PROTOCOL

STUDY TITLE: A Pilot Study to Evaluate the Safety and Efficacy of Oral Treprostinil in the Treatment of Calcinosis in Patients with Systemic Sclerosis

STUDY DRUG: Oral Treprostinil

SUPPORT PROVIDED BY: United Therapeutics

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1. Background:
Calcinosis cutis is the deposition of calcium in the skin and subcutaneous tissues. It is a common and potentially debilitating manifestation of systemic sclerosis (SSc), affecting almost one quarter of these patients. Several studies have found an association between calcinosis and vascular manifestations of SSc including digital ulcers (DU) [1] [2] and acro-osteolysis.[3] We confirmed this in a retrospective multi-center international cohort study of 5280 patients with SSc where DUs were the strongest predictor of calcinosis in multivariate analysis (OR 3.7, 95%CI 2.6-5.3, p<0.0001). Interestingly, we also found a novel association between calcinosis and osteoporosis (OR 3.9, 95%CI 2.1-7.4, *p*<0.0001).

Treprostinil delivered by continuous subcutaneous infusion was effective in both the healing and prevention of DU in patients with SSc in an open-label, single-center clinical trial of 5 (of 12) patients who were able to tolerate the medication.[4] A study of 148 subjects with DU showed that the administration of oral treprostinil up to 16 mg twice daily for 20 weeks was associated with a small but statistically insignificant reduction in net ulcer burden in comparison to placebo.[5] Additionally, preliminary observations in the Pulmonary Hypertension Assessment and Recognition of Outcomes in Scleroderma (PHAROS) registry have found that two patients with SSc-PAH and calcinosis treated with subcutaneous treprostinil for PAH experienced approximately 50% radiographic improvement in their calcinosis lesions after 6 months of therapy (Shapiro et al., unpublished data).

We developed and validated a novel radiographic scoring system to assess the severity of calcinosis affecting the hands of patients with SSc that accounts for area coverage, density, and anatomic location. This scoring system is feasible and was found to have excellent intra- and inter-rater reliability with intra-class correlation coefficients (ICC) of .93 (.89-.97) and .89 (.86-.92), respectively.[6]

Given that calcinosis is a frequent, debilitating complication of SSc associated with digital vascular ischemia with no effective therapies, a clinical trial, using novel outcome measures, testing the safety and efficacy of a powerful vasodilator for the treatment of calcinosis is warranted.

2. Primary/Secondary objectives:
Our main objective is to conduct an open-label proof-of-concept study with the following primary and secondary endpoints.
Primary endpoints:

- To assess the safety and tolerability of oral treprostinil using TID dosing in SSc patients with calcinosis affecting the hands.
- To assess the efficacy of oral treprostinil in stabilizing the calcinosis burden or reducing the radiographic progression of calcinosis over one year.

Secondary endpoints:

- To assess the effect of oral treprostinil on the change in the Scleroderma Health Assessment Questionnaire, the Cochin Hand Functional Scale, SF-36, Mawdsley Calcinosis Questionnaire, Raynaud Condition Score, and patient and physician assessment of calcinosis severity over one year.

- To assess changes in blood flow using SPY perfusion machine following treatment with treprostinil at 1 year compared to baseline.
- To assess changes in vascular and SSc-PAH associated biomarkers following treatment with treprostinil at 1 year compared to baseline.
- To assess bone markers P1NP and C-telopeptide levels at baseline and at 1 year
- To assess baseline cortical area and cortical porosity measured by HR-pQCT in SSc patients with calcinosis.
- To assess osteoclastogenesis using PBMCs cultures at baseline and at 1-year

3. Hypothesis:
We hypothesize that calcinosis is a result of microvascular injury and ischemic damage, and that therefore treprostinil may be beneficial in the treatment of calcinosis in patients with SSc.

4. Study design:
This prospective open-label trial will enroll 12 patients with SSc and at least one calcinotic lesion of the hands that is palpable on physical examination and also measureable on hand radiographs. Each subject will undergo a screening evaluation 4 weeks before treatment with the study drug is initiated. Each subject will receive treprostinil 0.125 mg TID orally, which will be increased by 0.125 mg TID every 3 to 4 days as tolerated. Follow-up evaluations will be performed every 3 months over a 12-month period of time.

5. Study population:
The population for this study will consist of adult SSc patients with evidence of at least one calcinotic lesion of the hands that is palpable on physical examination and also measureable on hand radiographs.

Key inclusion criteria:
- Signed written informed consent
- Age > 18 years of age
- Diagnosis of limited or diffuse cutaneous systemic sclerosis (SSc) according to the revised 2013 ACR/EULAR classification criteria for SSc[7]
- Radiological and physical examination evidence of at least one subcutaneous calcium deposition in the hands that is clinically apparent as part of routine clinical care.
- If female of childbearing potential, the patient must have a negative pregnancy test at screening and baseline visits
- Oral corticosteroids (≤ 10 mg/day of prednisone or equivalent) and NSAIDs are permitted if the patient is on a stable dose regimen for ≥ 2 weeks prior to screening and throughout the study
- Calcium channel blockers, alpha-1-antagonists, ACE-inhibitors, angiotensin receptor blockers, and protein-pump inhibitors are permitted as long as the doses are stable for 4 weeks prior to screening and throughout the study
- Women of childbearing potential must agree to use adequate contraception when sexually active with any combination of at least 2 effective methods of birth control (except for women who have a partner who is sterile, i.e. due to vasectomy)

Key Exclusion Criteria:

- Rheumatic disease other than SSc
- Patients with pulmonary arterial hypertension (PAH), NYHA Class III or IV, as determined by right heart catheterization or on PAH approved medications for PAH
- Patients with moderate or severe hepatic impairment (Child Pugh Class C), or transaminase elevation (ALT or AST) > 3 x the upper limit of normal at screening visit
- Patients with diverticulosis
- Hemoglobin < 75% of the lower limit of the normal range
- Systolic blood pressure < 95 mmHg or diastolic blood pressure < 50 mmHg
- Patients who are hemodynamically unstable, or have acute renal, cardiac or pulmonary failure, or any life-threatening condition.
- Concurrent malignancy except non-melanoma skin cancers
- Patients receiving specific (sildenafil, tadalafil) or unspecific phosphodiesterase-5 inhibitors (dipyridamole, theophylline), endothelin receptor antagonists, prostanoids, riociguat, or NO donors (nitrates) within 4 weeks of screening
- Patients receiving bisphosphonates, warfarin, colchicine, minocycline, intravenous immunoglobulins, or biological agents including abatacept or rituximab within 4 weeks of screening
- Patients receiving local treatments for calcinosis of the hands including surgical removal or intralesional steroid injections within 12 weeks of screening or throughout the study.
- Patients who have participated in another clinical trial of an investigative agent within 30 days of screening (or 5 half-lives of the investigational drug, whichever is longer)
- Pregnant or nursing women
- Patients with a history of drug or alcohol abuse within 6 months of screening
- Any medical condition that, in the opinion of the investigator, might interfere with the subject's participation in the study or poses an added risk for the subject
- Inability to comply with study and follow-up procedures

6. Description of the treatment:

United Therapeutics will provide treprostinil labeled for investigational use. The Sponsor/Investigator of the trial will ensure maintenance of complete and accurate records of the receipt, dispensation, and disposal or return of all trial drug in accordance with Title 21 Code of Federal Regulations (C.F.R.), Part 312.57 and 312.62 and United Therapeutics requirements.

