in restricted areas and locked filing cabinets, and by keeping all samples in restricted areas.

Retention of coded data and samples is needed until the end of the study to allow reassessment of information or assays as required to assist in the interpretation of individual outcomes and the overall results of the protocol.

Materials remaining after the planned analyses will stay in repositories, coded and identifiable only to the investigators intimately involved in the conduct and analysis of the study. To complete the studies described in the protocol, subjects' biologic samples coded with numbers only may be sent to collaborators, and samples will only be identifiable to those with restricted access to the database. Fully anonymous repository samples may be used for future research studies involving identification of autoimmunity-related genes or other markers that may help in improving understanding of the diagnosis, prognosis or pathogenesis of autoimmune disorders or for other research use beyond the scope of this study if subjects have agreed to that in the consents they have signed. If patients have not signed consent for anonymous research use beyond the scope of this study, we will seek appropriate IRB approval.

All data and any remaining materials will be returned to the PI at the completion of the study. All biological specimens will be frozen and stored in EAG laboratory freezers and/or in the NIEHS repository for research purposes as outlined in this protocol for as long as the protocol is active. We will track all collected data and specimens using local secure databases. When the protocol is closed all data and samples will be transferred to an appropriate long term data storage protocol.

Any loss or unanticipated destruction of samples or data (e.g., malfunction of a freezer) will be reported to the IRB. Additionally, a participant may decide at any point not to have their samples stored. In this case, the PI will destroy all known remaining samples and report this action to both the participant and to the IRB.

7. Study Personnel

Study personnel will perform the procedures as described below:

Principal Investigator: Adam Schiffenbauer, M.D., Investigator, Deputy Head, EAG EAG, NIEHS, NIH 10, Rm 6-C-432D, Bethesda, MD 20892: Will perform data collection, entry, and analysis of participant data, as well as with the recruitment and enrollment of subjects, as well as, planning and regulatory reporting to IRB, FDA and DSMB. Will oversee protocol development and implementation. Will consent patients.

Lead Associate Investigator: Lisa Rider, M.D., Head, EAG, NIEHS, NIH 10, Rm 6-5700, Bethesda, MD 20892: Will assist in the collection, entry, and analysis of participant data as well as with the recruitment and enrollment of subjects, and assist with planning and overseeing regulatory reporting and other aspects of the study protocol. Will consent patients.

Key Research Personnel

Associate Investigators:

Rachel Adam, M.S.N., R.N., NIHBC, ORSC, NIH: Working under the direction of the PI, will be assisting with safety monitoring of patients, recording and evaluating of adverse events, and data collection.

Julia Agafonova, M.S., Social & Scientific Systems, Inc., Silver Spring, MD: working under the direction of the PI, will serve as the protocol coordinator for this protocol. Her roles will include leading all efforts of regulatory coordination, study coordination, subject screening and registration, data entry and management, data analysis, specimen processing, laboratory repository management, study management, personally identifiable information management, DSMB coordination, and FDA regulatory submissions.

Nastaran Bayat, M.D., Social & Scientific Systems, Inc., Silver Spring, MD: working under the direction of the PI, will serve wide-ranging roles including regulatory coordination, study coordination, subject screening and registration, data entry and management, data analysis, specimen processing, laboratory repository management, study management, and personally identifiable information management.

Manfred Boehm, M.D., NHLBI, NIH: Will perform functional studies on peripheral blood cells of patients looking at pathways associated with calcinosis.

Stephanie Burrison, M.S.N,, R.N., NIHBC, ORSC, NIH: Working under the direction of the PI, will be assisting with safety monitoring of patients, recording and evaluating of adverse events, and data collection.

Marcus Chen, M.D., NHLBI, NIH: Will assist with the performance and evaluation of imaging studies.

Dawn Chin-Quee, Ph.D., M.P.H., Social & Scientific Systems, Inc., Durham, NC: Working under the direction of the PI, serves as an associate investigator with oversight of the SSS subcontracting team, and Reliance Agreements between SSS and the study. The SSS staff members working on this study, under the PI who will serve wide-ranging roles including regulatory coordination, study coordination, subject screening and registration, data entry and management, data analysis, specimen processing, laboratory repository, study management, personally identifiable information management, and clinical care.

Michael Collins M.D., NIDCR, NIH: Will perform evaluation of clinical samples and will assist clinically in evaluating calcinosis severity. Will also assist in monitoring for endocrine safety during the study including calcium-vitamin D homeostasis and osteoporosis evaluation. Alison Ehrlich, M.D., M.H.S., Foxhall Dermatology, LLC., Washington, D.C.: Will assist with dermatologic assessments.

Rachel Gafni, M.D., NIDCR, NIH: Will perform evaluation of clinical samples and will assist clinically in evaluating calcinosis severity and endocrine safety during the study including calcium-vitamin D homeostasis and osteoporosis evaluation.

Minal (Mina) Jain PT, Rehabilitation Medicine, CC, NIH: Will perform and assist in evaluation of strength testing, range of motion testing, and functional testing.

Anna Jansen, RN, C-NP, NIEHS, NIH: Will assist with patient scheduling, consenting, and facilitation of assessments. Will consent patients.

Galen Joe, M.D., Rehabilitation Medicine, CC, NIH: Will perform and assist in evaluation of strength testing, range of motion testing, and functional testing.

Shelley Kalsi, M.D., NHLBI, NIH: Will assist in clinical care and in recommendations about hematological safety in the study.

Mariana Kaplan, M.D., NIAMS, NIH: Will perform studies on patient blood samples examining net formation and net products.

Hanna Kim, M.D., NIAMS, NIH: Will serve wide-ranging roles including collection, entry and analysis of participant data, recruitment and enrollment of subjects, personally identifiable information management, clinical care, performance of research assessments, and recording of adverse events.

Jeffrey Kopp, M.D., NIDDK, NIH: Will assist with performance and evaluation of iothalamate clearance studies, as well as, assist in recommendations about renal safety during study.

Helen Looker, M.B.B.S., NIDDK, NIH: Will evaluate patient renal function data and assist with analysis of data related to kidney function.

Ashkan Malayeri, M.D., CC, NIH: Will evaluate and help to optimize patient MRIs and will assist with data collection/entry/analysis of such data.

Emilia Matthews, M.P.H, Social & Scientific Systems, Inc., Durham, NC: working under the direction of the PI, will serve wide-ranging roles including data entry and management, data extracts and analysis, programming, personally identifiable information management, and will serve as the lead to several SSS data entry and data management staff.

Corina Millo, M.D., CC, NIH: Will evaluate patient MRI/PET and assist with data collection/entry/analysis of such data.

Robert Nelson, M.D., Ph.D., NIH, NIDDK: Will process samples for iothalamate clearance and will assist in the determination of GFR from those samples.

James Reynolds, M.D., CC, NIH: Will evaluate patient DEXA scans and assist with data entry/analysis of such data.

Kakali Sarkar, PhD, NIEHS, NIH: Will assist with sample processing, managing the EAG biospecimen repository, and studies of gene expression in peripheral blood mononuclear cells, autoantibodies, and infectious agents.

Joseph Shrader, P.T., Rehabilitation Medicine, CC, NIH: Will perform and assist in evaluation of strength testing, range of motion testing, and functional testing.

Reed Thomas, B.S., NIDDK, CC, DEOB, Energy Metabolism Section: Working under the direction of the PI, will serve wide-ranging roles including performing DEXA scans, performing computational analysis of DEXA scans, and performing analysis of correlation of the DEXA scan data with other variables.

Rita Volochayev, PhD, CRNP, CPMN, CHT, MNLP, NIEHS, NIH: Will assist with patient scheduling, consenting, and facilitation of assessments. Will assist in the collection, entry, and analysis of participant data as well as with the recruitment and enrollment of subjects, and assist with regulatory reporting and other aspects of the study protocol. Will consent patients. Leslie Wehrlen, M.S.N., R.N., O.C.N., A.C.R.P-C.P., CC, NIHBC, ORSC, NIH: Working under the direction of the PI, will be assisting with safety monitoring of patients, recording and evaluating of adverse events, and data collection.

Jesse Wilkerson, B.S., Social & Scientific Systems, Inc., Durham, NC: Working under the direction of the PI, will serve wide-ranging roles including data entry and management, data analysis, personally identifiable information management, statistical analysis, study design, and serve as lead to several statisticians, data managers, and programmers.

Daniel Zaccaro, M.S., Social & Scientific Systems, Inc., Durham, NC: Working under the direction of the PI, will serve wide-ranging roles including data entry and management, data analysis, personally identifiable information management, statistical analysis, study design, and serve as lead to several statisticians, data managers, and programmers.

Non-Investigator Research Staff:

Collaborations have been established with the following collaborators and data or specimens will be sent to them to perform the specific analyses as described below.

NIH Collaborators:

Frederick Miller, M.D., Ph.D., NIEHS: Will assist in analysis of deidentified participant data.

Non-NIH Collaborators:

Ira Targoff, M.D., Oklahoma Medical Research Foundation, Oklahoma City, OK: Will evaluate serum for autoantibodies.

8. Study Statistical Considerations and Analytic Plan

8.1. Statistical Analyses

A single-arm, open-label study is proposed to evaluate the primary hypothesis that participants treated with sodium thiosulfate will experience greater improvement in calcinosis activity as assessed by a change in the calcinosis visual analogue scale (VAS) after treatment compared with any change in calcinosis activity visual analogue scale during the observation period prior to treatment with sodium thiosulfate.

A intention-to-treat statistical analysis will be performed where first, the primary outcome, the change in calcinosis activity VAS score from Week 0 to Week 10 compared with any change in calcinosis activity VAS score observed during the pre-treatment period (from Week -10 to Week 0), will be analyzed using a two-tailed paired t-test. This will be reported as absolute change for the primary outcome and calculated by taking value of the time 0 score minus the time -10 score and subtracting from that the value of the time

10 score minus the time 0 score. As secondary end points, we will also evaluate any changes as relative percent change and absolute percent change. Second, two-tailed paired t-tests will be performed to test for significant differences in the primary outcome for the adult and juvenile dermatomyositis patients separately. Multiple testing adjustments will be used to control for the multiple comparisons on the VAS score outcomes for the subgroup analysis.

Two-sided paired t-tests will be performed to test for significant differences in the relative percent change in VAS score, absolute percent change in VAS score, and the secondary outcome of change in quality of life score (Physical Function Score of CHQ-PF50 for children, SF-36 for adults) from Week 0 to Week 10, compared with any change in quality of life score observed during the pre-treatment period (from Week -10 to Week 0). The secondary outcomes of changes in the components of quality of life over time, changes in functional disability over time, change in muscle strength over time, and changes in gene expression associated with therapy will also be analyzed using paired t-tests. For the gene expression analysis, multiple testing adjustments will be used to control the false discovery rate. Categorical measures of improvement in calcinosis lesions, improvement in or stabilization of myositis activity, and incidence of adverse events will be analyzed using McNemar tests. Fisher exact tests will be used to test for differences between responders and non-responders to therapy. The severity of adverse events and quantitative measures of improvement in calcinosis lesions and myositis activity will be analyzed using paired t-test or regression to control for additional covariates. Generalized linear mixed models will be considered to analyze quantitative secondary outcomes across time. Descriptive statistics, such as mean, median and standard deviation, of the primary outcome and secondary outcomes of change in VAS, change in quality of life (QOL), change in functional disability, improvement in calcinosis lesions, and change in muscle strength are also of interest and will be used to plan further studies. Prior to statistical testing, the normality of quantitative measures will be evaluated by plotting the empirical distributions of the quantitative measures to look for deviations from normality as well as using QQ-plots. For non-normally distributed endpoints, normalizing transformations, such as the log transformation, will be applied. If normality cannot be achieved by transforming the data, analogous nonparametric tests, such as the Wilcoxon Signed Rank test, will be used in the analysis. The multiple comparison adjustment method that will be used is a stepwise Bonferroni-Class Method as published in Holm (39) and Hochberg (40).

The validity of the calcinosis assessment tool (CAT) will be assessed by focusing on the similarity between the CAT and other disease activity measures, like the Global Physician Score (GPhS) and Patient Reported Outcomes Measurement Information System (PROMIS®). Large disagreement between the CAT and other disease activity measures would suggest that the CAT tool is not measuring improvement in calcinosis but small differences may indicate that the CAT is capturing improvement in calcinosis that the other measures are not capturing. To assess the similarity between the disease activity measures, the Spearman rank correlation coefficient between the change in the CAT responses and the change in the other disease activity measure responses will be calculated. One can then test whether this correlation coefficient is different than zero, which would suggest an association between the two measures. A kappa statistic will be calculated to assess the agreement between discrete measures in the CAT and other discrete measures of disease activity. In the case where the number of categories observed by the CAT and other discrete measures of numbers of categories differ, the comparison will be accomplished by comparing the pooled scores for all categories of

the CAT to the pooled scores of each independent finding using a nonparametric Wilcoxon ranked sum statistic. Evaluation of the Mawdsley Calcinosis Questionnaire will be performed in the same manner as for the CAT. A cluster analysis will be considered to explore how well the change in VAS, along with other measures of disease activity, such as change in Global Physician Score, Patient Reported Outcomes Measurement Information System, MRI, CT scans, DEXA scans and vascular imaging, can distinguish between responders to intravenous sodium thiosulfate therapy and non-responders. Responders will be defined as those individuals who meet the primary end point and non-responders will be those individuals who fail to meet the primary end point. A Spearman rank correlation coefficient will be used to identify disease activity measures that are highly correlated and contain the duplicate information. This will aid in variable selection for future multivariate analyses to identify responders to therapy. We will also attempt to assess the responsiveness of these tools through their standardized response means.

8.2. Sample Size Considerations

Sample size estimates for this analysis are based on the following assumptions:

1. The primary outcome is the mean difference in the change in calcinosis activity during the pre-treatment period and the change in calcinosis activity during the treatment period.
2. The calcinosis activity will be measured by the calcinosis visual analogue scale (VAS) score. This score ranges from 0 to 100 mm.
3. Pre-treatment period change in calcinosis activity is calculated by subtracting the calcinosis VAS score at Week -10 from the calcinosis VAS score at Week 0. Treatment period change in calcinosis activity is calculated by subtracting the calcinosis VAS score at Week 0 from the calcinosis VAS score at Week 10.
4. The treatment period change in calcinosis activity subtracted from the pre-treatment period change in calcinosis activity is approximately normally distributed or can be transformed such that it will be approximately normally distributed.
5. The sample size estimate is the sample size required after attrition.

There are few prior data on the use of sodium thiosulfate for treatment of the calcinosis comorbidity in dermatomyositis patients. Therefore, there is limited information to estimate the necessary sample size to achieve adequate power. For the power analysis, a standard deviation ranging between 5 and 20 mm is assumed for the difference in the change in calcinosis activity between the treatment and pre-treatment periods based on previous studies that use visual analog scale measurements (Kelly 2001 and Demoly et al. 2013). The power analysis is based on two-sided, paired t-tests with a within subject correlation of 0.5 and 90% power. The sample sizes required for a range of significance levels, assumed standard deviations, and mean differences in the change in calcinosis activity VAS score between the pre-treatment and treatment period are given in Table 3.