Treprostinil

Treprostinil (Orenitram®) is a prostacyclin vasodilator indicated for the treatment of pulmonary arterial hypertension (WHO Group 1) to improve exercise capacity. The chemical name of Orenitram® is Acetic acid, 2-[[(1R,2R,3aS,9aS)-2,3,3a,4,9,9a-hexahydro-2-hydroxy-1-[(3S)-3-hydroxyoctyl]-1H-benz[f]inden-5-yl]oxy]-, complexed with 2,2'-iminobis[ethanol] (1:1). The molecular formula is $C_{23}H_{34}O_5C_4H_{11}NO_2$, and its molecular weight is 495.65. Orenitram® is an extended release osmotic tablet for oral administration, and it is formulated as the diolamine salt of treprostinil, a tricyclic benzindene analogue of prostacyclin. It is available in the following four strengths: 0.125 mg (white tablet imprinted with UT 0.125), 0.25 mg (green tablet imprinted with UT 0.25), 1 mg (yellow tablet imprinted with UT 1), and 2.5 mg (pink tablet imprinted with UT 2.5). The formulations also contain xylitol, maltodextrin, sodium lauryl sulfate, magnesium

stearate, cellulose acetate, triethyl citrate, polyvinyl alcohol, titanium dioxide, polyethylene glycol, and talc. In addition tablets may contain colorants FD&C Blue #2, iron oxide yellow, and iron oxide red. The imprinting ink contains shellac glaze, ethanol, isopropyl alcohol USP, iron oxide black, n-butyl alcohol, propylene glycol, and ammonium hydroxide.

Mechanism of Action
The major pharmacologic actions of treprostinil are direct vasodilation of pulmonary and systemic arterial vascular beds, inhibition of platelet aggregation, and inhibition of smooth muscle cell proliferation.

Storage
Treprostinil must be stored at 25°C (77°F); excursions 15°C to 30°C (59°F to 86°F). For further details, see the Investigator Brochure and the Orenitram® Package Insert.[8]

Dosage and Administration
Initiate treprostinil at 0.125 mg TID (every 8 ± 2 h), with dose escalation of an additional 0.125 mg TID every 3 to 4 days as tolerated. No dose changes will be allowed for 5 days before the month 12 visit. [9] Give with food, swallow tablets whole, and use only intact tablets; do not crush, split, or chew. The maximum dose, dose escalation or titration will be done at the Investigator's discretion based upon individual subject tolerability. Patients with mild hepatic impairment (Child Pugh Class A) should initiate treprostinil at 0.125 mg TID, and increment at 0.125 mg TID every 6 to 8 days.[8]

Criteria for Treatment Discontinuation
Treatment will be discontinued in the following circumstances:

- Patients who wish to withdraw from further participation
- Patients with a serious or life-threatening adverse event that in the opinion of the investigator is directly attributable to the study drug
- The patient deviated from the protocol
- The patient's behavior is likely to undermine the validity of his / her results
- Patients who have a positive pregnancy test during the study
- Patients who develop any condition that in the opinion of the investigator could be worsened by further treatment with the study drug

Criteria for Stopping Rules
The study may be stopped at any time if, in the opinion of the Investigator and / or Sponsor, continuation of the study represents a serious medical risk to the subjects. This may include, but is not limited to, the presence of serious, life-threatening, or fatal adverse events or adverse events that are unacceptable in nature, severity, or frequency. The Sponsor reserves the right to discontinue the study for any reason at any time.

Down titration of study medication
Following the 12-month study assessments, patients will be titrated off study drug. Subjects discontinuing treprostinil, upon exiting the study at the End of Study visit or at early termination, who are receiving doses greater than 2 mg TID, should be tapered in 25% increments every 24 hours based on their End of Study dose prior to discontinuation. Once a dose of 2 mg TID or less is

reached, the subject can discontinue treprostinil. For example, a subject who reached an End of Study dose between 6 and 12 mg TID would decrease by 2 mg TID every 24 hours, and a subject who reached an End of Study dose between 2 and 6 mg TID would decrease by 1 mg TID every 24 hours.

7. Estimated duration: 14 months

8. Time and events schedule and description of assessments:

Assessments:

Screening Visit (1 month prior to treatment):

- Informed consent
- Review of inclusion and exclusion criteria
- Complete medical history including scleroderma diagnosis
- Review of concomitant medications
- Complete physical examination including examination of calcinosis, digital ulcers, vital signs, height/weight, and Modified Rodnan skin score (MRSS)
- Electrocardiogram (EKG)
- Laboratory tests: CBC with differential, comprehensive metabolic panel, and urinalysis
- Urine pregnancy test (females of child bearing potential only)
- Review of adverse events

Baseline Visit (0 month):

- Review of concomitant medications
- Complete physical examination with vital signs, and MRSS
- Examination of calcinosis and digital ulcers
- Laboratory tests: CBC with differential, comprehensive metabolic panel, and urinalysis
- Urine pregnancy test (females of child bearing potential only)
- Questionnaires: SHAQ, Cochin hand functional scale, SF-36 assessment of health status, Mawdsley Calcinosis Questionnaire, Physician/Patient Global Assessment of calcinosis severity, and Raynaud's Condition Score.
- Review of adverse events
- Study drug dispense/return
- Radiological assessment of calcinosis
- Skin biopsy
- Blood for biomarker analyses
- SPY assessment of superficial blood flow
- Anthropometric measurements
- High resolution peripheral quantitative computed tomography (HRpQCT)
- Handgrip strength measurement

At 3 months visit:

- Review of concomitant medications
- Examination of calcinosis and digital ulcers
- Urine pregnancy test (females of child bearing potential only)
- Questionnaires: SHAQ, Cochin hand functional scale, SF-36 assessment of health status, Mawdsley Calcinosis Questionnaire, Physician/Patient Global Assessment of calcinosis severity, and Raynaud's Condition Score.