Table 3 Sample Sizes required, after attrition, to detect significant differences in the change in calcinosis activity between the pre-treatment and treatment periods. The estimates are based on two-sided, paired t-tests and 90% power where α

denotes the significance level and sd denotes the standard deviation of the differences[a].

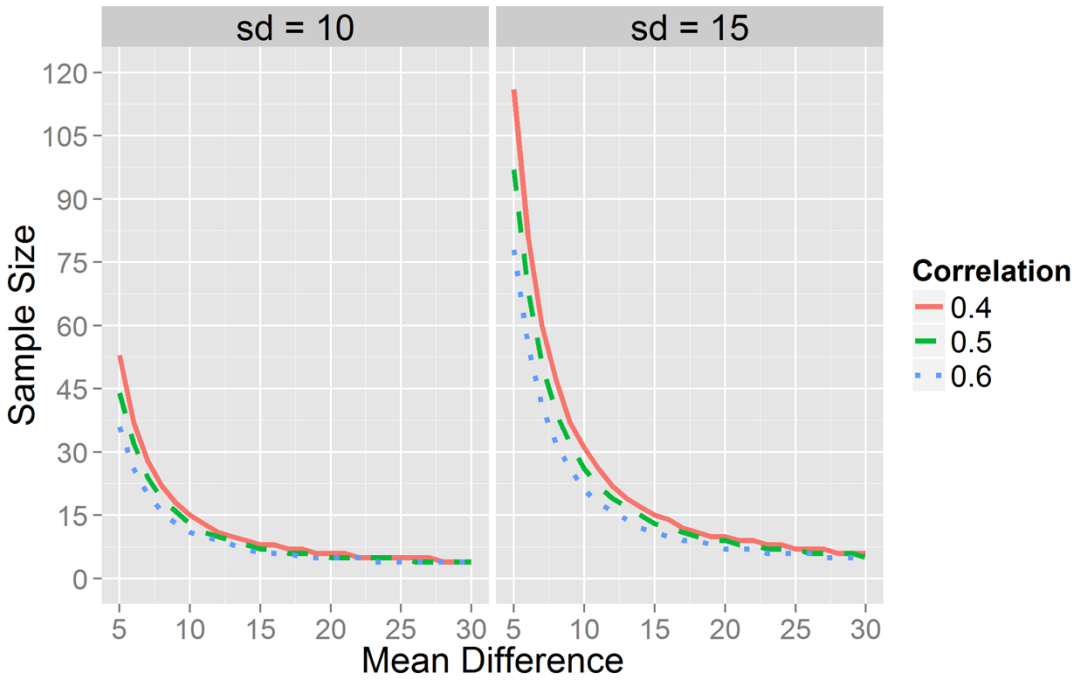
	α=0.025				α=0.05			
Mean Difference[b]	**sd=5**	**sd=10**	**sd=15**	**sd=20**	**sd=5**	**sd=10**	**sd=15**	**sd=20**
10	6	16	31	53	5	13	26	44
11	6	13	26	44	5	11	22	37
12	5	12	23	38	5	10	19	32
13	5	11	20	32	4	9	17	27
14	5	10	17	28	4	8	15	24
15	5	9	16	25	4	7	13	21
16	4	8	14	23	4	7	12	19
17	4	8	13	20	4	6	11	17
18	4	7	12	18	4	6	10	16
19	4	7	11	17	4	6	9	14
20	4	6	10	16	3	5	9	13
25	4	5	8	11	3	5	7	9
30	3	5	6	9	3	4	5	7

[a]In between-patient standard deviation of the differences in change in calcinosis activity score between the treatment and pre-treatment periods

[b]The mean of the differences in change in calcinosis activity between the treatment and pre-treatment periods

Assuming a standard deviation of 10 and a significance level of 0.05, a sample size of 13, after attrition, will provide at least 90% power to detect an improvement in calcinosis VAS score between the treatment and pre-treatment periods of at least 10 mm. If the standard deviation is 15, then a sample size of 13 will detect a mean difference of at least 15 mm. The sample size required depends on the assumed within subject correlation. Figure 2 illustrates how the required sample size will change depending on the assumed within subject correlation. Generally, the larger the assumed within subject correlation, the smaller the required sample size. For the subgroup analysis focusing on the juvenile and adult DM patients separately, a Bonferroni correction can be used to control the overall family-wise error rate to below 0.05 giving a significance level of 0.05/2=0.025 for each paired t-test in the subgroup analysis. Assuming that approximately the same number of juvenile and adult DM participants is enrolled in the study, a sample size of 13 for the combined group will result in at least 6 participants in each age subgroup. Assuming subgroup sizes of 6 and a standard deviation of 10, there will be at least 90% power to detect age group specific mean differences of at least 20 mm.

Figure 2 Sample sizes required, after attrition, to detect significant differences in calcinosis activity between the treatment and pre-treatment periods for standard deviation values of 10 and 15 and a range of within subject correlations. The estimates are based on two-sided, paired t-tests with a 0.05 significance level (α=0.05) and 90% power



8.3. Accrual Number

Based on the information provided in preliminary data, unpublished reports, other studies involving sodium thiosulfate therapy, and the power analysis, the targeted number of participants to complete this study is 13. Considering the involved schedule of this therapy balanced with the lack of alternative treatment options for these patients, we anticipate a dropout rate of approximately 25%. Thus, to achieve a final sample size of 13 participants completing the study, we plan to enroll 18 participants. As discussed initially completers will be patients who receive all infusions or who reach the primary end point by week 6. For patients who need medication escalation prior to week 10 we will attempt to enroll another patient to use for the primary analysis instead of them as long as the total enrollment is still 18 or less patients.

Based on our experience enrolling a similar population, we anticipate screening approximately 5-6 individuals for every 1 eligible participant. Thus, we anticipate screening approximately 100 individuals for this study. Screening will be done by phone or in person interview and review of medical records. If the patient has been involved in other research studies information from those studies may also be considered.

9. Human Subjects Protection

The Principal Investigator will submit the protocol, informed consent form, recruitment methods, and any other proposed participant materials to the IRB for review and approval. Participants will not be enrolled until the submission has been approved in writing by the IRB. Once the protocol is approved, the investigator will be responsible for obtaining IRB approval of the annual Continuing Review for the duration of the study.

The investigator will submit and obtain approval from the IRB for all amendments to the protocol, informed consent form, and other study documentation referenced above. Amendments will not be implemented without prior IRB approval, except where necessary to eliminate immediate hazards to the participants.

9.1. Consent/Assent Documents and Process

Informed consent is an ongoing, interactive process that is initiated when the discussion regarding study participation begins and continues throughout study participation. The Principle Investigator or other approved and properly trained study personnel will discuss the study's purpose, procedures, risks, benefits, and the rights of participants to help potential participants and/or their parents make an informed decision about enrollment. Potential participants and/or their parents will be given ample time to ask the investigator questions and obtain clarifications regarding the study prior to agreeing to enrollment. Consent will be obtained by Adam Schiffenbauer, Lisa Rider, Rita Volochayev or Anna Jansen, who have completed human subjects' research protection training, training on the protocol and informed consent, and conflict of interest/ethics training and clearance. In addition, these individuals have been approved to consent by the PI of the protocol. The process for obtaining informed consent from prospective subjects will be followed as described in NIH HRPP Policy 301 - *Informed Consent*. Other members of the study team may also discuss sections of the consent document with potential patients as part of the ongoing process of informed consent, but this will not substitute for a complete discussion and consent with one of the above named consenting individuals. All study members besides the PI and lead AI prior to being allowed to consent on their own will watch a patient be consented to this study by a member of the study team approved to consent and performed a consent while being monitored by a member of the study team already approved to consent.

After voluntarily agreeing to take part in the study, participants or the participants' parent/guardian will be asked to sign and date a current IRB-approved informed consent form (ICF) that is compliant with 21 CFR 50, and assent will be obtained from minors. The signed informed consent form will be obtained prior to the initiation of any study procedures. When a document that is in electronic format is used for the documentation of consent, this study will use the iMed platform, which is 21 CFR, Part 11 compliant, to obtain the required signatures. During the consent process, participants and investigators will view the same approved consent document simultaneously in their respective locations through an approved telehealth platform. Both the investigator and the participant will sign the document with a hand signature using a finger, stylus, or mouse. Under the requirements set forth in 21 CFR 50.55 (e)(1), the permission of one parent is sufficient for this study in which the research is greater than minimal risk but presents the prospect of direct benefit (45 CFR 46.405, 21 CFR 50.52). Participants will be informed that they may withdraw consent at any time throughout the study. During the course of the study, participants and/or their parents will be informed of any changes

in the design of the study or any changes in the risks of participation, and their consent for ongoing participation will be obtained.

The acquisition of informed consent will be documented in the participant's medical record, including the date that consent is obtained. A copy of the signed and dated ICF will be provided to the participant and/or their parents. The original version of the ICF will be retained in the medical records department at the NIH Clinical Center.

For minors, additional steps will be taken as part of the consenting process. Efforts will be made for minor patients to sign an assent to the study if they are able to do so.

The protocol will be explained to each child as detailed as possible as appropriate to their age and level of understanding; a parent or guardian will be present to witness this discussion, and the child's questions about the research study will be addressed. Written assent will be obtained for child participants under the age of 18 years, as appropriate to each child's level of maturity and understanding. Children 14-17 years of age will be verbally consented and sign on the assent line of the consent document, while children 7 – 13 years of age will obtain written assent in a separate assent document for this age group. If a child is unable to understand the written consent or assent document, the content will be verbally explained to the child as best we can at an age-appropriate level. If a child is unable to sign the document, but is agreeable to participation, this will be documented in CRIS.

When a pediatric subject reaches age 18, continued participation (including ongoing interactions with the subject or continued analysis of identifiable data) will require that consent be obtained from the now adult with the standard protocol consent document to ensure legally effective informed consent has been obtained.

If reconsent is not feasible, we request waiver of informed consent to continue to use data and/or specimens for those individuals who become lost to follow up or who have been taken off study prior to reaching the age of majority.

Requirements for Waiver of Consent consistent with 45 CFR 46.116(f)(3):

(1) The research involves no more than minimal risk to the subjects.

   a. Analysis of samples and data from this study involves no additional risks to subjects.

(2) The research could not practicably be carried out without the waiver or alteration.

   a. Considering the length of time between the minor's last contact with the research team and their age of majority, it will likely be very difficult to locate them again. A significant reduction in the number of samples analyzed is likely to impact the quality of the research.

(3) As the research involves using identifiable private information or identifiable biospecimens, the research could not practicably be carried out without using such information or biospecimens in an identifiable format.

   a. Though the purpose of future studies cannot yet be known, they often involve the correlation of clinical outcomes and clinical interventions with laboratory studies. Such information would be unavailable if access to medical record numbers was unavailable.

(4) The waiver or alteration will not adversely affect the rights and welfare of the subjects.

a. Retention of these samples or data does not affect the welfare of subjects.

(5) Whenever appropriate, the subjects will be provided with additional pertinent information after participation.

a. We only request a waiver of consent for those subjects who have been lost to follow-up or who have been taken off study prior to reaching the age of majority.

In the case of minors where there are custody considerations that are beyond the scope of NIH HRPP Policy 402 - *Children in Research*, an NIH Ethics consultation will be requested before proceeding with the consent process. In situations where there are custody considerations and both parents of a minor patient are unable to come to NIH in person informed consent may be obtained by telephone. In this case, relevant consent documents will be mailed to prospective participants and a phone call date and time will be scheduled. The PI Adam Schiffenbauer; the LAI, Lisa Rider.; the AI, Anna Jansen, will execute the consent process in a telephone call, seeking the individual's consent, and then instruct prospective subjects to mail back consent documentation to the study team. A local adult witness, who may be a family member, also signs the consent form as a witness to the subject's signature. Often the consent process begins in prior informational phone calls with the prospective subject, which may be conducted by a clinician who is an AI or PI on the protocol, the EAG research nurse, or the protocol coordinator. These telephone calls will include providing information about the study and study procedures, discussing risks of participation and answering all patient questions. Email, texting, or electronic communications such as Skype or WebEx, may also be used as part of this consent process. All consent contacts from study staff, will be documented; specifically, the name of the caller, who was reached, the date of the call or contact, and a general comments section for what was discussed and which questions were asked will be recorded. At the time of consent signing the final documentation of consent will occur in CRIS.

We do not plan or anticipate the enrollment of non-English speaking subjects. However, they are not excluded from participation either. If there is unexpected enrollment of a research participant for which there is no translated extant IRB-approved consent document, the Principal Investigator and/or those authorized to obtain informed consent will use the short form consent process as described in MAS Policy M77-2, NIH HRPP Policy 301 - *Informed Consent*, 21 CFR 50.27 and 45 CFR 46.117 (b) (2. The summary that will be used is the English version of the extant IRB-approved consent document. We will notify the IRB at the time of continuing review of the frequency of the use of the short form.

9.2. Rationale for Subject Selection

Children: Potential participants age 7 or older will be considered for enrollment in this protocol. Children are included because the complication of calcinosis in dermatomyositis is more common in pediatric patients than adults. Due to the volume of

blood being drawn for this study and the requirement to remain still and cooperative for several assessments in this study (e.g., MRI, DEXA and CT scans, clinical assessments), children under the age of 7 or less than 26 kg are not included in the study population.

Pregnant and Breastfeeding Participants: Sodium thiosulfate for one-time use with cyanide poisoning is classified as Pregnancy Category C. There are no well-controlled studies of its use in pregnant women and therefore, women who are pregnant are excluded from participation in the study. It is not known whether sodium thiosulfate is excreted in breast milk and therefore, nursing mothers are also excluded from participation in this study. Participants of reproductive potential will be asked if they are pregnant or breastfeeding. Any participant found to be pregnant at the screening visit will not be eligible for enrollment until they are no longer pregnant, at which time they would undergo a second abbreviated screening visit. Pregnancy testing will be used to safeguard against accidental enrollment of participants who are pregnant but do not yet know it. Female participants of reproductive potential will receive a urine or serum pregnancy test at all study visits and during the infusion period of 0 to 10 weeks on a weekly basis, they will also be asked if they are breastfeeding at each of these time points; both are assessed as part of clinical care to eliminate any risks to an unborn fetus or nursing child. The pregnancy tests will be performed at the NIH Clinical Center laboratory. All participants will be required to use birth control during study participation. All participants will be told about the importance of using approved birth control methods (see section 9.2). Pregnant women are excluded from enrollment, but if a participant becomes pregnant after week 0 they will complete the study except for receiving additional doses of drug and those study procedures, ionizing radiation in particular, felt to be dangerous to the fetus. See section 5.5 for additional information on pregnancy and breastfeeding status.

Prisoners: Prisoners and adults who are or may not be able to consent will not be enrolled due to uncertainty surrounding coercion in participating in clinical research, and the inability to freely complete all study parameters.

NIH Employees: While there is no plan to enroll NIH staff as subjects, we will allow NIH employees to enroll should the event arise, and all safeguards mentioned in NIH HRPP Policy 404 - *Policy-Research Involving NIH Staff as Subjects*, will be followed.

9.3. Recruitment Plan

Potential participants will be recruited, screened, and enrolled without regard to gender, race, or ethnicity.

Subjects will be recruited by advertisements *[IRB-approval for which will be obtained based on the requirements in* NIH HRPP Policy 302 - *Policy - Subject Recruitment and Compensation]* placed in medical journals, on websites/email list-servers of medical professionals*,* in patient support group or registry mailings*, and/or in a* variety of patient support group literature and websites, including new patient communication sites, such as Facebook, MySpace, Twitter, Google Adwords and making podcasts for distribution as allowed by NIH policies. The study will also be advertised by direct mailings to professional society members, by announcements at meetings and development of a NIEHS recruitment website

We anticipate recruitment of 18 fully eligible participants will take up to approximately 3 years from study opening to complete.

10. Evaluation of Risks/Discomforts and Benefits Ratio

10.1.1. Benefits

The potential benefits to participants include thorough clinical assessment (including physical exams, diagnostic assessments, and laboratory tests) by experts in the field, which may be used to guide recommendations for medical care to be used by their primary physician(s). Participants and/or their parents will be informed of any findings that require medical attention or any other relevant health findings, and participants and/or their parents will have the opportunity to discuss the findings with a study physician.

As there is no therapy that has proven to be highly effective in the treatment of calcinosis associated with dermatomyositis, participation in this study offers patients suffering from this condition the potential for therapeutic relief from the debilitating effects of their condition. Additionally, participants have the opportunity to contribute to the medical community's understanding of calcinosis, dermatomyositis, and potential treatments, which may provide positive feelings of helping others.

The potential knowledge gained from this research may help define therapeutic strategies for dermatomyositis-associated calcinosis, and will contribute needed safety data on the longer-term use of intravenous sodium thiosulfate in the context of a prospective clinical trial. This study would also lead to the development of a calcinosis assessment tool, and potentially to new imaging modalities for calcinosis and biomarkers, which could be used to assess efficacy of therapeutic agents for calcinosis in future clinical trials. Finally, the study may help improve our understanding of the immunologic, endothelial, and genetic factors important in the pathogenesis of calcinosis and dermatomyositis, and in responses to therapy.

10.1.2. Risks/Discomforts

Participants in this study will undergo multiple inpatient assessment and/or treatment periods lasting approximately 3 days up to 10 weeks. This poses a significant disruption to the lifestyle and daily schedules of the participants. The study staff will work with the participant/parent to find the optimum timing for study visits that minimize the impact of visits for the participant/parent while meeting the requirements of the protocol. Patients will be allowed to go on pass when they do not have assessments and there is not a need to monitor them inpatient for safety reasons. Recreation therapy will be consulted for every participant to help inpatient stays be more enjoyable.

Risks associated with specific study procedures are described in the sections below.

10.1.2.1. Sodium Thiosulfate Treatment

Sodium thiosulfate injection solution, USP 12.5 g/50 mL [FDA Application Number: (NDA) 203923] has been approved in the US since 1992 and is indicated for sequential use with sodium nitrite for treatment of acute cyanide poisoning that is judged to be life-threatening (41). There have been no controlled clinical trials conducted to systematically assess the adverse events profile of sodium thiosulfate, and thus AE information reported in the medical literature was not collected using consistent

monitoring or methodologies. Side effects that have been reported for sodium thiosulfate include osteoporosis, dental injury, hypotension, nausea, transient hypernatremia, transient hypertension and metabolic acidosis. This listing of adverse events involves a literature review, conversations with principal investigators involved in the studies described in table 2 where we were able to reach them, and from Adherex's briefing "Sodium Thiosulfate Injection, USP, 25% Briefing Document for The Pediatric Subcommittee of the Oncologic Drugs Advisory Committee" from November 1, 2011 presented to the FDA. Specific targeted monitoring for these side effects will be performed (see Section 14.3). Medications may be administered as needed to help alleviate these side effects. In particular, for participants who suffer from nausea with infusions, an option for pre-treatment with medications in order to prevent their nausea will be available. Patients experiencing fever, chills or hypotension may be premedicated with acetaminophen and/or Benadryl.

The frequency of occurrence of adverse events cannot be definitively assessed, but some estimates can be made. Many times, in the literature sodium thiosulfate is given with cisplatin or other chemotherapy agents so it is difficult to ascertain whether side effects are due to the chemotherapeutic agents or to sodium thiosulfate. Neuwelt et al. (26) assessed adverse events in patients receiving sodium thiosulfate with chemo therapy. Out of 12 children receiving sodium thiosulfate only one child experienced an elevated serum sodium level the day after receiving therapy and that child had Fanconi's kidney secondary to prior ifosfamide therapy. This is the only side effect he attributes out of 132 courses to sodium thiosulfate that reached a grade of 2 or greater. He also notes with premedication there were no episodes of nausea that rose to being greater than grade 1. In his earlier paper (42), in adults, he notes nausea requiring premedication in most adults receiving 16-20 g/m^2 sodium thiosulfate. For the transient hypernatremia, he notes at 20 g/m^2 the serum sodium increased 10-15% above baseline during infusion and was associated with a 10-15% increase in blood pressure. He also shows this transient hypernatremia peaked immediately post transfusion and was down on average to only 7% above baseline 30 minutes post infusion. Based on this information, as well as discussions with the investigators from other studies, as well as the short half-life of sodium thiosulfate, the peak electrolyte abnormalities are believed to occur in under an hour post discontinuation of medication and are unexpected to occur post treatment after week 10. The Adherex's briefing "Sodium Thiosulfate Injection, USP, 25% Briefing Document for The Pediatric Subcommittee of the Oncologic Drugs Advisory Committee" from November 1, 2011 presented to the FDA lists transient hypernatremia as happening in 21-100 out of every 100 children, nausea and vomiting happening in 5-20 out of every 100 children, and hypotension and contact dermatitis happening in less than 5 out of 100 children. Metabolic acidosis was reported as a side effect in a case report. Osteoporosis is a theoretical risk based on the drugs mechanism of action, but has never been reported.

Sodium thiosulfate is substantially excreted by the kidney, and the risk of toxic reactions may be greater in patients with impaired renal function. Thus, individuals with renal impairment (GFR less than 30 representing severe renal disease) or kidney stones have been excluded from participation. All adult patients will receive an infusion of 1 liter of normal saline following each infusion as tolerated. Pediatric patients who can tolerate it will receive 5-10 cc/kg IV normal saline (up to maximum 1 liter) following sodium thiosulfate infusion. Oral hydration between infusions will be encouraged.

Sodium thiosulfate for this study will be given intravenously. To achieve this patients may have several types of IV access placed including, but not limited to a peripheral IV, a PICC line, a port, or a central line. The safest and least disruptive option for the patient will always be selected. The risks of this line placement include bruising, infection, bleeding, and calcification at the site of line placement. Prior to the placement of any IV access route besides a peripheral IV additional consent for line placement will be obtained. There is the potential for sodium thiosulfate extravasation or contact with skin as a risk in this study. Sodium thiosulfate due to its chelation properties has been used to treat extravasation of chemotherapy medications (43) and based on this and our experience we do not expect any significant complications from extravasation. One patient we managed who received IV sodium thiosulfate had extravasation into her arm and developed local pain for ~10 minutes after the IV was placed that then resolved. Sodium thiosulfate is also used topically for off-label purposes and has not been reported to have caused skin irritation. In the event of extravasation, the administration of the medication will be stopped and the patient assessed for side effects. Once the patient has no complaints from the site of extravasation, we will attempt to reestablish IV access and complete the infusion.

In our experience with this medication we have also experienced significant electrolyte abnormalities in calcium, magnesium, sodium, phosphorus, and potassium. Potassium abnormalities are thought to be due to the dextrose solution and rapid changes with insulin in serum potassium. The others are thought secondary to study drug. These levels are monitored for safety and appropriate corrective actions are administered as needed.

10.1.2.2. Dextrose Diluent

Dextrose will be used as the diluent for the sodium thiosulfate. With the use of dextrose the patient may experience hyperglycemia during the infusions. Hyperglycemia can cause the following thirst, polyuria, weight loss, blurry vision, mental status changes, obtundation, seizures, coma, abdominal pain, nausea, and vomiting. The risk of hyperglycemia is increased in patients with diabetes. If hyperglycemia becomes severe the study team will take actions to control the hyperglycemia. In patients with diabetes or with glucose intolerance additional monitoring may take place to increase safety of dextrose administration including, but not limited to increased fingerstick blood glucose monitoring or continuous glucose monitoring. Patients with hyperglycemia secondary to this may also need to have changes made to their diabetes regiment that can include, but not be limited to increased insulin dose, increased insulin frequency, and/or an insulin drip with infusions. Patients with diabetes will receive consults from the hospital blood glucose management team prior to initiating sodium thiosulfate and will be monitored by them during their periods at the NIH clinical center.

Dextrose infusion can also cause confusion, loss of consciousness, dehydration, hypervolemia, hypokalemia, and potential allergic reaction.

10.1.2.3. Muscle Strength and Function Testing

The risks of muscle strength and function assessments are similar to the risks of physical activity for this patient population. Mobility is often limited and painful for these patients, and testing may exacerbate their discomfort or condition, or could result in musculoskeletal injury. Staff performing the assessments will be qualified to administer

the tests, including any methods to use during testing to minimize the chance of adverse outcomes.

10.1.2.4. MRI

For the period of January 1st 2011 to January 1st 2012, according to the Manufacturers and Users Device Events (MAUDE) FDA database, 161 adverse events involving MRI were reported and for 2014, according to the MAUDE database, there were only 11 incidents involving a magnetic resonance scanner making the risk of an adverse event from a MRI negligible. It is possible for MRI to cause injury to patients with metal in their bodies, however, most surgically-placed metal is safe. Subjects will be screened thoroughly, and any subject with a metal implant deemed unsafe will not be eligible for the study. The MRI procedure may be unpleasant due to the confined space and loud noise. Precautions such as protective ear equipment and allowing the patients to communicate with the operator during the study will be taken to minimize this discomfort. No contrast agent will be used. MRI offers additional information not obtainable by DEXA or CT scan. These modalities allow the evaluation of inflammation, by visualizing water content. This allows us to determine the amount of edema, presumed to be associated with inflammation, around areas of calcinosis and also in muscle. MRI is the gold standard modality for assessing for muscle inflammation and thus would be expected to be used to assess for any muscle benefit for the study drug. In addition, we have seen patients with liquefied calcinosis where the CT and DEXA scans have difficulty detecting the liquefied portion, but these areas are readily visible on MRI.

10.1.2.5. CT Scan

Non-contrast CT scan is a routinely performed procedure; however, it is associated with ionizing radiation. The hazard of radiation from a CT scan will be explained to patients prior to the test. All adult patients will receive up to 2.5 rem/scan with children receiving 0.76 rem/scan and as part of this study will receive one research scan and up to two clinical CT scans. The research radiation from this test is below the 5 rem/year recommended limit set by radiation safety for adults and above the 0.5/rem/year recommended limit set by radiation safety for pediatric patients. There is increased risk from the radiation. However, there is the potential for direct benefit. The information on the extent and location of calcinosis in these patients helps to determine disease burden to guide therapy, has located previously unknown areas of calcinosis that explain vague patient complaints that otherwise would have led to additional evaluations, and for those patients contemplating surgery, can help guide surgical resection. There is also a research benefit to performing these CT scans as the best way to define disease burden and quantitate change in calcinosis over time. It also serves as our gold standard for identifying calcinosis in evaluating the ability of DEXA scan to determine calcinosis burden. Risks of radiation exposure are detailed in the consent form, using wording provided by the NIH Radiation Safety Committee. In some participants, going through the CT scanner may cause claustrophobia. Pregnancy testing will be implemented prior to any female participant undergoing CT scan to reduce the chance of exposure of a developing fetus to ionizing radiation. For 2014 the MAUDE database recorded 3 incidents involving a computerized tomography scanner, which given the national number of CT scans performed, makes the risk of acute mechanical injury negligible.

All clinically indicated scans that take part during this study will be performed on a specific clinical scanner using a research based method to reduce radiation exposure,

instead of on a clinical scanner with normal software interpretation. Adult patients will receive a maximum of 2.5 rem from a clinical CT scan and pediatric patients will receive a maximum of 0.76 rem using the research algorithm, which is lower than the radiation of an equivalent CT scan on a clinical scanner not using this algorithm at the NIH Clinical Center. In addition, this clinical scan using the research algorithm will provide more information and in particular allow for better 3D reconstruction than a traditional clinical CT scan.

There are limited measurements to objectively monitor calcinosis and one of these methods is through imaging. Due to the depth of many of these lesions, their range of consistencies and the often overlying bone, ultrasound based imaging is not able to perform a full assessment of areas of calcinosis and often has an interfering echo shadow density that obscures the measurement of calcinosis. A full body x-ray survey would not be able to image all areas with overlying bone, would not be possible in many of the patients with restricted range of motion, is harder to reproduce at different time points, is often not very sensitive in detecting smaller lesions, and still involves a significant amount of radiation. We have already seen patients where by x-ray they were diagnosed as having lung lesions, and on CT it was discovered they were paravertebral muscle calcification. For all of these reasons, we believe plain radiographs are of limited use in assessing calcinosis in this setting. MRI is poor at imaging actual calcinosis lesions and insensitive in their detection, and MRI/SPEC has been unable to reveal information about lesions due to their high density. Due to this, it is our experience that MRI is too limited in what it can reveal about calcinosis lesion size and morphology to be useful for that purpose. DEXA scan we believe poses the ability to offer a low radiation method for evaluating calcinosis, but suffers from many of the same issues of full body x-ray survey in that it cannot image lesions that overly bone. Nuclear imaging techniques such as ^{18}FDG PET offer insights into calcinosis metabolism and activity, but still must be correlated with a CT scan to get a structural correlation and do not pick up all calcinosis lesions. Based on this, we believe CT is the only way to obtain reliable full body imaging of calcinosis lesions, despite the significant amount of radiation associated with it.