- Blood for biomarker analyses
- Review of adverse events
- Study drug dispense/return

At 6 months visit:

- Review of concomitant medications
- Examination of calcinosis and digital ulcers
- Complete physical examination with vital signs, and MRSS
- Laboratory tests: CBC with differential, comprehensive metabolic panel, and urinalysis
- Urine pregnancy test (females of child bearing potential only)
- Questionnaires: SHAQ, Cochin hand functional scale, SF-36 assessment of health status, Mawdsley Calcinosis Questionnaire, Physician/Patient Global Assessment of calcinosis severity, and Raynaud's Condition Score.
- Review of adverse events
- Study drug dispense/return

At 9 months visit:

- Review of concomitant medications
- Examination of calcinosis and digital ulcers
- Urine pregnancy test (females of child bearing potential only)
- Questionnaires: SHAQ, Cochin hand functional scale, SF-36 assessment of health status, Mawdsley Calcinosis Questionnaire, Physician/Patient Global Assessment of calcinosis severity, and Raynaud's Condition Score.
- Review of adverse events
- Study drug dispense/return

At 12 months visit:

- Review of concomitant medications
- Examination of calcinosis and digital ulcers
- Urine pregnancy test (females of child bearing potential only)
- Questionnaires: SHAQ, Cochin hand functional scale, SF-36 assessment of health status, Mawdsley Calcinosis Questionnaire, Physician/Patient Global Assessment of calcinosis severity, and Raynaud's Condition Score.
- Review of adverse events
- Study drug dispense/return
- Complete physical examination with vital signs, and MRSS
- Laboratory tests: CBC with differential, comprehensive metabolic panel, and urinalysis
- Radiological assessment of calcinosis
- Skin biopsy
- Blood for biomarker analyses
- SPY assessment of superficial blood flow
- EKG
- High resolution peripheral quantitative computed tomography (HRpQCT)

At 13-months visit:

- Review of concomitant medications
- Examination of calcinosis and digital ulcers

- Review of adverse events
- Study drug return
- Complete physical examination with vital signs

Study Flowchart:

Study phase	Pre-treatment phase	Treatment phase					Follow-up phase
Visit	Screening	Baseline	1	2	3	End	Follow-up
Month	-1	0	3	6	9	12	13
Informed consent	X						
Inclusion/exclusion criteria	X						
Medical history including SSc	X						
Concomitant medications	X	X	X	X	X	X	X
Physical examination	X	X		X		X	X
Examination of calcinosis and digital ulcers	X	X	X	X	X	X	X
Vital signs	X	X		X		X	X
Height/Weight	X						
EKG	X					X	
Laboratory tests*	X	X		X		X	
Urine pregnancy test	X	X	X	X	X	X	
Radiological assessment of calcinosis		X				X	
Scleroderma Health Assessment Questionnaire (SHAQ)		X	X	X	X	X	
Cochin Hand Functional Scale		X	X	X	X	X	
Mawdsley Calcinosis Questionnaire		X	X	X	X	X	
SF-36 assessment of health status		X	X	X	X	X	
Raynaud's Condition Score		X	X	X	X	X	
Physician/patient global assessment of calcinosis severity		X	X	X	X	X	
MRSS	X	X		X		X	
Skin biopsies		X				X	
Blood for biomarker analyses		X	X			X	
PBMC		X				X	
P1NP and C-telopeptide		X				X	
SPY		X				X	
XtremeCT II scans		X				X	
Adverse events	X	X	X	X	X	X	X
Study drug dispense/return		X	X	X	X	X	X

*CBC, CMP, UA

Description of assessments:

Medical history including SSc: Significant past or present illnesses, current prescription or nonprescription medications (including vitamins and herbal products), and history of allergies or idiosyncratic responses to drugs should be noted.

Physical examination: A complete physical examination will be conducted by a physician at Screening, 6 months, and at end of study. Any significant changes to the subject's medical condition, physical examination, and concomitant medications should be documented throughout the course of the study. Any untoward medical experience should be recorded as an adverse event.

Examination of calcinosis and digital ulcers: Investigator will perform a thorough clinical examination looking for calcinotic deposits at each visit. We will use case report forms with picture images of the hands to record calcinosis and digital ulcers at each evaluation (Appendix A). We will define digital ulcer as an area with visually discernable depth and a loss of continuity of epithelial coverage in the volar aspect of the finger and distal from the proximal interphalangeal joints, which could be denuded (active) or covered by a scab or necrotic tissue (indeterminate). At each visit, the status of each digital ulcer will be rated as "A," a current active digital ulcer, "H", a completely healed ulcer, or "I," an ulcer with indeterminate status, as previously defined.[10]

Vital Signs: Systolic and diastolic blood pressure, heart rate, respiratory rate, and temperature (°C) will be measured prior to assessments and after sitting for 5 minutes at screening, baseline, 6 months, and 12 months. Vital signs should also be assessed in the case of abnormal clinical signs and symptoms.

Electrocardiogram (ECG): Twelve-lead ECGs will be recorded after at least 5 minutes rest in the semi-recumbent position at screening and at the end of the study at 12 months. Recordings should include lead II as a rhythm strip and contain at least 5 QRS complexes. ECG parameters collected (after at least 5 minutes rest) include heart rate, and PR interval, QT interval, QRS duration and any clinically significant abnormalities.

Laboratory tests: Blood and urine samples for the measurement and evaluation of CBC, CMP, and urinalysis (UA) will be collected at screening, 6 months and at the end of the study, and analyzed at Stanford laboratory.

Urine pregnancy test: urine beta-HCG will be measured in all female in reproductive age at all visits.

Radiological examination of calcinosis: Patients will have plain radiographs of the hands at baseline and at 1 year. Dr. Kathryn Stevens and Dr. Lorinda Chung will serve as the blinded X-ray assessors.

Scleroderma Health Assessment Questionnaire (SHAQ): The SHAQ is a patient self-administered instrument, which has been previously validated in SSc and demonstrates meaningful clinical changes in the course of the disease over time. It is comprised of the HAQ-DI, a self-administered 20-question instrument that assesses a patient's level of functional ability and five scleroderma visual analogue scale (VAS) measurements to evaluate symptoms specific to SSc (Appendix B). [11-13]

Cochin Hand Function Scale (CHFS): CHFS is a questionnaire derived from 18 validated questions to assess functional disability and handicap due to hand involvement in rheumatoid arthritis. Each answer is scored on a scale of 0 (no difficulty) to 5 (impossible to do), with a maximum score of 90. A higher score indicates worse disability or handicap. The CHFS has been demonstrated as a reliable and valid assessment of hand function at the activity level in persons with SSc (Appendix C).[14, 15]

Mawdsley Calcinosis Questionnaire: The content of this questionnaire is patient-generated and includes 17 questions related to the impact of calcinosis rated from 0 (no limitation) to 10 (worst limitation possible) (Appendix D).