Having a method to evaluate the calcinosis lesions radiographically instead of simply by physical exam is critical for several reasons. First, many areas of calcinosis are not visible or palpable on exam. Thus, they cannot be assessed for response to therapy except by imaging. It is unclear if all areas of calcinosis respond the same to treatment, so we feel it is not appropriate to make assumptions on what can be large unobservable lesions based on the changes we observe in superficial lesions. Second, calcinosis lesions can move as part of their course which has to be differentiated from the formation of new lesions in evaluating disease therapy. As an example, in the last case we present from our personal experience on treatment with sodium thiosulfate, the patient developed on surface exam many more superficial calcinosis lesions. However, from CT scans we were able to see that these were not new lesions, but old deep lesions coming to the surface. Thus, without the CT, she would likely have been assessed as worsening, while with CT evidence she was assessed as having improvement. Third, CT is an objective measurement compared to many of the subjective measures we are using in this study due to the difficulty in assessing calcinosis and the scans will be read by observers blinded to the treatment in the protocol. Having an objective measurement to monitor alongside the more subjective measurements will allow it to provide support that those measures are due to true change and not placebo effect. Fourth, CT scan can be used to track changes in disease

burden even with minimal clinical findings. In a clinical setting sodium thiosulfate is likely to be best given in an episodic manner and CT imaging would be a way to assess for increasing disease burden and potentially the need for retreatment before the patient had significant new symptoms. Fifth, CT will help us to determine cause for improvement. As previously discussed there are multiple proposed mechanisms of action in addition to the role of sodium thiosulfate as a calcium chelator. Significant improvement in other areas without a noticeable change on CT could help demonstrate that one of the main mechanisms of action of sodium thiosulfate is something other than its role as a calcium chelator. Sixth, CT scan can help determine if symptoms are due to calcinosis or other conditions. For example, a patient with left hip pain may have that pain due to calcinosis or simply muscle strain or hip synovitis or avascular necrosis. On physical exam, a calcinosis lesion may not be appreciated, but it does not rule out a deep calcinosis lesion, which a CT scan could rule in or out and thus better allow one to differentiate if the symptoms are due to calcinosis or not. Based on the importance of having CT scans as part of the assessment of calcinosis as stated above, we feel the added risk of the research radiation associated with CT scan is worth the direct benefit to patients.

We will also perform up to two clinical CT scans in calcinosis patients using the research CT algorithm. Since using the research algorithm allows us to reduce the radiation dose of clinical CT scans below the normal level used for CT imaging, we feel that having a clinically indicated CT scan done as part of this protocol using this algorithm provides patients with reduced risk for the research CT scan compared to a standard clinical CT and thus the benefits of having these clinically indicated CT scans using the research algorithm outweigh the risks.

10.1.2.6. MRI/PET

An MRI/PET offers the potential benefit of understanding the activity of a patient's calcinosis. The ability to observe not just morphologic changes, but metabolic activity of these areas of disease offers the potential to better understand the benefit of sodium thiosulfate therapy and guide future treatment off of these findings. The hazard of radiation exposure from a MRI/PET will be explained to patients prior to this test. This study involves all of the risks of a MRI and in addition the risks associated with the radioactive agent and the placement of an IV line. The adult patients will receive 0.67 rem of radiation which is less than one tenth the radiation exposure of a normal diagnostic CT scan and based on BEIR V estimates would increase their lifetime risk of cancer death by 0.0504% (The standard radiation exposure of a diagnostic CT scan is significantly higher than the radiation dose of the CT scans being used in this study due to our use of a research algorithm to reduce radiation exposure). Pediatric patients will receive less than 0.69 rem of radiation based on age and body habitus. For January 1st 2011 to January 1st 2012 according to the Manufacturers and Users Device Events (MAUDE) FDA database, 161 adverse events involving MRI were reported making the risk of an adverse event from a MRI negligible. This procedure also involves the placement of an intravenous catheter which can lead to complications such as infection or bleeding. The patients will be screened in an interview with a physician prior to undergoing a MRI/PET for any contraindications to this test. Prior to the scan the patients will receive a blood glucose check to ensure they have a blood sugar below 200 mg/dl, and all female patients of child bearing potential will be given a pregnancy test to ensure they are not pregnant. In addition, immediately prior to undergoing the scan the patient or their guardian will complete a standardized radiology checklist for potential

risks, which will be reviewed by the radiology staff prior to the patient being scanned. The patients will be given oral hydration prior to the scan and encouraged to void afterwards to reduce radiation exposure. This procedure involves the placement of an intravenous catheter which can lead to complications such as infection or bleeding. Individuals with contraindications to this test will be excluded from receiving the procedure, including pregnant women.

As for MRI, this test involves a risk of claustrophobia and the patient has to lay still for a prolonged period. To help make this easier for patients, we will offer them the ability to watch TV shows or movies during the MRI/PET if they so choose.

This study represents an increase over minimal risk with potential for direct benefit. From our initial imaging studies now ongoing we are noticing different PET activity patterns for various calcinosis lesions. Some calcinosis lesions are not PET avid, sometimes the calcinosis itself is PET avid, and in some the surrounding tissue is PET avid. This allows us to determine if a calcinosis lesion is active or not. It has been our experience that all calcinosis lesions in patients do not respond the same to sodium thiosulfate. Some of this is likely due to size and location, but this does not explain all of the differences observed. We believe some of this is due to the metabolic state of the calcinosis itself. By observing lesions with PET before treatment, we will be able to determine if certain metabolic levels of lesions are more prone to responding to therapy. This may allow future patients to receive a PET scan to better determine their ability to respond. For those patients in the study, it will let us know which of their lesions are likely to respond to additional therapy based on their MRI/PET scans. Looking at metabolic activity of the calcinosis lesions will also allow us to determine if there is a change in activity with treatment. An increase in metabolic activity in calcinosis lesions post treatment may represent an early sign of calcinosis reformation or resolution. By monitoring for this change in metabolic activity, we may be able to tell patients where they are most likely to have calcinosis reoccurrence and those areas could be targeted for additional therapy. We have also seen differences in the longevity of patients' response to therapy and feel PET imaging can help give insight into determining which patients are likely to have a sustained response and which ones will not. The direct benefit involves the ability to better understand the activity of a patient's disease, which can help guide therapy. It can be difficult on traditional MRI to distinguish active disease from damage in an area with prior damage and adding the PET scan component improves the ability to do this. Due to the need to stay still during the scan to obtain optimal images, we will not perform the scan in patients under 7 years of age and will not perform the scan in patients we feel would be unable to remain still for the scan.

10.1.2.7. Thermography and Vascular Imaging

The risks of thermography and capillaroscopy are similar to that of photography. Patients may experience some discomfort from having to hold still for a period of time. To minimize risk, patients will be asked if they are tolerating holding still during the procedure, and if they are experiencing discomfort, they will be given a break in image acquisition during which time they will be allowed to move.

10.1.2.8. DEXA

This research study requires a small amount of radiation from a maximum of 6 DEXA scans in one year. Periodically, one or two of the DEXA scans may need to be repeated.

Even with these extra scans, the added radiation dose is minimal. The NIH Radiation Safety Committee has reviewed the use of radiation in this research study and has approved this use as involving minimal risk and necessary to obtain the research information desired.

Using the standard way of describing radiation exposure one DEXA scan has an effective dose of less than one thousandth of one Roentgen equivalent man (rem). By comparison the average person in the United States receives this much radiation every day from natural background sources, such as the sun, outer space, and from radioactive materials that are found naturally in the earth's air and soil. In this scan the only part of the body exposed is the skin, which is less vulnerable to radiation than most other parts of the body. The chance anyone has of eventually dying of cancer in their lifetime is 1 in 4. After receiving this study, for all practical purposes, the chance will remain 1 in 4.

Pregnant women will be excluded.

10.1.2.9. Renal Ultrasound

Renal ultrasound is a non-invasive procedure. There are no known safety risks to the patient associated with this procedure, however, some individuals may experience very mild discomfort due to the application of the gel to the skin (e.g., if the gel is cold) or due to pressure of the transducer on areas of the abdomen that are sensitive (e.g., near an area with calcinosis).

10.1.2.10. Blood Drawing

The risks and discomforts of blood drawing are those associated with venipuncture and include pain, development of a bruise at the venipuncture site, a vasovagal reaction, and a very small chance of infection. All children seen at the NIH Clinical Center will be offered EMLA® cream (2.5% lidocaine, 2.5 % pribcaine) or ELAMAX® cream, to be applied to the site of blood draw 15-60 minutes prior to the needle stick to minimize pain.

The amount of blood drawn from study participants will be within the limits allowed by the NIH Clinical Center (Medical Administrative Policy 95-9: Guidelines for Limits of Blood Drawn for Research Purposes in the Clinical Center). Participants will also be informed about the amount of blood taken for this study. The exact blood tubes and volumes may change based on changes in NIH Clinical Center procedures, but will be monitored and remain under the guideline based limits.

10.1.2.11. Urine Collection

There are no specific risks associated with urine collection. Some participants may experience feelings of self-consciousness when providing the sample.

10.1.2.12. Iothalamate Clearance

There is the potential for allergic reaction to the iothalamate and patients with a history of true allergic reaction will be excluded from this assessment to minimize risk. There is a small risk of complications due to the placement of the venous line. Risks of venipuncture and steps to minimize discomfort in children are the same as described in Section 10.1.2.10.

10.1.2.13. Durometry

No adverse reactions to durometry are anticipated. The procedure involves applying pressure to the skin with the durometer. In cases where the area is sensitive, the participant may feel slight discomfort consistent with that of normal palpation in the course of a physical examination.

10.1.2.14. Calcinosis Aspiration

There is a small risk of complications due to the insertion of a needle into the area of calcinosis, including pain, swelling, and potential infection. There is also risk of re-accumulation of the fluid. Risks of the needle insertion and steps to minimize discomfort in children are the same as described in Section 10.1.2.10 for venipuncture. Topical ELA-MAX cream will also be offered as local anesthetic. In addition, there is the risk of reformation of the calcinosis after aspiration.

10.1.2.15. Neutrophil Extracellular Traps (NETs) Analysis

There are no risks to the participant associated with the conduct of the NET analyses other than those associated with blood drawing, which are described in Section 10.1.2.10.

10.1.2.16. Microparticle and Other Endothelial Biomarker Evaluation

There are no risks to the participant associated with the conduct of microparticle and other endothelial biomarker evaluations other than those associated with blood drawing, which are described in Section 10.1.2.10.

10.1.2.17. Center for Human Immunology Research

The predominant risks of this research are those associated with the blood draw as stated in Section 10.1.2.10. It is unlikely that any data obtained from this analysis could prove to be harmful or stressful to the patient. In addition, we do not plan to share these research tests performed in a non-CLIA certified lab with patients.

10.1.2.18. Questionnaire Assessments

Questionnaires may be uncomfortable for the participants and filling out these questionnaires may cause or worsen anxiety and/or depression for the participant. In more severe cases it may involve thoughts of self-harm. If participants experience these complications, we plan to follow the NIH Clinical Center procedure.

10.1.2.19. Breach of Confidentiality

In any clinical trial, there is the potential for breach of confidentially as a result of inadvertent disclosure of information collected for research purposes. The steps that will be taken to protect confidentiality are described in Section 11.

10.1.2.20. Overall Radiation Assessment

CT scan, DEXA scan, and MRI/PET involve radiation. The total radiation participants will be exposed to per protocol from all study procedures is described in Table 9 (adults) and Table 10 (juvenile participants). Adult patients will receive up to 3.84 REM from research studies per year and juvenile patients will receive up to 1.45 REM per year from research studies. Due to the radiation exposure from the study as well as the unknown reproductive risks of sodium thiosulfate patients will be required to be on effective birth control if they are of reproductive potential throughout the study. The research radiation involved in this protocol is a significant amount and is over the recommended guidelines by the NIH clinical center for pediatric patients. All possible ways to reduce radiation dosage for this study have been undertaken. As described for the specific studies we feel the benefits for each of these outweigh the risks. The different techniques involving radiation exposure reveal significantly different beneficial data for patients and for generalizable knowledge about the disease in question. One could argue two studies could be done one using DEXA and CT and one using MRI/PET in order to reduce the radiation to any specific individual. However, co-localization of the MRI/PET and CT scan will allow us to spatially orient the PET data to significantly improve the information it provides about the enhancement pattern of specific calcinosis lesions. Also, performing both imaging modalities in one study allows us to reduce the risk associated with the non-imaging parts of the study to one group of patients instead of performing this study with only MRI/PET or CT/PET and then performing a second study with the other modality. Thus, not also individually, but as a whole for the study we feel the radiation risk is outweighed by the benefit.

10.1.2.21. CT/PET

CT/PET was used for a single adult patient once in place of a MRI/PET. CT/PET would be used in up to two more adult patients in place of MRI/PET if MRI/PET is unavailable. This would be done for the scientific integrity of the protocol in the setting that a MRI/PET could not be obtained. For these individuals their total research radiation dose would be 4.01 rem, which is 0.17 more than they would receive had they had a MRI/PET. This is still below the recommended research threshold of 5 rem/year in adult patients.

Using the PET component of the CT/PET will obtain the similar information as to the MRI/PET (Section 10.1.2.5). The PET component of the scan will allow us to determine if a calcinosis lesion is active or not. By observing lesions with PET before treatment, we will be able to determine if certain metabolic levels of lesions are more prone to responding to therapy. This may allow patients to receive a PET scan to better determine their ability to respond. Looking at metabolic activity of the calcinosis lesions will also allow us to determine if there is a change in activity with treatment. An increase in metabolic activity in calcinosis lesions post treatment may represent an early sign of calcinosis reformation or resolution. By monitoring for this change in metabolic activity, we may be able to tell patients where they are most likely to have calcinosis reoccurrence and those areas could be targeted for additional therapy. We have also seen differences in the longevity of patients' response to therapy and feel PET imaging can help give insight into determining which patients are likely to have a sustained response and which ones will not. The direct benefit involves the ability to better understand the activity of a patient's disease, which can help guide therapy. It can be

difficult on traditional MRI to distinguish active disease from damage in an area with prior damage and adding the PET scan component improves the ability to do this.

10.1.2.22. Summary/Classification of Risk: Benefit for the Study as a Whole

All participants and/or their parents will be fully informed of all pertinent study procedures, volunteer responsibilities, and the risks and benefits of participating in this protocol by informed consent procedures. The study will also obtain pediatric assent whenever possible, dependent on participant maturity, to ensure proper understanding.

Adults and children participating in this study may receive direct benefit for their calcinosis from treatment with the product under investigation (sodium thiosulfate). The procedures included in this study are widely used. The CT scan and MRI/PET aspects of this study represent an increase over minimal risk with the potential for direct benefit. The other procedures in this study pose a minor increase over minimal risk to the participant. The participants in this study will receive a therapeutic compound at an unapproved dose for an unapproved indication, thus this study as a whole is considered to pose more than minimal risk. However, since there are currently no known alternative therapeutic options for these patients to effectively improve their calcinosis, the potential risk is reasonable in relation to the potential clinical benefit they may derive from participating in the study.

The study is likely to yield generalizable knowledge about the impact of sodium thiosulfate on calcinosis associated with dermatomyositis, the safety of IV sodium thiosulfate, and could enhance our understanding of the pathogenesis of calcinosis associated with dermatomyositis.

This study is classified as Category 2 research for children, because it involves greater than minimal risk but presents the prospect of direct benefit to the individual child.

11. Protection of Participants' Privacy and Confidentiality

The investigator will ensure the protection of the participant's privacy and the confidentiality of identifiable data collected during research in accordance with NIH HRPP Policy 107 - *Privacy and Confidentiality*. Safeguards of privacy will include to the extent possible: providing private areas for obtaining consent, collecting information, and conducting examinations and research procedures; limiting the presence of research staff or others present during procedures; limiting information collected to that pertaining to the stated research objectives; and limiting information collected about those who are not the subject of research (e.g., family members or other potential participants). Procedures that will be used to maintain the confidentiality of data and medical records are described in Section 17.