The Medical Outcomes Study 36-Item Short Form Health Survey (SF-36): The SF-36 is one of the most widely used instruments to assess quality of life in patients with systemic illnesses. It is a self-administered questionnaire covering eight areas: physical function, physical role, bodily pain, general health, vitality, social function, emotional role, and mental health. For each area, the score ranges from 0 (poorer health status) to 100 (better health status) (Appendix E). [16-24]

Patient and Physician global assessments: Patients and physicians will each independently rate the severity of calcinosis on a 10 cm VAS. The term "severity" will be used to measure the extent of disease activity and associated disability or discomfort the patient experiences during the indicated time period (Appendix F).[25]

Raynaud's Condition Score (RCS): We will measure changes in Raynaud's phenomenon (RP) by using the RCS, a self-assessment of RP activity using a 0–10 ordinal scale. The RCS incorporates the cumulative daily frequency, duration, severity, and impact of RP attacks (Appendix G).[25]

Modified Rodnan Skin Score (mRSS): The mRSS measures skin tightness and is the sum of scores from 17 surface anatomic areas (fingers, hands, forearms, arms, feet, legs, and thighs bilaterally, and face, chest, and abdomen singly) rated on a 0–3 scale (0=normal skin; 1=mild thickness; 2=moderate thickness; 3=severe thickness with inability to pinch the skin into a fold). The skin score will be the sum of the individual skin assessment scores and ranges from 0 (best possible outcome) to 51 (worst possible outcome) (Appendix H). [26-28]

Skin biopsies: We will collect two side-by-side skin samples to assess for vascular changes on histopathology and gene expression changes following treatment with treprostinil: one at baseline and one at the end of the study. This part of the study will be optional for participating patients. Skin biopsies will be obtained from a standard site on the forearm (extensor surface of forearm approximately 10 cm proximal to the olecranon, which is chosen for its uniform

involvement in patients with SSc). Two adjacent 5 mm punch biopsies (taken full thickness to subcutaneous fat) will be harvested from the site. This involves selecting an area to biopsy and wiping the skin with alcohol. Next, 1-2 cc of lidocaine with 1:100,000 epinephrine is introduced into the skin using a 30G needle. Two punch biopsies will then be taken and processed as described in appendix I. Next, the wound will be sutured with 1-2 4.0 nylon sutures. Wounds will be dressed with polysporin and a bandage. Verbal wound care instructions will be given. The first biopsy will be bisected: one half will be flash frozen in liquid nitrogen and half will be embedded in paraffin. The second biopsy will also be bisected: one half will be placed in RNA later, and the other half will be placed in a cryo-tube. (See appendix I for details)

Research blood: We will collect serum and peripheral blood samples to assess changes in vascular and SSc-associated biomarkers following treatment with treprostinil. Blood samples for the assessment of biomarkers will be drawn at baseline, 3 months, and end of study visit. Please see appendix for a complete list of biomarkers (Appendix J).

P1NP and C-telopeptide: Procollagen Type I Intact N Terminal Propeptide (P1NP), a marker of bone formation, and C-telopeptide, a marker of bone resorption, will be measured at baseline and 12 months to provide information about the rate of bone turnover, and to determine whether patients with calcinosis have high turnover rates.

PBMCs: PBMCs will be obtained for each participant at baseline to assess for abnormal osteoclastogenesis. A recent publication investigated whether acro-osteolysis (bone resorption of distal phalanges), a clinical feature often associated with digital ulcers and calcinosis[3], was associated with abnormal osteoclastogenesis in SSc patients. The authors found that hypoxia may enhance the formation of osteoclasts particularly in SSc patients with acro-osteolysis. We hypothesize that SSc patients with calcinosis will similarly demonstrate increased osteoclastogenesis, resulting in enhanced propensity to osteoporosis/bone loss. [29] We will obtain PBMCs at baseline and 12 months.

SPY: The SPY Near-Infrared Perfusion Assessment System (distributed by LifeCell Corp., Branchburg, N.J.; manufactured by Novadaq Technologies Inc., Richmond, BC, Canada) is an imaging technology that utilizes indocyanine green (ICG), and allows real-time visual assessment of superficial blood flow.[30]

XtremeCT II scan: The latest-generation high-resolution peripheral quantitative computed tomography (HR-pQCT) XtremeCT II scan is a novel technology with dramatically improved spatial resolution. It is able to define the cortical and trabecular surfaces of the bones in a three-dimensional fashion, and therefore provides information on bone microarchitecture as well as bone density. The XtremeCT II machine at Stanford University is one of 10 in the United States. We will perform HR-pQCT at baseline and at 12 months to assess for changes in cortical area and cortical porosity.

Safety: During the study, the primary assessment of safety will be changes in vital signs, clinical laboratory parameters, EKG, and the development of adverse events.

9. Safety monitoring and reporting

Definitions:
Adverse Event (AE): An AE is any untoward medical experience occurring to a subject during a clinical trial whether or not it is related to the study drug. An AE may include an intercurrent illness, injury, or any other concomitant impairment of the subject's health, as well as abnormal laboratory findings if deemed to have clinical significance. AEs may also include worsening of an existing symptom or condition or post-treatment events that occur as a result of protocol-mandated procedures.

Severe Adverse Event (SAE): A SAE is an AE occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening AE
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability / incapacity
- A congenital anomaly / birth defect

In addition, important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the subject and require medical / surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in hospitalization, or the development of drug dependency or drug abuse. Life threatening means that the subject was, in the view of the Investigator, at immediate risk of death from the event as it occurred. It does not mean that the event, had it occurred in a more severe form, might have caused death.

Reporting Responsibilities:
In the event of an adverse event, the first concern will be for the safety of the patients. Investigators are required to collect and document all adverse events (AEs) and non-related serious adverse events (SAEs). All SAEs, regardless of expectedness or causality, must be reported to the Sponsor by fax (+ 1 919-313-1297 or other appropriate number) within 24 hours of awareness. A completed SAE report form along with any relevant hospital records and autopsy reports should be faxed to the Drug Safety Department at United Therapeutics Corporation. A follow-up SAE report form must be forwarded to the Drug Safety Department at United Therapeutics Corporation within 48 hours of the receipt of any new / updated information. The Investigator must also promptly notify their Investigational Review Board (IRB) or Ethics Committee (EC) of the SAE, including any follow-up information, in accordance with applicable national regulations and guidelines set forth by the IRB or EC. All documents related to AEs (serious, non serious, related, or not) will be readily available for review, should the need arise.

An AE or SAE occurring during the study must be documented in the subject's source documents and on the appropriate CRF page. Information relating to the AE such as onset and cessation date and times, intensity, seriousness, relationship to study drug, and outcome is also to be documented in the CRF. Where possible, AEs should be recorded using standard medical terminology. If several signs or symptoms are clearly related to a medically defined diagnosis or

syndrome, the diagnosis or syndrome should be recorded on the CRF page, not the individual signs and symptoms.

All AEs should be followed until either resolution (or return to normal or baseline values), until they are judged by the Investigator to no longer be clinically significant, or for at least 4 weeks if the AE extends beyond the final visit. All SAEs that occur during the study will be followed until resolution, death, or the subject is lost to follow-up even if they are ongoing more than 4 weeks after completion of the final visit. Supplemental measurements and / or evaluations may be necessary to investigate fully the nature and / or causality of an AE or SAE. This may include additional laboratory tests, diagnostic procedures, or consultation with other healthcare professionals. CRF pages should be updated.