12. Study Agents/Interventions

This section describes the study treatment (i.e., administration of sodium thiosulfate); all other study interventions are described in Section 4.1.

12.1. Supply and Preparation of Sodium Thiosulfate

Sodium thiosulfate will be supplied by Hope Pharmaceuticals (Scottsdale, Arizona, USA) in 50-mL vials containing 12.5 grams of sodium thiosulfate at a concentration of 250 mg/mL. This solution has an osmolarity of 2,250 mOsm/L. Study drug will be prepared as 12.5 grams of sodium thiosulfate in 450 mL of D5W for an osmolality of 538 mOsm/L. In patients who have problems with the volume of fluid or if there is a shortage of D5W then sodium thiosulfate can also be prepared at concentrations of 27.8 mg/mL to 71.4 mg/ml in D5W for osmolalities of 538 mOsm/L to 899mOsm/L. All medication will be received by the NIH pharmacy. Stability experiments have been run and the medication is stable at these concentrations for at least 24 hours.

12.2. Intravenous Administration of Sodium Thiosulfate

Sodium thiosulfate will be administered intravenously to participants at a dose of 16 g/m^2 per each infusion. The infusion will be administered over a period of approximately 3 hours; for patients experiencing nausea, the rate can be slowed up to half of the normal rate. In addition, for patients where the drug volume is felt to be unsafe to give over a 3 hour period the infusion time can be increased to up to 6 hours.

After the infusion, adult participants who can tolerate it will receive up to 1 L of normal saline to promote urinary excretion of calcium. Pediatric patients who can tolerate it will receive 5-10 cc/kg to a maximum of 1 L of IV normal saline post sodium thiosulfate infusion. Ability to tolerate additional infusion will be based on clinical exam at time of infusion, the patient's medical history, tolerance of prior infusions, and laboratory results.

During the treatment period, infusions will be administered 3 times weekly for 10 weeks.

Sodium thiosulfate will always be administered under the supervision of a medical practitioner who is licensed to give intravenous therapy.

If new osteoporotic fractures develop during treatment participants will have study drugged stopped, but will continue with all assessments. If a patient develops a fracture prior to starting study drug, but after enrollment they may return in 1 year or longer period of time to have a second -10 visit and then continue with the study based on that date. During the 1 year or longer weight the patient would be allowed to start a bisphosphonate.

Patients have been reported to have nausea with infusions of sodium thiosulfate. Nausea may be treated by a physician, including pretreatment if needed, with each infusion with anti-emetics, such as Zofran.

As described above, IV normal saline may be administered following each infusion, and participants will be encouraged to maintain oral hydration status between infusions. It is thought that increasing patient hydration will allow more of the calcium thiosulfate to be excreted. Patients experiencing fevers, chills or hypotension will be pre-medicated with Tylenol and Benadryl. Patients experiencing high serum phosphate levels during the treatment period may receive sevelamer.

12.3. Justification for Selection of Dose and Frequency of Sodium Thiosulfate

We have chosen a dose of 16 g/m2 for this study. Based on the previously done human studies and existing animal data this dose should be able to be administered without serious adverse events. Based on cases we have accumulated this appears to be a dose where patients tend to respond, with poor response to low dose treatment. Based on the case reports we have accumulated this appears to be the minimal dose with a significant beneficial effect. With the published side effect data, there is also a trend towards greater electrolyte abnormalities at larger doses so we tried to keep dosing below 20 g/m^2.

We would like to maximize drug frequency in order to minimize the time it takes patients to get all of their infusions. In a study of sodium thiosulfate for cisplatin toxicity patients received medication 3 times a week with minimal side effects except for nausea (NCT00716976 unpublished data). They noted that some electrolyte abnormalities in these patients could persist for 24 hours, but very rarely beyond that. Based on this there is a concern that infusions more frequent than three times a week will not give patients enough time to completely correct any transient hypernatremia or hypocalcemia and thus they could accumulate a larger change eventually becoming clinically dangerous. In those patients who had electrolyte abnormalities beyond 24 hours it was often elevated phosphorus. We will monitor patients for this and in those with elevated phosphorus we will initiate sevelamer to reduce their phosphorus. In the cases, we have heard those patients who were on twice a week dosing tended to have stabilization of their calcinosis, but not improvement so we felt there was a need for at least three times a week infusions.

12.4. Acquisition and Accountability

If any investigational product remains in the NIH Clinical Center Pharmacy after the last dose of sodium thiosulfate has been administered per protocol, the remaining investigational product will be disposed of according to the guidance in the Investigational Product Disposition SOP and Pharmacy's policy.

12.5. IND Information

Sodium thiosulfate is not currently indicated for the treatment of calcinosis and thus an IND will be filed prior to the conduct of this study. NIEHS will serve as the sponsor for this IND. This study has received an IND of number 135039. The date of receipt by the FDA was April 13, 2017.

13. Adverse Event and Unanticipated Problem Reporting

13.1. Definitions

Adverse events, non-compliance both serious or continuing, protocol deviations both major and minor, as well as unanticipated problems will be reported to the IRB as defined and described by the NIH Office of Human Subjects Research Protection policy #801. In order to compare events across the length of the protocol we plan to continue to

report to the FDA, DSMB, Clinical Director (CD), and Hope Pharmaceuticals according to the prior regulations and terminology as described below. If an event occurs that is reportable in a more expedited manner or at a higher level of reporting under NIH policy #801, then that policy's timelines will take priority. The following terms are defined as described in NIH HRPP SOP 16 ("Reporting Requirements for Unanticipated Problems, Adverse Events and Protocol Deviations"): Adverse Events (AE), Serious Adverse Event (SAE), Protocol Deviation (PD), serous, Unanticipated Problem (UP), and Unanticipated Adverse Device Effect (UADE).

All AEs occurring during the study, including those observed by or reported to the research team, will be recorded. The PI will ensure events are reported according to the requirements of NIH HRPP SOP 16.

In reference to the timings described below, the applicable institutional review board (IRB) for this protocol is the NIH Central IRB and the applicable Clinical Director (CD) for this protocol is the NIEHS CD.

13.2. Serious Events

Reporting for *serious events* will be as follows:

- The PI will report serious UPs, serious PDs, and UADEs to the IRB and CD as soon as possible, but not more than 7 days after first learning of the event.
- The PI will report any SAEs immediately according to requirements of 21 CFR 312.64(b).
- All events will also be reported along the same time frame to Hope Pharmaceuticals.

13.3. Not Serious Events

Reporting *<u>not</u> serious events* will be as follows:

- The PI will report all UPs that are not serious to the CD no more than 14 days after first learning of the event.
- The PI will report all PDs that are not serious no more than 14 days after first learning of the event.
- Non-serious protocol deviations will only be reported (within 14 days after the PI first learns of the event) if they represent a departure from NIH policies for the conduct of human subjects research, adversely affect the health care of the subject(s) or compromise the interpretation or integrity of the research. Non-serious protocol deviations that result from normal subject scheduling variations or technical issues associated with sampling that does not impact the health of the subject or the interpretation of the study data will not be reported.

By definition, all UADEs are "serious" and will be reported as described in Section 13.2.

13.4. Deaths

All deaths that are possibly, probably or definitely related to the research will be reported to the IRB within 24 hours after the PI first learns of the event. The PI will report all

deaths to the CD as soon as possible, but not more than 7 days after first learning of the event.

13.5. Reporting at Time of Continuing IRB Review

At the time of continuing IRB review, the PI will provide the IRB an aggregated summary of all UPs, all PDs, all UADEs, and all AEs.

The following anticipated non-UP adverse events are expected to occur with high frequency, and they generally cause only minimal distress and no permanent damage:

We expect a high frequency of the risks and/or discomforts of phlebotomy, which are a common occurrence that generally causes only minimal distress and no permanent damage.

We expect a high frequency of the risks and/or discomforts of obtaining IV access, which are a common occurrence that generally causes only minimal distress and no permanent damage.

Variations in the amount of blood drawn, as the amount may be based upon clinical indication and the participant's weight and other recent blood testing completed. However, the maximums of whole blood collected for research purposes in children will not exceed 5 mL/kg in a single day or 9.5 mL/kg in an eight-week period, and in adults will not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over 8 weeks.

Due to blood draw limits based on size it may not be possible to draw all blood tests for a given day. In this situation, efforts will be made to draw those tests missed at the next available scientifically logical opportunity, but some blood tests may need to be skipped. A priority of blood draw is indicated in Appendix B where blood draws are listed in order of priority. Due to the commonality of this problem in smaller children, we will not plan to report these protocol deviations.

In some instances, there are errors with phlebotomy where not all blood work ordered is drawn. If this occurs we will attempt to draw those missed tests at the next available scientifically logical opportunity if for research purposes only, but they may be skipped. If they are needed for safety monitoring purposes they will be drawn immediately.

Some blood work must be done fasting and certain urine studies must be collected at specific points. As patients often accidently eat and/or fail to properly collect their urine, we will not report samples missed due to these situations, and we will work to collect them at the next available scientifically logical opportunity, but some samples may be missed and this will not be reported as a protocol deviation.

Although some blood tests require the participant to be fasting, in the event that the participant is unable to fast, tests may occur in the non-fasting state and/or fasting labs will not be collected.

14. Data and Safety Monitoring

The clinical and laboratory procedures used in this study represent a minor increase over minimal risk for study participants. However, repeat-dose IV administration of sodium thiosulfate is not approved in the US for any indication, and this drug is not approved for the treatment of calcinosis associated with dermatomyositis; therefore, we plan to utilize a Data and Safety Monitoring Board (DSMB), adverse event monitoring, and ongoing site monitoring.

14.1. Use of Data and Safety Monitoring Board

To further protect the rights and welfare of participants in this trial, which will enroll from a vulnerable population (i.e., children), and because safety information on the long-term use of sodium thiosulfate is limited, we plan to use a Data and Safety Monitoring Board for this study. The DSMB will have complete independence from the study team.

During the course of the study, the DSMB will review cumulative study data biannually to evaluate safety, efficacy, study conduct, and scientific validity and integrity of the trial. As part of this responsibility, DSMB members must be satisfied that the timeliness, completeness, and accuracy of the data submitted to them for review are sufficient for evaluation of the safety and welfare of study participants. The DSMB may also convene as needed if any safety issues arise that the Principal Investigator and/or NIEHS Clinical Director or designee would like the DSMB to address. DSMB member will receive all reports of serious adverse events within 48 hours of the occurrence. The DSMB will operate under a charter and its membership and charter will be approved by the NIEHS Clinical Director. The DSMB will report its written findings from each meeting to the NIEHS Clinical Director and the study PI.

The DSMB will consist of three members minimum. It will include at least one pediatric physician, one adult physician, and one individual with biostatistical experience. The members of the board should include individuals with expertise in auto-immune diseases and individuals familiar with managing calcinosis. The DSMB chair will have prior experience sitting on a DSMB.

The principal investigator will submit the written DSMB recommendations to the IRB upon receipt.

14.2. Subject Monitoring

Tests used to monitor participant safety are described in Section 14.3 below.

Throughout the treatment period, inpatient participants will be monitored daily while in the clinical center and participants who elect to stay locally and come to the NIH for treatments will be monitored 3 times weekly. Additional visits occurring approximately 14 weeks and 52 weeks after the treatment period ends will allow for an assessment of any delayed safety or efficacy issues. Any new medical issues that arise during treatment will be addressed and managed.

Criteria for individual participant withdrawal from the treatment phase of the study include:

- Inability to comply with the requirements of the protocol (including inability to tolerate the sodium thiosulfate therapy)
- Breastfeeding or pregnancy detected prior to Week 10
- Serious adverse event necessitating discontinuation of the sodium thiosulfate therapy.
- Common Terminology Criteria for Adverse Events level 3 or greater event thought to be related to study drug, besides nausea, prior to week 10.
- Nausea that cannot be controlled by slowing rate of infusion and adding anti-emetic medications prior to week 10.

- A new fracture prior to week 10. If a patient develops a fracture prior to week 0 if the patient would meet enrollment criteria in a year or greater time they may have a second -10 visit and continue from there with the normal study process.
- Patient request to withdraw from the study.
- Patients who have new medical problems develop during the study not thought to be related to study drug will be treated per standard of care medicine. If it is felt that the new condition makes it unsafe for the patient to continue in the study, then they will be withdrawn.
- Withdrawn patients will continue to be followed according to the schedule of the protocol and return for all assessment visits as tolerated and safe.

If patients have a history of diabetes the NIH endocrinology and/or blood glucose management team will be consulted for their ongoing care and to follow them during their treatment phase. If the blood sugar reaches a level that either of these teams feels warrants pausing treatment, treatment will be paused. Similarly, if CTCAE criteria are reached of level 3 or greater for hyperglycemia treatment will be paused. If blood sugar is reported as a value greater than 600 further doses will not be given until the consult team specifically reviews that result.

If patients have a history of anemia or develop anemia during the study hematology consult service will be consulted. If the consult service feels the patient's treatment should be paused it will be paused. If the patient's HgB value goes below 9 treatment will be paused until specific additional input from that value can be given by the hematology consult service.

14.3. Adverse Event Monitoring

At each study contact, participants and/or their parents will be asked about any adverse events they have experienced. Any adverse events reported will be coded based on the Common Terminology Criteria for Adverse Events and logged.

The clinical site investigator will determine which events are associated with the use of study drug. For reporting purposes, an AE should be regarded as possibly related to the use of the investigational product if the investigator believes:

- There is a clinically plausible time sequence between onset of the AE and sodium thiosulfate administration; and/or
- There is a biologically plausible mechanism for sodium thiosulfate causing or contributing to the AE; and
- The AE cannot be attributed solely to concurrent/underlying illness, other drugs, or procedures.

An AE is deemed associated with the use of the study drug "if there is a reasonable possibility that the AE may have been caused by the drug" (21 CFR 312.32)

Known side effects of sodium thiosulfate include osteoporosis, hypotension, nausea, prolonged bleeding time, and metabolic acidosis. The development of kidney stones is a theoretical risk with the use of this medication. During treatment with sodium thiosulfate, we will perform specific targeted monitoring for these side effects as follows:

- Osteoporosis will be monitored by DEXA scan at week 0, 6, 10, 24, and 62 for changes in bone mineral density. Height will also be measured for loss of stature.
- Blood pressure will be monitored before and after infusions to evaluate for alterations of blood pressure. It will be monitored every 30 minutes during the infusion and if the patient develops any symptoms.
- Nausea will be evaluated by patient reports.
- Prolonged bleeding time will be monitored by weekly laboratory evaluation of prothrombin time (PT) and partial thromboplastin time (PTT) from weeks 0 to 10 during the infusions.
- Metabolic acidosis will be evaluated by electrolyte and venous blood gas monitoring once per week from weeks 0 to 10 during the infusions. This has been attributed to mild metabolic acidosis immediately following infusion that potentially after several infusions without chance for the participant to equilabrate their acid base balance to baseline, could reach more clinically significant levels. This complication will be monitored more frequently in the initial 2 patients to ensure this remains true in our study population.
- Renal ultrasound will be used to monitor for the development of renal stones (when no CT scan is available), with assessments at weeks 6, 24, and 62.

14.4. Study-Stopping Criteria

Stopping criteria for individual participants are described in Section 14.2. If three or more patients experience serious AEs, study entry will be paused for review by the DSMB and a decision made about continuing or stopping the study.

15. Clinical Monitoring Plan

The NIEHS will designate a clinical monitor to carry out monitoring visits at the NIH Clinical Center. The purpose of the visits is to ensure that the rights of human participants are protected, that the study is implemented in accordance with the protocol, NIEHS standards, and International Conference on Harmonization (ICH) Good Clinical Practice (GCP), and that the integrity of study data is maintained. The monitor will review various aspects of the study including, but not limited to:

- Continued acceptability of facilities and staff
- Documentation of informed consent
- Compliance to the protocol
- Documentation and reporting of adverse events and protocol deviations

The initial monitoring visit will be scheduled for approximately 1 year after the first subject receives the first dose of study medication (i.e., sodium thiosulfate), and additional monitoring visits will occur approximately once per year thereafter. The monitor will work with the investigator to schedule the visits at a time that is mutually agreeable. The monitor will document the date and time of all visits on a monitoring log that will be stored in the study regulatory binder at the NIH Clinical Center. During the visits, the investigator will provide the monitor with workspace and access to source documents, research records, and the Clinical Trials Database (CTDB). The investigator

will also be available to meet with the monitor to discuss findings and reply to inquiries. After the visit, the monitor will produce a report to document all findings, discussions, and solutions. The report or a follow-up letter summarizing the contents of the report will be sent to the Principal Investigator. This report will be reviewed promptly and any noted unanticipated problems, adverse events, protocol deviations, and issues of non-compliance will be reported to the appropriate parties on a timeline as in accordance with Section 13 of this protocol. These findings will also be summarized at the time of continuing review as outlined in Section 13 of this protocol. Additional follow-up will be conducted by email and telephone as needed.

16. Alternatives to Participation or Alternative Therapies

There are currently no established therapeutic alternatives known to be highly effective in the treatment of calcinosis associated with juvenile or adult dermatomyositis. The alternative to participating in this study is to not participate, and to receive conventional care, which in our clinic consists of aggressive control of their underlying muscle disease and treatment with bisphosphonates. In some situations, alternative therapy can also include surgery.

17. Data/Records Management

All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives may inspect all documents and records required to be maintained by the Investigator, including but not limited to medical records. All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified by a coded number only, in order to maintain participant confidentiality. All records will be kept in restricted, locked areas. Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the NIDDK/NIAMS IRB, the FDA, the NIEHS, or the OHRP. The investigator will inform participants that study monitors and other representatives of the NIH may inspect research records. Participants will also be informed that study monitors and other NIH representatives are bound by agreement and the law to maintain participant privacy and confidentiality.

The results of the study may be presented in reports, published in scientific journals or presented at medical meetings. However, participants will not be identified in any study reports.

Study information will be kept secure and private in two locations as follows:

- Hard copies of the signed consent forms will be kept in a locked study file cabinet in the medical records room.
- Electronic records with PII (e.g., name, address, and phone number) and a schedule of the participant's visits will be stored in password-protected study management databases and in the NIH CRIS for purposes such as reminder phone calls and participant reimbursement.

Clinical information will not be released without written permission of the participant, except as necessary for monitoring by the NIDDK/NIAMS IRB, NIEHS, FDA and the Office for Human Research Protections (OHRP). The investigator will inform participants and/or their parents that study monitors and other representatives of the NIH and FDA may inspect research records. Participants and/or their parents will also be informed that

study monitors and other NIH representatives are bound by agreement and the law to maintain participant privacy and confidentiality.

18. Compensation

Volunteers will be compensated for time and research-related inconveniences. They will receive $40 per day spent as an inpatient, outpatient, or completing phone evaluations. Payments will be authorized within one week of the end of each study visit or earlier corresponding to approximately week -10, week -4, week 10, week 24, and week 62. Participants will have travel and lodging covered as part of the protocol. Participants who travel to the study visits in their own vehicle will be reimbursed 40 cents per mile. For pediatric patients, we will also pay for travel and lodging for 1 guardian. For the first visit travel and lodging will be covered for 2 guardians for pediatric patients.

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Appendix A. List of Appendices

The following appendices are included within this protocol document:

Appendix A List of Appendices

Appendix B Time and Events Tables

Appendix C Summary of Research Assessments Performed that Use Ionizing Radiation

Appendix D Work Flow Sheet

Appendix E Quality of Life Questionnaires

Appendix F Screening Evaluation Form

Appendix G Script for Assessing Adverse Events for Weeks 10-24

Appendix H Brief Summary of Goals of Imaging Techniques

Appendix B. Time and Events Tables

Table 4 Overview of Study Procedures Performed by Study Week

	Pre-treatment				Treatment										Follow-up	
	Week															
	Screening	-10	-4	0	1	2	3	4	5	6	7	8	9	10	24	62
Inclusion/exclusion criteria assessment	X	X														
Sodium thiosulfate treatment (3x/week) [a,b]					X	X	X	X	X	X	X	X	X	X		
Check for rapid responder and termination of treatment										X						
Medical history	X	X		X		X				X	X			X	X	X
Medication history and concomitant medications [c]	X	X		X		X				X				X	X	X
Review of systems every 2 weeks during infusion		X		X		X		X		X		X		X	X	X
Physical exam	X	X		X		X		X		X		X		X	X	X
Pregnancy test for women of child-bearing potential	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Myositis clinical activity and damage assessments (Table 7)	X	X		X		X		X		X		X		X	X	X
Muscle function and strength testing (see Table 7 for details)	X	X		X		X		X		X		X		X	X	X
Skin Assessments (see Table 7 for details)	X	X		X		X		X		X		X		X	X	X
MRI (whole body)		X								X					X	X[p]
CT scan without contrast (all extremities)				X										X		X
CT scan without contrast (trunk)				X										X		X
MRI/PET				X[q]										X[n,q]		X [m,q]
Infrared photography		X		X		X				X				X	X	X
Nailfold capillaroscopy		X		X		X				X				X	X	X
Photography [d]		X		X						X				X	X	X
Laser Speckle Contrast Imaging		X		X		X				X				X	X	X

	Pre-treatment				Treatment										Follow-up	
	Week															
	Screening	-10	-4	0	1	2	3	4	5	6	7	8	9	10	24	62
DEXA for safety monitoring and calcium scoring [e]	X[o]	X		X						X				X	X	X
Renal ultrasound		X		X[l]						X				X[l]	X	X [l]
Iothalamate clearance		X [f]		X						X				X	X [g]	X [g]
24-hour urine [h]		X		X						X				X	X	X
Blood collection (see Table 5 and Table 6 for details) [i]	X[o]	X		X	X	X	X	X	X	X	X	X	X	X	X	X
Calcinosis aspiration (as required, and if <15 cc)										X				X	X	X
Calcinosis aspiration (major)														X	X	X
Adverse event monitoring [j]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Questionnaire completion (see Table 7 below for details)	X	X	X	X		X		X		X		X		X	X	X
IV access [k]		X		X	X	X	X	X	X	X	X	X	X	X	X	X
Durometry		X		X						X				X	X	X

a. Subjects will receive once-weekly assessments between study weeks 0 and 10
b. Sodium thiosulfate treatments are administered 3 times/week, starting at Week 0 and continuing through Week 10
c. At Week -10, Week 0, Week 24, and Week 62, medication history and concomitant medications will be reviewed with the subject in detail; at all other visits, any changes occurring since the last evaluation will be assessed.
d. Photography will be performed more frequently than indicated if there's a noted change
e. If a DEXA scan has been performed within 6 months prior to initiation of the study, that will be used as the baseline DEXA evaluation
f. Iothalamate clearance not performed for children at Week -10 visit
g. Iothalamate clearance is optional at Week 24 and Week 62 if normal previously
h. Urine assessment will includes calcium, phosphorus, pyrophosphate, creatinine, citrate, and urate; while receiving sodium thiosulfate infusions, evaluations for renal function and urinary excretion of calcium will be performed weekly. They will use a split collection with Cr, Na, CA, Mg, P, and urate coming from the non-acidified container. Cr, citrate, and oxalate will be collected from the acidified container.
i. While receiving sodium thiosulfate infusions, evaluations for renal function, metabolic acidosis, PT/PTT, and serum calcium will be performed weekly

	Pre-treatment				Treatment										Follow-up	
	Week															
	Screening	-10	-4	0	1	2	3	4	5	6	7	8	9	10	24	62

j. These are the times for formal review for adverse events. In addition, patients, will have contact information to reach the study team to contact them for any expected adverse events they experience at other times.
k. If needed, a PICC or Port-A-Cath line will be inserted to maintain IV access for multiple infusions and this may occur prior to a scheduled visit.
l. Only performed if the CT scan during that visit does not include the kidneys.
m. If a patient has become pregnant or is breastfeeding and is unable to receive a MRI/PET at this visit they will instead receive a whole body MRI.
n. At week 10 adults patients will receive a MRI/PET while pediatric patients will receive a whole body MRI instead.
o. May be performed by their home physician
p. If the MRI/PET is not performed at this point a whole body MRI if safe will be performed instead.
q. If the MRI/PET machine is non-operational at this time point for the adult patients enrolled, the patients will instead have a CT/PET and a whole body MRI. Only three patients in the study may get CT/PET in place of MRI/PET.

Table 5 Type of Blood Test Performed or Sample Collected by Study Week in order of priority – <u>Adult</u> Subjects

Blood Test/Sample	Collection Tube	Required Volume	Week Collected								
			Screening *	-10	0	1-5	6	7-9	10	24	62
Acute care chemistry panel, mineral panel, hepatic panel, CK, LDH, total protein, Quantitative Immunoglobulins, C-Reactive Protein-High sensitivity, Pro Brain Natriuretic Peptide, CK-MB Immunoassay, uric acid, cystatin C, Thyroid screen (TSH, FT_4)	Green/yellow Lithium Heparin Tube	One 4.0 mL tube	X	X	X	X[d]	X[d]	X[d]	X[d]	X	X
Iothalamate (optional at week 24 and 62 if previously normal)	Heparinized Tube	Six 3 mL tubes		X[e]	X[e]		X[e]		X[e]	X[b,e]	X[b,e]
Venous Blood Gas, Ionized Magnesium Whole Blood, Ionized Calcium Whole Blood	Marquest Gaslyte Sampler	3 mL	X		X	X[d]	X[d]	X[d]	X[d]		
Aldolase	Red/yellow SST	One 4.0 mL tube	X	X	X		X		X	X	X
ANA, ENA, JO-1	Red/yellow SST	4 mL		X							
Complete blood count and differential	Light lavender top	One 3 mL tube	X	X	X		X		X	X	X
Erythrocyte Sedimentation Rate (ESR)	Black top	One 1 mL tube	X	X	X		X		X	X	X
Vitamin D, 25-Hydroxy, Vitamin D, 1,25-Dihydroxy	Red/yellow SST	One 4 mL tube		X	X		X		X	X	X

Blood Test/Sample	Collection Tube	Required Volume	Week Collected								
			Screening *	-10	0	1-5	6	7-9	10	24	62
Parathyroid Hormone	Light Lavender	3 mL		X	X		X		X	X	X
IgE	Red/Yellow SST	4 mL		X	X	X[d]	X[d]	X[d]	X[d]	X	X
Bone Alkaline Phosphatase, Serum	Red-Yellow SST	0.6 mL		X	X		X		X	X	X
Thrombomodulin	Light Blue	4.5 mL		X	X		X		X	X	X
Hepatitis, HIV, HTLV-I serologies	Red/Yellow SST	8 mL	X	X	X						
PT,PTT, vWF panel, Factor VIII Activity	Blue-black	3 mL	X	X	X	X[c]	X	X[c]	X	X	X
HLA typing	Yellow ACD-A	Two 8.5 mL tubes		X							
Myositis autoantibody testing	Red/yellow SST	6 mL		X							
CHI RNA gene expression analysis	PAXgene Blood RNA	One 2.5 mL tube		X	X	X[a]	X		X	X	X
Endothelial activation markers	Blue-black	10-20 mL		X	X		X		X	X	X
CHI proteomics/ Cytokine Analysis	Red/yellow top (Greiner Vacuette serum tube with gel)	One 8 ml tube		X	X	X[a]	X		X	X	X
CHI Flow Cytometry	Green Top (BD Vacutainer)	Three 10 mL tubes		X	X	X[a]	X		X	X	X

Blood Test/Sample	Collection Tube	Required Volume	Week Collected								
			Screening *	-10	0	1-5	6	7-9	10	24	62
NIH Clinical Center Flow Cytometry,	Purple top	Two 3 mL tubes		X			X		X	X	X
Blood IFG23	Red-Yellow SST	1.5 mL	X	X	X		X		X	X	X
Long-term DNA storage	PAXgene DNA	Three 8 mL tubes		X					X		X
Long-term RNA storage	Tempus RNA	Three 3 mL tubes		X	X		X		X	X	X
Long-term serum storage	Red/yellow SST	Two 8 mL tubes		X	X		X		X	x	X
Spot urines UA, urine ca/cr ratio	Sterile urine collection cup		X	X	X	X	X	X	X	X	X
First morning void urines during infusions: Urine sodium, Ca, Cr, eGFR, phosphorous	Sterile urine collection cup			X	X	X	X	X	X	X	X
Urine pregnancy	Sterile urine collection cup		X	X	X	X	X	X	X	X	X

X[a]= These will be collected at week 2 during the week 1-5 period to assess for rapid response to treatment. For pediatric patients, we will calculate at week 0 whether these blood draws will put them over the NIH limits for safe research blood draws and will not draw these bloods if this is the case. Not being able to have these bloods drawn will not impact study eligibility

X[b]= Iothalamate clearance is optional at Week 24 and Week 62 if normal previously
X[c]= PT and PTT will be collected at Weeks 1-5 and Weeks 7-9
X[d]=The samples will be collected twice weekly.
X[e=]Iothalamate testing may not be performed if logistical reasons preclude the study from being able to take place.
*All blood and urine tests for the screening visit may be done with an outside physician.