10. Statistical Considerations

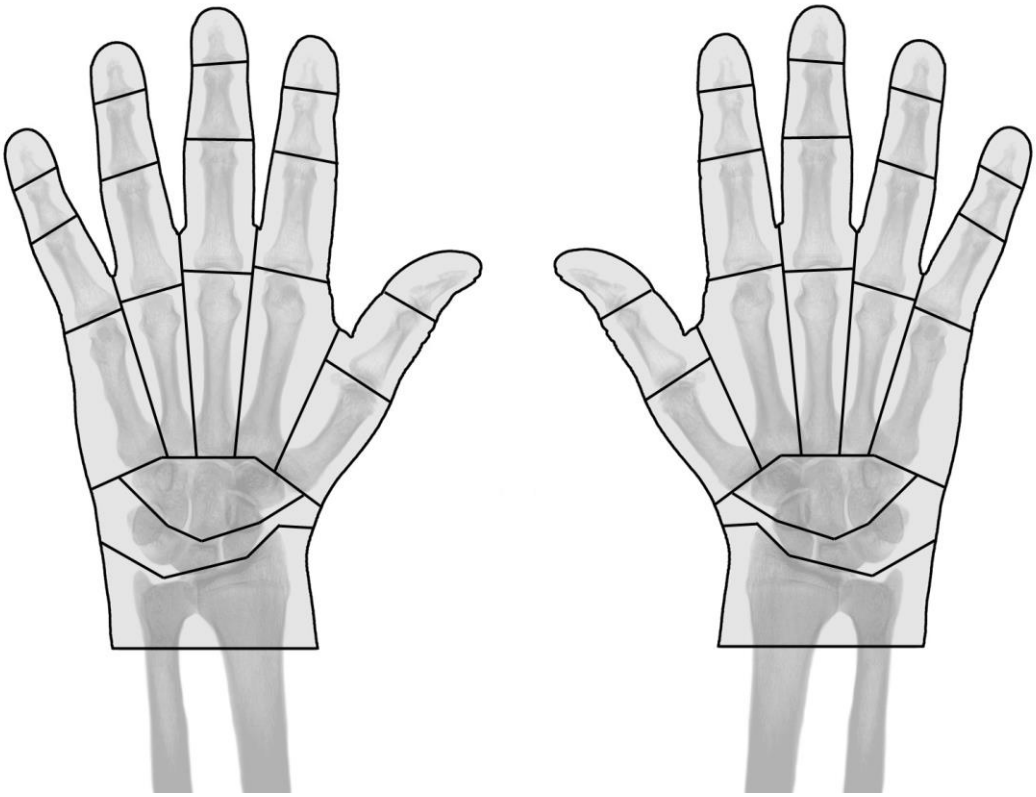
Statistical analysis:

Descriptive statistics and frequency distributions of all variables of interest will be reported as proportions (%) for categorical variables and as mean ± standard deviation or median (range) for continuous variables. Baseline to 12-month differences and 95% confidence intervals will be calculated for scored outcomes (x-ray score, MRSS, physician's global assessment by VAS, and quality of life measurements). For the primary efficacy endpoint, mean change and standard deviation in calcinosis burden assessed by radiograph from baseline to 12-month visit, we will use Student's t-test. The mean rate of change of calcinosis in radiograph will be calculated with the following formula: (Year 1 XR score – Baseline XR score)/time. XR score is defined as: sum of scores for 22 weighted areas affecting each hand: %area coverage (0-100) X density (1-3) X weight for each area.[6]

Sample size calculation:

We will need 9 paired radiographs (9 patients) at 1-year to provide >80% power to detect a mean change in x-ray score of 12.0 (equivalent to a minimally significant change in score of 25%) with a SD of 8.2 with moderate correlation r=0.5 using a two-sided test at alpha 0.05 level. We estimate a 15% dropout rate based on prior clinical trials. Hence, the total number of subjects needed to obtain 9/0.85 subjects who complete the study is 11 subjects. Also, due to the small sample size, we plan to use Wilcoxon signed rank test to analyze the effects. Thus, we anticipate losing about 10% of power due to using a non-parametric test. As a result, the sample size is further adjusted to 11/0.9 = 12. Statistical significance will be defined as $p \leq 0.05$.

Appendix A. CRF for assessment of digital ulcers and calcinosis



Appendix B. Scleroderma Health Assessment Questionnaire (SHAQ)

In this section we are interested in learning how your illness affects your ability to function in daily life. Please feel free to add comments.

Please check the one response that best describes your usual abilities

IN THE PAST SEVEN DAYS:

	Without **ANY** difficulty	With **SOME** difficulty	With **MUCH** difficulty	**UNABLE** to do
DRESSING & GROOMING				
Are you able to:				
- Dress yourself, including tying shoelaces and doing buttons	_____	_____	_____	_____
- Shampoo your hair?	_____	_____	_____	_____
ARISING				
Are you able to:				
- Stand up from an armless straight chair?	_____	_____	_____	_____
- Get in and out of bed?	_____	_____	_____	_____
EATING				
Are you able to:	_____	_____	_____	_____
- Cut your meat?				
- Lift a full glass to your mouth?	_____	_____	_____	_____
- Open a new milk carton?	_____	_____	_____	_____
WALKING				
Are you able to:				
- Walk outdoors on flat ground?	_____	_____	_____	_____
- Climb up five stairs?	_____	_____	_____	_____

Please check any **AIDS or DEVICES** that you usually use for any of these activities:

_____ Cane
_____ Walker
_____ Crutches
_____ Wheelchair
_____ Devices for dressing (button hook, zipper pull, etc.)
_____ Built up or special utensils
_____ Special or built-up chair
_____ Other (specify:_____)

Please check any categories for which you usually need **ASSISTANCE FROM ANOTHER PERSON:**

_____ Dressing and grooming
_____ Arising
_____ Eating
_____ Walking

_____ _____
Patient Signature Date

	Without **ANY** difficulty	With **SOME** difficulty	With **MUCH** difficulty	**UNABLE** to do
Are you able to:				
- Wash and dry your entire body?	_____	_____	_____	_____
- Take a tub bath?	_____	_____	_____	_____
- Get on and off the toilet?	_____	_____	_____	_____
Are you able to :				
- Reach and get down a 5 pound object (such as a bag of sugar) from just over your head?	_____	_____	_____	_____
- Bend down and pick up clothing off the floor?	_____	_____	_____	_____
Are you able to:				
- Open car doors?	_____	_____	_____	_____
- Open jars that have been previously opened?	_____	_____	_____	_____
- Turn faucets on and off?	_____	_____	_____	_____
Are you able to:				
- Run errands and shop?	_____	_____	_____	_____
- Get in and out of a car?	_____	_____	_____	_____
- Do chores such as vacuuming or yardwork?	_____	_____	_____	_____

Please check any **AIDS or DEVICES** that you usually use for any of these activities:

_____ Raised Toilet Seats _____ Bathtub Bar _____ Bathtub Seat

_____ Long-Handled Appliances for Reach

_____ Jar Opener (for jars previously opened)

_____ Long-Handled Appliances in Bathroom

_____ Other (specify:_____)

Please check any categories for which you usually need **HELP FROM ANOTHER PERSON:**

_____ Hygiene

_____ Gripping and Opening Things

_____ Reach

_____ Errands and Chores

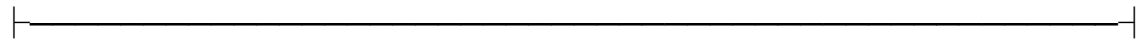
_____ _____

Patient Signature Date

We are also interested in learning whether or not you are affected by pain because of your illness. You do not have to answer the questions that do not apply to you.

How much pain have you had because of your illness **IN THE PAST WEEK ?**
PLACE A MARK ON THE LINE TO INDICATE THE SEVERITY OF THE PAIN.

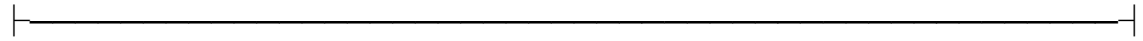
NO PAIN VERY SEVERE PAIN



0 100

IN THE PAST WEEK, how much have your intestinal problems interfered with your daily activities?
PLACE A MARK ON THE LINE TO INDICATE THE LIMITATION OF ACTIVITY.

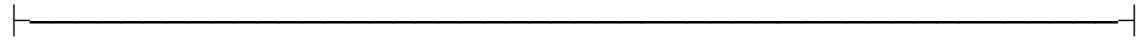
INTESTINAL PROBLEMS DO NOT LIMIT ACTIVITIES VERY SEVERE LIMITATION



0 100

IN THE PAST WEEK, how much have your breathing problems interfered with your daily activities?
PLACE A MARK ON THE LINE TO INDICATE THE LIMITATION OF ACTIVITY.

BREATHING PROBLEMS DO NOT LIMIT ACTIVITIES VERY SEVERE LIMITATION

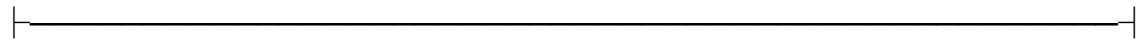


0 100

IN THE PAST WEEK, how much has Raynaud's interfered with your daily activities?
PLACE A MARK ON THE LINE TO INDICATE THE LIMITATION OF ACTIVITY.

RAYNAUD'S DOES VERY SEVERE

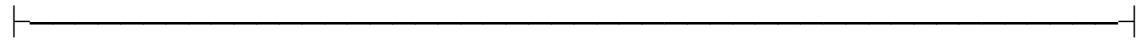
NOT LIMIT ACTIVITIES LIMITATION



0 100

IN THE PAST WEEK, how much have your finger ulcers interfered with your daily activities?
PLACE A MARK ON THE LINE TO INDICATE THE LIMITATION OF ACTIVITY.

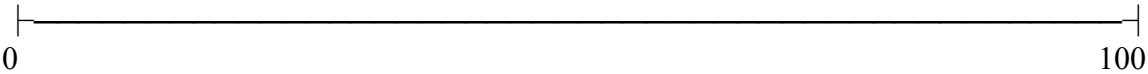
FINGER ULCERS DO NOT LIMIT ACTIVITIES VERY SEVERE LIMITATION



0 100

Overall, considering how much pain, discomfort, limitations in your daily life and other changes in your body and life, how severe would you rate your disease today?
PLACE A MARK ON THE LINE TO INDICATE THE LIMITATION OF ACTIVITY.

NO DISEASE VERY SEVERE LIMITATION



0 100

_____ _____

Patient Signature Date

Appendix C. Cochin Hand Functional Scale

Answers to the questions
0 = yes, without difficulty
1 = yes, with a little difficulty
2 = yes, with some difficulty
3 = yes, with much difficulty
4 = nearly impossible to do
5 = impossible to do

In the kitchen

1. Can you hold a bowl?
2. Can you grasp a full bottle and raise it?
3. Can you hold a plate full of food?
4. Can you pour liquid from a bottle into a glass?
5. Can you unscrew the lid from a jar that has been opened before?
6. Can you cut meat with a knife?
7. Can you prick things well with a fork?
8. Can you peel fruit?

Dressing

9. Can you button your shirt?
10. Can you open and close a zip?

Hygiene

11. Can you squeeze a new tube of toothpaste?
12. Can you hold a toothbrush efficiently?

At the office

13. Can you write a short sentence with an ordinary pen?
14. Can you write a letter with an ordinary pen?

Other

15. Can you turn a round door knob?
16. Can you cut a piece of paper with scissors?
17. Can you pick up coins from a table top?
18. Can you turn a key in a lock?

_____ _____

Subject signature Date

Appendix D. Mawdsley Calcinosis Questionnaire

Patient Name or Reference_____
Current geographical location _____
Hemisphere/season_____
Month/Day _____

Part A.
1. a. How many calcinosis lesions (open or closed) do you ACTUALLY have today? _____
1. b. How many calcinosis do you FEEL that you have today? _____
2. a. How many digital ulcers do you have today? _____
2. b. How many of these digital ulcers do you think are related to calcinosis? ____

Part B.
In the past TWO WEEKS, what is the worst degree that…

1. Your Raynaud's has interfered with daily activities?

No Limitation — Maximum limitation/ Worst possible

0 ——— 10

2. Your DIGITAL ULCERS interfered with daily activities?

No Limitation — Maximum limitation/ Worst possible

0 ——— 10

3. You experienced PAIN from your calcinosis?

No Pain — Worst Possible Pain

0 ——— 10

4. You felt any areas of your calcinosis getting TIGHTER or having more pressure?

Not at all — Maximum

0 ——— 10

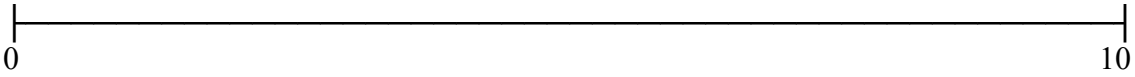
5. You felt any areas of your calcinosis GROWING under your skin?

Not at all — Maximum

0 ——— 10

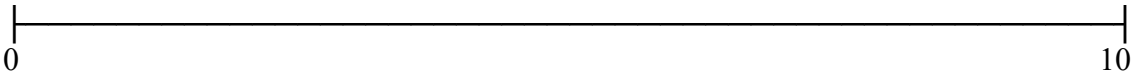
6. You felt any areas of your calcinosis THROBBING?