**Weekly blood and urine test will be completed during weeks 1-5, and weeks 7-9.

Table 6 Type of Blood Test Performed or Sample Collected by Study Week in order of priority– <u>Pediatric</u> Subjects

Blood Test/Sample	Collection Tube	Required Volume	Week Collected								
			Screening *	-10	0	1-5	6	7-9	10	24	62
Acute care chemistry panel, mineral panel, hepatic panel, CK, LD, total protein, Quantitative Immunoglobulins, C-Reactive Protein-High sensitivity, Pro Brain Natriuretic Peptide, CK-MB Immunoassay, uric acid, cystatin C, Thyroid screen (TSH, FT_4)	Green/yellow Lithium Heparin Tube	One 4.0 mL tube	X	X	X	X[d]	X[d]	X[d]	Xd	X	X
Venous Blood Gas, Ionized Magnesium, Ionized Calcium	Marquest Gaslyte Sampler	3 mL	X		X	X[d]	X[d]	X[d]	X[d]		
Iothalamate (optional at week 24 and 62 if previously normal)	Heparinized tube	Six 3 mL tubes			X[e]		X[e]		X[e]	X[b,e]	X[b,e]
Aldolase	Red/yellow SST	One 4.0 mL tube	X	X	X		X		X	X	X
ANA, ENA, JO-1	Red/White SST	2.5 mL		X							
Complete blood count and differential	Light lavender top	One 3 mL tube	X	X	X		X		X	X	X
Erythrocyte sedimentation rate (ESR)	Black top	One 1 mL tube	X	X	X		X		X	X	X
Vitamin D, 25-Hydroxy, Vitamin D, 1,25-Dihydroxy	Red/yellow SST	One 4 mL tube		X	X		X		X	X	X

Blood Test/Sample	Collection Tube	Required Volume	Week Collected								
			Screening *	-10	0	1-5	6	7-9	10	24	62
Parathyroid Hormone	Light Lavender	2 mL		X	X		X		X	X	X
IgE	Red/Yellow SST	4 mL		X	X	X[d]	X[d]	X[d]	X[d]	X	X
Bone Alkaline Phosphatase, Serum	Red/Yellow SST	0.6 mL		X	X		X		X	X	X
Hepatitis, HIV, HTLV-I serologies	Red/White SST	4 mL	X	X	X						
PT,PTT, vWF panel, Factor VIII Activity	Blue-black	3 mL	X	X	X	X[c]	X	X[c]	X	X	X
HLA typing	Yellow ACD-A	One 8.5 mL tube		X							
Myositis autoantibody testing	Red/yellow SST	6 mL		X							
CHI RNA gene expression analysis	PAXgene Blood RNA	One 2.5 mL tube		X	X	X[a]	X		X	X	X
Endothelial activation markers	Blue-black	10-20 mL		X	X		X		X	X	X
CHI proteomics/ Cytokine Analysis	Red/yellow top (Greiner Vacuette serum tube with gel)	Two 4 mL tubes		X	X	X[a]	X		X	X	X
CHI Flow cytometry	Green Top (BD Vacutainer)	Three 10 mL tubes		X	X	X[a]	X		X	X	X

Blood Test/Sample	Collection Tube	Required Volume	Week Collected								
			Screening *	-10	0	1-5	6	7-9	10	24	62
NIH Clinical Center Flow Cytometry,	Purple Top	Two 3 mL tubes		X			X		X	X	X
Blood IFG23	Red/Yellow SST	1.5 mL		X	X		X		X	X	X
Thrombomodulin	Light Blue	1.7 mL		X	X		X		X	X	X
Long-term DNA storage	PAXgene DNA	Three 8 mL tubes		X					X		X
Long-term RNA storage	Tempus RNA	One to three 3 mL tubes		X	X		X		X	X	X
Long-term serum storage	Red/yellow SST	Two 8 mL tubes		X	X		X		X	X	X
Spot urines UA, urine ca/cr ratio	Sterile urine collection cup		X	X	X	X	X	X	X	X	X
First morning void urines during infusions: Urine sodium, Ca, Cr, eGFR, phosphorous	Sterile urine collection cup			X	X	X	X	X	X	X	X
Urine pregnancy	Sterile urine collection cup		X	X	X	X	X	X	X	X	X

X[a]= These will be collected at week 2 during the week 1-5 period to assess for rapid response to treatment. For pediatric patients, we will calculate at week 0 whether these blood draws will put them over the NIH limits for safe research blood draws and will not draw these bloods if this is the case. Not being able to have these bloods drawn will not impact study eligibility.

X[b]= Iothalamate clearance is optional at Week 24 and Week 62 if normal previously

X[c]= PT and PTT will be collected at Weeks 1-5 and Weeks 7-9

X[d]=The samples will be collected every week and twice on weeks 6, and 10.
X[e=]Iothalamate testing may not be performed if logistical reasons preclude the study from being able to take place.
*All blood and urine tests for the screening visit may be done with an outside physician.

**Weekly blood and urine test will be completed during weeks 1-5, and weeks 7-9.

Table 7 Schedule of Quality of Life and Myositis Assessments by Study Week

Assessment	Completed By…										
		Screening	**-10**	**-4**	**0**	**2**	**4**	**6**	**8**	**10**	**24**	**62**
CHQ-PF50 (if pediatric) or SF-36 (if adult)	Patient AND Parent of participant (for CHQ); Participant (for SF-36)		X	X	X	X	X	X	X	X	X	X
Physician Calcinosis Visual Analogue Scale	MD	X	X		X	X	X	X	X	X	X	X
Calcinosis Type and Extent Form	MD	X(optional)	X		X			X		X	X	X
Calcinosis Sentinel Lesion Form	MD		X		X			X		X	X	X
IMACS Activity Core Set Measures:												
Physician Global Activity	MD	X	X		X	X	X	X	X	X	X	X
Patient/Parent Global Activity	Participant and parent of participant	X	X	X	X	X	X	X	X	X	X	X
CHAQ (if pediatric) or HAQ (if adult),	Parent of participant and pediatric participant (for CHAQ); Participant (for HAQ)	X	X	X	X	X	X	X	X	X	X	X
Childhood Myositis Assessment Scale (CMAS)	MD	X (optional)	X		X	X	X	X	X	X	X	X
Myositis Disease Activity Assessment Tool (MDAAT)	MD	X	X		X	X	X	X	X	X	X	X
IMACS Disease Damage Core Set Measures:												
Myositis Damage Index (MDI)	MD		X		X	X	X	X	X	X	X	X
Physician Global Assessment of Disease Damage	MD	X	X		X			X		X	X	X
Patient/Parent Global Assessment of Disease Damage	Participant and parent of participant		X	X	X			X		X	X	X

Assessment	Completed By…										
		Screening	**-10**	**-4**	**0**	**2**	**4**	**6**	**8**	**10**	**24**	**62**
IMACS Assessment of Study Outcome Form	MD, Participant, and parent of participant			X[a,b]	X[b]			X		X	X	X
Disease Activity Score (DAS)	MD		X		X	X	X	X	X	X	X	X
Calcinosis Assessment Tool (CAT), including Pain VAS	Participant and parent of participant, reviewed by MD, MD	X	X	X[a]	X	X	X	X	X	X	X	X
Mawdsley Calcinosis Questionnaire	Participant and parent of participant, reviewed by MD, MD		X	X[a]	X	X	X	X	X	X	X	X
PROMIS® Questionnaire	Participant and parent of participant (pediatric pts)		X	X[a]	X	X	X	X	X	X	X	X
Skindex-29+3	Participant and parent of participant (pediatric pts)		X	X	X	X	X	X	X	X	X	X
Adult Myopathy Assessment Tool (AMAT)	Rehab Medicine Department		X		X			X		X	X	X
Motor Function Measurement (MFM)	Rehab Medicine Department		X		X			X		X	X	X
Functional Index 3 (FI3)	Rehab Medicine Department, MD		X		X	X	X	X	X	X	X	X
Passive Range of Motion (ROM)	Rehab Medicine Department		X									
Targeted Passive Range of Motion	Rehab Medicine Department				X	X	X	X	X	X	X	X
Sit to stand, 6-minute walk, timed up and go	Rehab Medicine Department or MD		X		X	X	X	X	X	X	X	X
Quantitative muscle testing including grip and Key Pinch Strength	Rehab Medicine Department		X		X			X		X	X	X
Manual Muscle Testing (MMT)	Rehab Medicine Department, MD	X	X		X	X	X	X	X	X	X	X
Cutaneous Assessment Tool	MD		X		X	X	X	X	X	X	X	X
CDASI	MD		X		X	X	X	X	X	X	X	X

[a]Patient questionnaires only at week -4

[b]At time -4 and 0 this will be compared to week -10, at all other times it will be compared to week 0.

Table 8 Targeted Safety Monitoring Assessments

	Inpatient Period - STS Infusions Given 3x Weekly			**Follow-up Visits**	
	Week 0	**Week 6**	**Week 10**	**Week 24**	**Week 62**
DEXA for osteoporosis	X[a]	X	X	X	X
Blood Pressure	X – taken before and after each infusion from Week 0 to Week 10				
Nausea	X – evaluated continually by patient report from Week 0 to Week 10				
PT/PTT	X – performed once per week from Week 0 to Week 10				
Renal Ultrasound		X		X	X
Electrolytes, venous blood gas	X – performed once per week from Week 0 to Week 10				

a. If a DEXA scan has been performed within 6 months prior to initiation of the study, that will be used as the baseline DEXA evaluation

Appendix C. Summary of Research Assessments Performed that Use Ionizing Radiation

Table 9 Total Ionizing Radiation Exposure Per Protocol for Adult Participants in REM

Assessment	Radiation Effective Dose Per Assessment	Maximum Total Times Given Per Protocol	Times given per year	Maximum Yearly Exposure	Total Exposure Per Protocol
DEXA	0.000181	6	5	0.000905	0.001086
CT Whole-body	2.5	1	1	2.5	2.5
MRI/PET	0.67	3	2	1.34	2.01

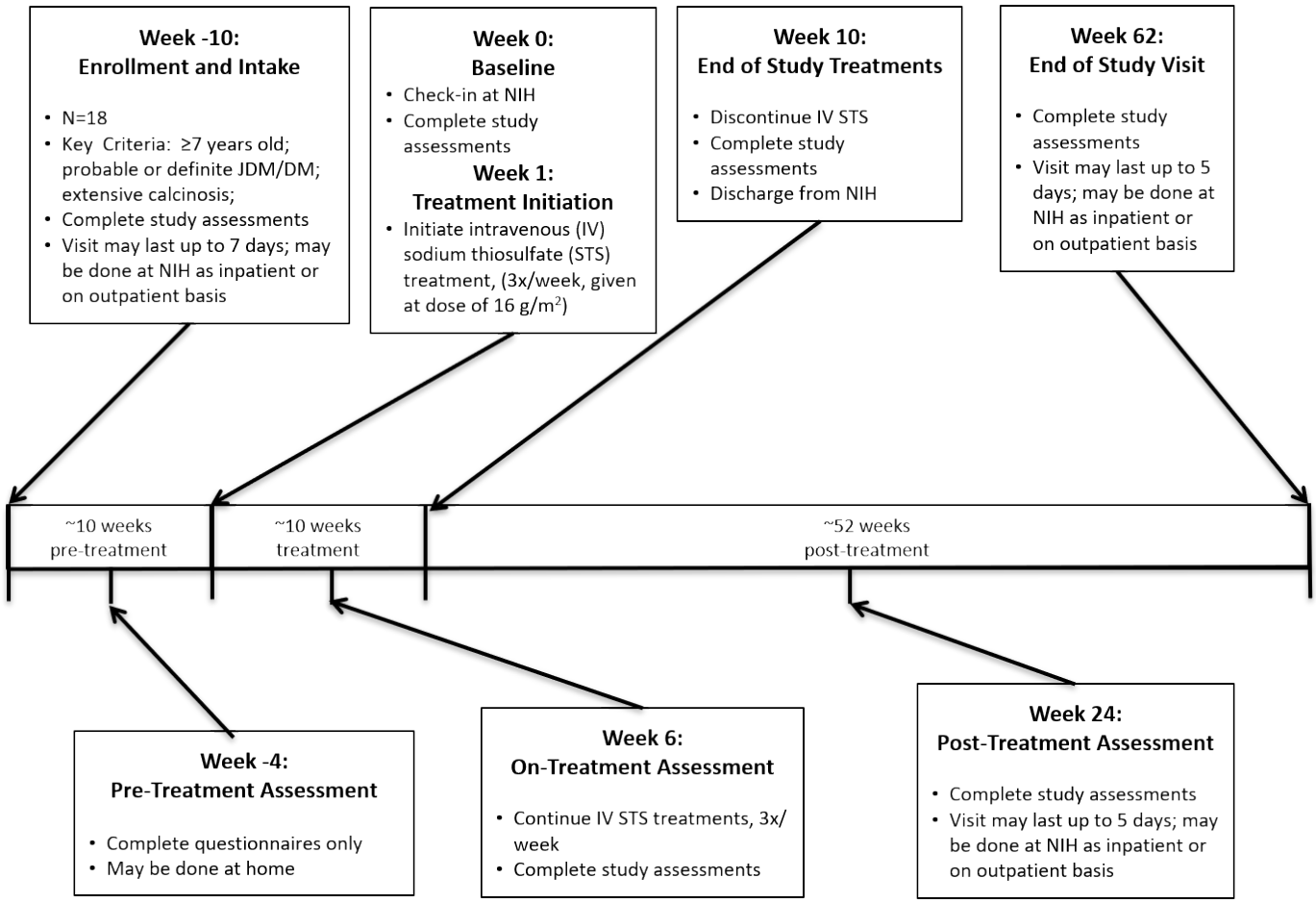
See Table 4 for timings of assessments

Table 10 Total Ionizing Radiation Exposure Per Protocol for Juvenile Participants in REM

Assessment	Radiation Effective Dose Per Assessment	Maximum Total Times Given Per Protocol	Times given per year	Maximum Yearly Exposure	Total Exposure Per Protocol
DEXA	0.000181	6	5	0.000905	0.001086
CT Whole-body	0.76	1	1	0.76	0.76
MRI/PET	0.42-0.69	2	1	0.42--0.69	0.84-1.38

See Table 4 for timings of assessments

Appendix D. Work Flow Sheet



* Patients at week 6 who reach the rapid response criteria may choose to stop active treatment with STS.

** Not pictured in the schematic is a screening visit that occurs prior to study initiation at which time study eligibility will be determined.

Appendix E. Quality of Life Questionnaires

The following QoL questionnaires will be administered as part of this study, and copies of the questionnaires will be attached as an appendix of the protocol:

- Child Health Questionnaire - Parent Form 50 (CHQ-PF50; for pediatric participants) *or* Medical Outcomes Study Short Form Health Survey (SF-36; for adult participants)
- QoL questionnaires from within the IMACS activity and disease damage core set measure assessments include:
    - Childhood Health Assessment Questionnaire (CHAQ; for pediatric participants) *or* Health Assessment Questionnaire (HAQ; for adult participants)
- Calcinosis Assessment Tool (CAT), including Pain VAS
- Mawdsley Calcinosis Questionnaire
- The Patient Reported Outcomes Measurement Information System (PROMIS®) questionnaire will be used to capture daily life functions and health-related quality of life
- Skindex-29

Appendix F. Screening Evaluation Form

The Screening Evaluation Form is included with the protocol submission to the IRB as a separate attachment.

SE

Sodium Thiosulfate for Calcinosis

SCREENING EVALUATION (SE)

For internal use only

COMPLETION LOG

A2. Date Received __ __ / __ __ __ / __ __ __ __

DD MMM YYYY

A4. Visit (Evaluation) Date: _____/_____/_____
DD MMM YYYY

I. STUDY INFORMATION

1. Participant type: a) ☐ Adult

b) ☐ Minor

2. Myositis type: a) ☐ Dermatomyositis

b) ☐ Juvenile Dermatomyositis (< 18 years at diagnosis)

II. DEMOGRAPHICS

1. Gender: a) ☐ Male b) ☐ Female

2. Date of birth __ __/__ __ __/__ __ __ __
DD MMM YYYY

III. COMMENTS

1. Comments on the case? ☐ Yes ☐ No

a. IF YES, please specify:

_____

_____

_____

IV. INCLUSION CRITERIA

For these questions if the date is not known please leave the date field blank.

1.	Does the potential participant have documented symmetric proximal muscle weakness on exam, and if so what was the first date this was documented?	O Yes 1.____/_____ MMM YYYY	O No	O N/A
2.	Has the potential participant had an EMG? (skip question 2a if No)	O Yes	O No	
2a.	If so, does the potential participant have documented EMG evidence for myopathy, and if so what was the first date this was documented?	O Yes 2a.___/____ MMM YYYY	O No	O N/A
3.	Does the potential participant have documented elevations of any of the following muscle enzymes (CK, LDH, AST, ALT or aldolase), and if so what was the first date this was documented?	O Yes 3.____/_____ MMM YYYY	O No	O N/A
4.	Does the potential participant have documented muscle biopsy evidence of polymyositis or dermatomyositis, and if so what was the first date this was documented?	O Yes 4.____/_____ MMM YYYY	O No	O N/A
5.	Does the potential participant have documented Gottron's papules or sign or Heliotrope rash on exam, and if so what was the first date this was documented?	O Yes 5.____/_____ MMM YYYY	O No	O N/A
6.	Considering 1-5 above, does the potential participant have documented criteria for probable or definite DM or JDM by the criteria of Bohan and Peter? (See criteria below this table.)	O Yes	O No	O N/A
7.	Is the potential participant at least 7 years of age?	O Yes	O No	
8.	Does the potential participant have extensive calcinosis (involving at least 2 extremities or the torso)?	O Yes	O No	O N/A
9.	Does the potential participant have a calcinosis activity VAS score of ≥3.5 cm (out of 10 cm)?	O Yes	O No	
10.	Is the potential participant able and willing to undergo all study procedures and assessments, including receiving IV infusions?	O Yes	O No	
11.	a. What is the patient/parent global disease activity VAS score for this potential participant? _____ b. What is the physician global disease activity VAS score for this potential participant? _____ c. Are the potential participant's lab values for CK, LDH, aldolase, AST, and ALT all ≤2X ULN? O Yes O No d. In consideration of (a), (b), and (c), does the potential participant have myositis disease activity that is stable (i.e., both VAS scores <4 cm and all available lab values ≤2X ULN)?	O Yes	O No	
12.	If taking any medications for myositis, has this treatment been stable for at least 6 weeks prior to study entry? (If not taking medications for myositis, mark N/A in right-hand column.)	O Yes	O No	O N/A
13.	If of reproductive potential, is the potential participant willing to use a reliable form of birth control for the 62-week duration of the study? (If not of reproductive potential, mark N/A in right-hand column.)	O Yes	O No	O N/A
14.	Is the potential participant or the potential participant's parent/legal guardian able and willing to give consent for the study?	O Yes	O No	

Bohan and Peter Criteria:

1. Symmetric, often progressive, proximal muscle weakness
2. Characteristic electromyographic (EMG) triad
   - Short duration, small, low amplitude polyphasic potentials
   - Fibrillation potentials, seen even at rest
   - Bizarre high frequency repetitive discharges
3. Elevations of serum levels of muscle associated enzymes
   - - Creatine kinase (CK)
   - - Aldolase
   - - Lactate dehydrogenase (LD)
   - - Transaminases (ALT/SGPT and AST/SGOT)
4. Evidence of chronic inflammation in muscle biopsy:
   - - Necrosis of type I and type II muscle fibers
   - - Degeneration and regeneration of myofibers with variation in myofiber size
   - - Focal collections of interstitial or perivascular mononuclear cells

5. Characteristic rashes of dermatomyositis:
   - Scaly erythematous palpable eruptions over the metacarpophalangeal
   - or interphalangeal joints, knees, elbows or medial malleoli (Gottron's papules)
   - - Erythematous macules over the metacarpophalangeal or interphalangeal joints, knees, elbows or medial malleoli (Gottron's sign)
   - - Periorbital or upper eyelid purplish discoloration (Heliotrope rash)

Definite disease	=	For DM any 3 of the first 4 criteria plus the rash
Probable disease	=	For DM any 2 of the first 4 criteria plus the rash
Possible disease	=	For DM any 1 of the first 4 criteria plus the rash
Juvenile disease	=	For DM any 2 of the first 4 criteria plus the rash
Adult disease	=	For DM any 1 of the first 4 criteria plus the rash

V. EXCLUSION CRITERIA

1.	Is the potential participant pregnant or breastfeeding? (If not a female of reproductive potential, mark N/A in right-hand column.)	O Yes	O No	O N/A
2.	Does the potential participant have any known allergies to sodium thiosulfate or its components?	O Yes	O No	O N/A
3.	a. What is the patient/parent global disease activity VAS score for this potential participant? _____ b. What is the physician global disease activity VAS score for this potential participant? _____ c. In consideration of (a) and (b), does the potential participant have severe myositis activity (i.e., either VAS score >4 cm)?	O Yes	ONo	
4.	Has the potential participant had an escalation of immunosuppressive therapy in the past 2 months, including the addition of a new agent to treat the underlying disease or an increase in dose of an existing medication used to treat the patient's disease (other than an adjustment for weight or body surface area in children)? (If not taking immunosuppressive therapy, mark N/A in right-hand column.)	O Yes	O No	O N/A
5.	Does the potential participant have a current malignancy or a history of malignancy within 5 years of their diagnosis for JDM/DM (except for benign skin lesions or basal cell carcinoma)?	O Yes	O No	
6.	Does the potential participant have a known or suspected history of alcohol or drug abuse in the prior 6 months?	O Yes	O No	
7.	Does the potential participant have systemic lupus erythematosus, scleroderma, or a disease besides JDM/DM that is associated with calcinosis as a complication?	O Yes	O No	
8.	Has the potential participant had a change in any medications for the treatment of their calcinosis in the past 2 months? Medications include, but are not limited to alendronate, etidronate, pamidronate, probenecid, colchicine, diltiazem, thalidomide, and aluminum hydroxide. (If not taking medications for calcinosis currently or in the past 2 months, mark N/A in right-hand column.)	O Yes	O No	O N/A
9.	Has the potential participant taken any of the following medications in the past 2 months: • Probenecid; • Diltiazem; • Aluminum hydroxide; • Hydrochlorothiazide?	O Yes	O No	

10.	Does the potential participant have current or a history of any of the following: • Heart failure; • Renal impairment (GFR less than 30 representing severe renal disease); • Liver disease (Child-Pugh class C); • Arrhythmias (that are symptomatic or are concerning for progression to symptomatic arrhythmias); • Recurrent kidney stones (more than one episode of symptomatic kidney stones separated by at least 1 month)?	O Yes	O No	
11.	a. If ≥18 years of age, does the potential participant have a T-score at or below -2.5 and one or more fractures? O Yes O No b. If <18 years of age, does the potential participant have a Z-score below -2 and one or more fractures? O Yes O No c. In consideration of (a) or (b), does the potential participant have severe osteoporosis (A yes for either answer above) or has he/she had a bone fracture in the past year?	O Yes	O No	
12.	a. Does the participant have a psychiatric illness or a history of medical noncompliance? O Yes O No (mark N/A in right-hand column) b. In consideration of (a), if yes, does the study team feel this will make the patient unlikely to complete the study?	O Yes	O No	O N/A
13.	a. Does the potential participant have dysphagia? O Yes O No (mark N/A in right-hand column) b. In consideration of (a), if yes, is it to a severity where non-oral feeding alternatives are needed to maintain adequate nutrition?	O Yes	O No	O N/A
14.	Does the potential participant require supplemental oxygen?	O Yes	O No	
15.	Has the potential participant experienced >3 episodes of cellulitis requiring IV antibiotics related to calcinosis within a year prior to enrollment, or had cellulitis within the prior month?	O Yes	O No	
16.	Has the potential participant ever taken sodium thiosulfate (previously or currently receiving)?	O Yes	O No	
17.	a. Is the potential participant currently taking oral prednisone or an equivalent oral corticosteroid dose? O Yes O No (mark N/A in right-hand column) b. In consideration of (a), if yes, is the dose more than 1 mg/kg/day?	O Yes	O No	O N/A

18.	Does the potential participant have any chronic infections that may make assessment of muscle disease difficult, including, but not limited to, hepatitis, HIV, HTLV1, or HTLV 2?	⭘ Yes	⭘ No	
19.	a. Complete Section VI (list of heath conditions). b. In reference to conditions listed in Section VI, does the potential participant have any health conditions that, in the opinion of the investigator, significantly increase the risk of taking sodium thiosulfate or participating in any of the study procedures?	⭘ Yes	⭘ No	⭘ N/A
20.	a. What is the potential participant's weigh (in kg)? _____ b. In consideration of (a), does the participant weigh less than 26 kg? (Note: We will attempt to enroll participants greater than 28 kg; participants weighing 26-28 kg may enroll, but will only complete some of the research blood work.)	⭘ Yes	⭘ No	
21.	a. Is the potential participant taking a regimen of pulse steroids or IVIG? ⭘ Yes ⭘ No (mark N/A in right-hand column) b. In consideration of (a), if yes, is this being taken at an interval other than every 1, 2, or 5 weeks?	⭘ Yes	⭘ No	⭘ N/A
22.	a. If all inclusion criteria in Section IV have been met, and no other exclusion criteria in Section V have been met, then complete Section VII (list of concomitant medications). b. In reference to concomitant medications listed in Section VII, and following a thorough assessment by the study PharmD, are any of the potential participant's concomitant medications thought to alter sodium thiosulfate's effects or pharmacokinetics? (If not taking any concomitant medications, mark N/A in right-hand column.)	⭘ Yes	⭘ No	⭘ N/A

Eligibility Determination:

If the potential participant is at least 7 years of age, the criteria for probable or definite dermatomyositis are met, the responses to applicable inclusion criteria questions 6-16 are YES and all applicable exclusion criteria are NO, then the participant is eligible to participate.

VI. LIST OF HEALTH CONDITIONS

In the space provided below, list all current health conditions of the potential participant and any additional comments (e.g., onset, severity, etc.).

Condition	Comments

VII. LIST OF CONCOMITANT MEDICATIONS

In the space provided below, list all current medications the potential participant is taking, the dosing, and any additional comments (e.g., start date, duration of treatment, etc.). Include prescription and over-the-counter medications or dietary/nutritional supplements.

Medication, Dose	**Comments**
Prescription Medications:	
Non-Prescription Medications:	

For Iothalamate Clearance? (if yes to either question, unable to undergo iothalamate clearance)

Is the patient allergic to Iodine?

Yes	**No**	**N/A**

Does the patient have a history of asthma?

Yes	**No**	**N/A**

VIII. ENROLLMENT OUTCOME

1.	Participant is eligible for the study - all entry criteria met and no exclusions?	o Yes	o No	o N/A

Investigator Printed Name:_____

Signature of Investigator:_____

Appendix G. Script for Assessing Adverse Events for Weeks 10-24

For adult participants, the following questions will be asked. If the answer is an affirmative additional information will be asked for to obtain more details including the timing of any events, severity, and the associated outcomes.:

Since you were last at the NIH have you had to go to the hospital for any reason and if so why?

Since you were last at the NIH have you had any fractures or broken bones?

Since you were last at the NIH have you had any nausea or lightheadedness?

Since you were last at the NIH have you developed any pain when you urinate?

Since you were last at the NIH have you had any headaches?

Since you were last at the NIH have you experienced any episodes of disorientation or confusion?

Since you were last at the NIH have you had any episodes were you had difficulty stopping bleeding?

FOR FEMALE PARTCIPANTS ONLY: Since you were last at the NIH have you become pregnant?

FOR MALE PARTICIPANTS, ONLY: Since you were last at the NIH have any of your sexual partners become pregnant?

Since you were last at the NIH have you developed or been diagnosed with any new medical problems?

Since you were last at the NIH have you had any surgeries?

Have there been any changes in your medical condition that we have not asked about that you believe we should know about and if so what are they?

Since you were last at the NIH have you had any side effects from the sodium thiosulfate ?

Is there anything else you think we should be aware of for the study in terms of your health?

For pediatric participants, the following questions will be asked. If the answer is an affirmative additional information will be asked for to obtain more details including the timing of any events, severity, and the associated outcomes.:

Since your child was last seen at the NIH has he or she had to go to the hospital for any reason and if so why?

Since your child was last seen at the NIH has he or she had any fractures or broken bones?

Since your child was last seen at the NIH has he or she had any nausea or lightheadedness?

Since your child was last seen at the NIH has he or she developed any pain when you urinate?

Since your child lwas ast seen at the NIH has he or she had any headaches?

Since your child was last seen at the NIH has he or she experienced any episodes of disorientation or confusion?

FOR FEMALE PARTCIPANTS ONLY: Since your child last seen at the NIH has she become pregnant?

FOR MALE PARTICIPANTS, ONLY: Since your child was last seen at the NIH have any of his sexual partners become pregnant?

Since your child was last seen at the NIH has he or she had any episodes were he or she had difficulty stopping bleeding?

Since your child was last seen at the NIH has he or she developed or been diagnosed with any new medical problems?

Since your child was last seen at the NIH has he or she had any surgeries?

Have there been any changes in your child’s medical condition that we have not asked about that you believe we should know about and if so what are they?

Since your child was last seen at the NIH has he or she had any side effects from the sodium thiosulfate ?

Is there anything else you think we should be aware of for the study in terms of your child’s health?

Appendix H. Brief Summary of Goals of Imaging Techniques

PET	MRI	CT	DEXA	Ultrasound
Evaluation of energy metabolism of calcinosis lesions and associated soft tissue and fluid collections.	Identification of muscle and soft tissue edema.	Localization and structural information of calcinosis deposits.	Safety evaluation for bone density.	Safety screening for kidney stones.
Differentiation of active from inactive lesions.	Localization of fluid collections associated with calcinosis lesions.	Quantification of calcinosis	Evaluation of fat distribution.	
	Identification of liquefied calcinosis lesions.	Evaluation of density of fluid associated with calcinosis deposits.	Localization of calcinosis lesions.	
	Coupled with CT identification of structures impacted by calcinosis.	Coupled with MRI identification of structures impacted by calcinosis.	Quantification of calcium content of targeted calcinosis lesions.	
		Screening for kidney stones.		
		Changes in bone with treatment.		