Not at all Maximum



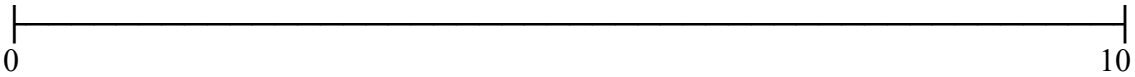
7. Your calcinosis has been TENDER to TOUCH?

Not at all Maximum



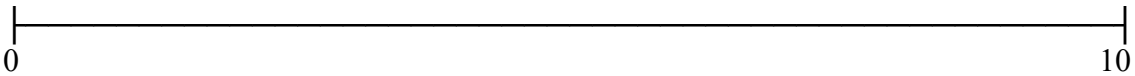
8. You felt the need to PROTECT areas of your calcinosis?

Not at all Maximum



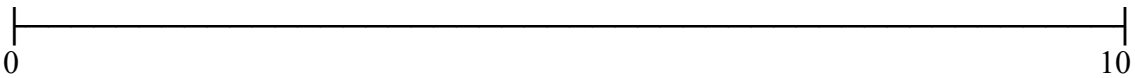
9. You have been FEARFUL or WORRIED that any of your calcinosis areas are INFECTED?

Not at all Maximum



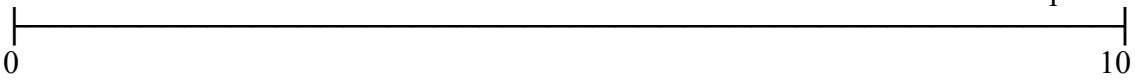
10. You have been worried that a calcinoisis wound MIGHT NOT HEAL?

Not at all Maximum



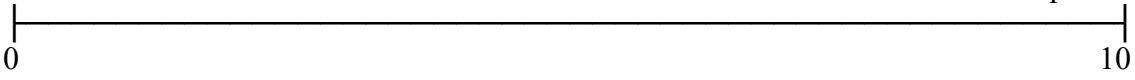
11. Your calcinosis interfered with ability to CARE FOR SELF?

No Limitation Maximum limitation/
Worst possible



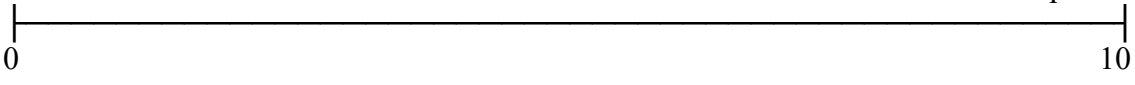
12. Your calcinosis interfered with your ability to USE YOUR HANDS?

No Limitation Maximum limitation/
Worst possible



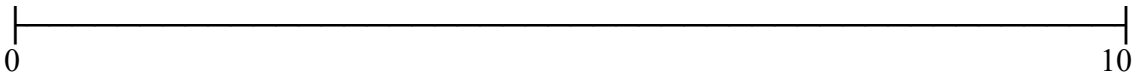
13. Your calcinosis interfered with WALKING?

No Limitation Maximum limitation/
Worst possible



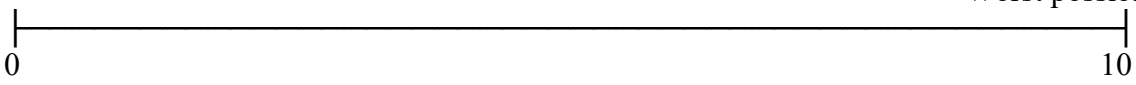
Test question: Your calcinosis interfered with your ability to USE YOUR HANDS or to WALK?

No Limitation Maximum limitation/
Worst possible



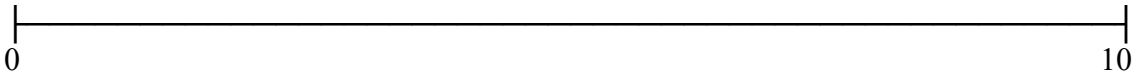
14. Your calcinosis interfered with your ABILITY TO WORK (paid or unpaid)

No Limitation — Maximum limitation/ Worst possible



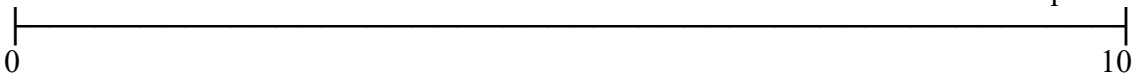
15. Your calcinosis made you FEEL DOWN, DEPRESSED or HOPELESS?

Not at all — Maximum



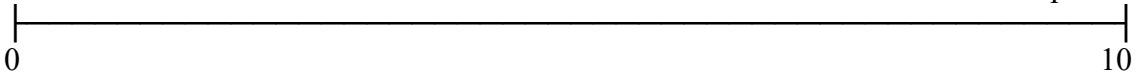
16. Your calcinosis interfered with your ability to enjoy and 'be there' for your FRIENDS and FAMILY?

No Limitation — Maximum limitation/ Worst possible



17. Your calcinosis interfered with your ability to enjoy RECREATIONAL ACTIVITIES (hobbies, sports, etc)?

No Limitation — Maximum limitation/ Worst possible



Appendix E. Medical Outcomes Study 36-Item Short Form Health Survey (SF-36)

Instructions for completing the questionnaire: Please answer every question. Some questions may look like others, but each one is different. Please take the time to read and answer each question carefully by filling in the bubble that best represents your response.

1. In general, would you say your health is:

- ❑ Excellent
- ❑ Very good
- ❑ Good
- ❑ Fair
- ❑ Poor

2. Compared to one year ago, how would you rate your health in general now?

- ❑ Much better now than a year ago
- ❑ Somewhat better now than a year ago
- ❑ About the same as one year ago
- ❑ Somewhat worse now than one year ago
- ❑ Much worse now than one year ago

3. The following items are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf?
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

c. Lifting or carrying groceries.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

d. Climbing several flights of stairs.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

e. Climbing one flight of stairs.
- ❑ Yes, limited a lot.
- ❑ Yes, limited a little.
- ❑ No, not limited at all.

f. Bending, kneeling or stooping.
❑ Yes, limited a lot.
❑ Yes, limited a little.
❑ No, not limited at all.

g. Walking more than one mile.
❑ Yes, limited a lot.
❑ Yes, limited a little.
❑ No, not limited at all.

h. Walking several blocks.
❑ Yes, limited a lot.
❑ Yes, limited a little.
❑ No, not limited at all.

i. Walking one block.
❑ Yes, limited a lot.
❑ Yes, limited a little.
❑ No, not limited at all.

j. Bathing or dressing yourself.
❑ Yes, limited a lot.
❑ Yes, limited a little.
❑ No, not limited at all.

4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

a. Cut down the amount of time you spent on work or other activities?
☐ Yes ☐ No

b. Accomplished less than you would like?
☐ Yes ☐ No

c. Were limited in the kind of work or other activities
☐ Yes ☐ No

d. Had difficulty performing the work or other activities (for example, it took extra time)
☐ Yes ☐ No

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

a. Cut down the amount of time you spent on work or other activities?
☐ Yes ☐ No

b. Accomplished less than you would like
☐ Yes ☐ No

c. Didn't do work or other activities as carefully as usual
☐ Yes ☐ No
6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbors, or groups?

- ❑ Not at all
- ❑ Slightly
- ❑ Moderately
- ❑ Quite a bit
- ❑ Extremely

7. How much bodily pain have you had during the past 4 weeks?

- ❑ Not at all
- ❑ Slightly
- ❑ Moderately
- ❑ Quite a bit
- ❑ Extremely

8.During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

- ❑ Not at all
- ❑ Slightly
- ❑ Moderately
- ❑ Quite a bit
- ❑ Extremely

9.These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks.

a. did you feel full of pep?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

b. have you been a very nervous person?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

c. have you felt so down in the dumps nothing could cheer you up?
- ❑ All of the time
- ❑ Most of the time

- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

d. have you felt calm and peaceful?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

e. did you have a lot of energy?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

f. have you felt downhearted and blue?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

g. did you feel worn out?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

h. have you been a happy person?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time
- ❑ None of the time

i. did you feel tired?
- ❑ All of the time
- ❑ Most of the time
- ❑ A good bit of the time
- ❑ Some of the time
- ❑ A little of the time

❑ None of the time

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

❑ All of the time
❑ Most of the time
❑ Some of the time
❑ A little of the time
❑ None of the time

11.How TRUE or FALSE is each of the following statements for you?

a. I seem to get sick a little easier than other people
❑ Definitely true
❑ Mostly true
❑ Don't know
❑ Mostly false
❑ Definitely false

b. I am as healthy as anybody I know
❑ Definitely true
❑ Mostly true
❑ Don't know
❑ Mostly false
❑ Definitely false

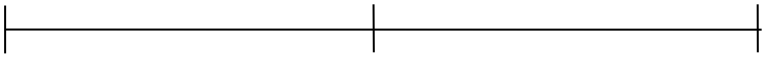
c. I expect my health to get worse
❑ Definitely true
❑ Mostly true
❑ Don't know
❑ Mostly false
❑ Definitely false

d. My health is excellent
❑ Definitely true
❑ Mostly true
❑ Don't know
❑ Mostly false
❑ Definitely false

Appendix F. Patient and physician calcinosis global assessment

Patient Calcinosis Global Assessment

Please rate the overall activity of your **calcinosis** today by placing a mark on the line below.



0 5 10

No evidence of calcinosis — Extremely active or severe calcinosis

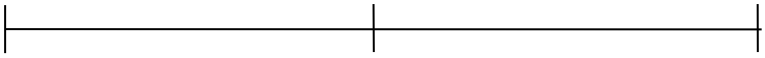
☐☐☐ cm

_____ _____

Patient Signature Date

Physician Calcinosis Global Assessment

Overall, considering how much pain, discomfort, limitations in your patient's daily life, and other changes in his/her body and life, how severe would you rate your patient's **calcinosis** today? Please place a mark on the line below.



0 5 10

No evidence of calcinosis — Extremely active or severe calcinosis

☐☐☐ cm

_____ _____

Physician Signature Date

Appendix G. Raynaud condition score

RAYNAUD'S CONDITION SCORE (RCS): The Day's RCS or Raynaud's Condition Score is the patient's rating of how much difficulty you had with your Raynaud's **TODAY**. Consider how many attacks you had and how long they lasted. Consider how much pain, numbness, or other symptoms the Raynaud's caused in your fingers (including painful sores) and how much the Raynaud's alone affected the use of your hands today.

No Difficulty Extreme Difficulty

|——————————————————|

0 10

Input number from 1.0 to 10.0, in increments of 0.10

Appendix H. Modified Rodnan Skin Score (MRSS)

SCLERODERMA Score from 0 (normal thickness) to 3 (severe thickening)		
Face		
Anterior chest		
Abdomen		
	LEFT	RIGHT
Fingers		
Dorsum of hands		
Forearms		
Upper arms		
Thighs		
Lower legs		
Dorsum of feet		
TOTAL:		

_____ _____

Evaluator's Signature Date

Appendix I. Skin tissue sample collection instructions

Snap freezing, or flash freezing, is the process by which samples are lowered to temperatures below -80°C very rapidly liquid nitrogen. Snap freezing achieves the same endpoint as slow rate-controlled freezing, but at a much faster rate. This procedure is intended to ensure that tissue samples collected will be frozen in a safe and efficient manner while eliminating the risks of contamination and variation in molecular integrity. The following protocol describes a general procedure for snap-freezing. For nonstandard sample types, always refer to the tissue – specific protocols.

This procedure describes the sample collection, storage and transport of tissue collected for RNA testing.

Materials

A. CryoStor™CS10 freezing medium (Stem Cell Technologies Cat#07930)
B. **2ml** CryoVials or **2ml** sample tube
C. 4mm Acu-Punch biopsy tool
D. Disposable gloves
E. Isopropanol filled freezing vessel
F. -80°C Freezer
G. Liquid nitrogen
H. Liquid nitrogen canister
I. Cutting board/surface
J. Long metal forceps
K. Scalpel/razor blades
L. Shipping Log
M. Sample Storage Box

Safety implications

Adhere to proper use of PPE. General precautions for biohazardous materials are to be observed. All disposable supplies should be disposed per the institution's waste protocol.

Procedure

Collecting Biopsy

1. Properly label (1) **2ml** sample tube AND (1) Cryo-tube with **1ml** CryoStor™ CS10 freezing medium.
2. Using a 4mm Acu-Punch biopsy tool , collect tissue from the non-dominant arm or upper buttock area.

For ATAC-seq

3. Dissect a small sample from the biopsy (approximately 1 cubic mm).

4. Place in prelabeled Cryo-tube with **1ml** CryoStor™
5. Freeze by placing in an isopropanol filled freezing vessel
6. Place vessel in -80°C freezer*.
   *Goal is 1 degree decrement per hour
7. Store at -80ºC for short term storage or liquid nitrogen for long term storage.

For RNA extraction

8. Place remaining biopsy in prelabeled **2ml** sample tube.
9. Snap freeze the biopsy by holding sample tube with long metal forceps and immersing approximately halfway down the height of the liquid nitrogen in the canister for at least 30 seconds.
10. Transfer sample tube to -80°C freezer for storage.
11. It is preferable to ship the samples on dry ice as soon as possible after collection.

Appendix J. Biomarkers list

Ang-1
Ang-2
MMP-2, MMP-9
NT-proBNP, PIGF
VEGFR1, sRAGE
GLUT-1
Ficolin-1
MBL
H62 plex (includes VEGF, PDGFBB, bFGF,IL-13, HGF, IL-6, IL-1β, IL-2, IL-4, IL-5, IL-8, IL-10, IL-12, IL-13,[TNF]-α,[IFN]-γ)

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