CLINICAL RESEARCH PROJECT **Protocol # 12-H-0110**

Protocol Title: *An Open-label, Non-randomized, Single-arm Pilot Study to Evaluate the Effectiveness of Etidronate Treatment for Arterial Calcifications due to Deficiency in CD73 (ACDC)*

Short Title: *Etidronate for ACDC*

Keywords: NT5E, Bisphosphonates, Vascular calcifications, Joint capsule calcifications, inherited disease

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Subjects of study:	**Number**	**Sex**	**Age range**
	20	M, F	18-80

Sponsor: NHLBI Office of the Clinical Director

Sponsor's Authorized Representative: Alessandra Brofferio, MD

Project involves ionizing radiation:	Yes
Off-site project:	No
Multi-Institutional project:	No
DSMB involvement:	No
Tech Transfer: CRADA	No
MTA	Yes

Investigational Device Exemption (IDE)
The test article is a non-significant risk medical device eligible for abbreviated IDE requirements of 21 CFR 812.2(b). No IDE license number is applicable.

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1. Précis

We have recently identified a novel genetic disease affecting nine known adults in whom de novo vascular calcifications develop in the lower extremity arteries and juxta-articular joint capsules of the fingers, wrists, ankles and feet. This rare disease results from bi-allelic mutations in the gene ecto-5-prime-nucleotidase (NT5E), encoding the CD73 protein. CD73, an enzyme involved in the extracellular ATP metabolic pathway, converts extracellular AMP to adenosine and inorganic phosphate. The clinical symptoms of this rare disease, termed ACDC (Arterial Calcifications due to Deficiency in CD73), include claudication of the calves, thighs, and buttocks, chronic ischemic pain of the feet at rest with threat of potential limb loss, and debilitating rheumatoid pain in the wrists and hands. Radiological and histological evaluations do not resemble classic atherosclerotic vascular calcification, since the calcification and dysplasia in ACDC occur in the medial portion of the arterial blood vessel wall. Data from patient-specific cell lines indicate increased activity of tissue non-specific alkaline phosphatase (TNAP), a key mediator of pathological ectopic tissue calcification, and thus reveals a potential therapeutic target.

To date, no effective therapy exists for ACDC patients. However, since bisphosphonates are potent competitive inhibitors of TNAP activity and are widely used to modulate bone metabolism, they may beneficially alter vascular calcification. In addition, our preliminary in vitro studies demonstrate the effectiveness of etidronate, a nitrogen-containing bisphosphonate, in lowering TNAP activity in cells isolated from ACDC patients. Etidronate, and bisphophonates in general, have proven safe and well tolerated by most patients.

This protocol provides for the administration of etidronate to ACDC patients, for whom no alternative treatment is available. Patients will be examined at the NIH Clinical Center bi-annually for 3 years. The primary objective of this clinical study is to test the effectiveness of etidronate in attenuating the progression of lower extremity arterial calcification and vascular blood flow based on CT calcium score and Ankle–brachial index (ABI).

2. Objectives

2.1 Primary Objective

This study will examine the effectiveness of an oral bisphosphonate, etidronate, in attenuating the progression of lower extremity arterial calcification and improved vascular blood flow in subjects diagnosed with the rare disease, ACDC, over a three-year period, based on CT calcium score and Ankle- brachial index (ABI).

2.2 Secondary Objectives

The secondary objectives include:

- Functional improvement in treadmill test results
- Decrease in hand pain based on Rheumatoid Arthritis assessment tool
- Changes in hand joint calcification based on hand x-ray
- Analysis of the composition of collected surgical tissue with calcification/inflammation

3. Background

3.1 Pathophysiology

We recently identified the molecular and enzymatic basis of a novel disease, ACDC, in nine patients with three different mutations in the NT5E gene.[1] Individuals with ACDC form de novo vascular calcifications with chondro-osseous histology in the lower extremity arteries. The affected arteries are tortuous, and calcification appears to be localized to the medial layer (Fig. 2). Interestingly, other vascular beds appear unaffected. Definitive diagnosis of ACDC is based on genetic studies demonstrating mutations in NT5E. Preliminary data based on quantitative CT scans conducted at the NIH Clinical Center under NHLBI protocols 10-H-0126 Cardiovascular Disease Discovery Protocol or 02-H-0050 Technical Development of Cardiovascular Magnetic Resonance Imaging, or the NHGRI 76-HG-0238 Undiagnosed Disease Protocol from 3 subjects shows a rate of lower extremity calcium progression averaging 9% per year (range 6.5–13.8%) (unpublished observation).

Deficiency of CD73 impairs production of adenosine, and the lack of extracellular adenosine signaling in patients with CD73 deficiency fosters calcification in lower extremity arteries. We found that lack of adenosine signaling in ACDC patient fibroblasts resulted in increased expression and activity of tissue non-specific alkaline phosphatase (TNAP). TNAP is a key enzyme in bone mineralization and is highly expressed by osteoblasts. CD73-deficient fibroblasts exhibit increased TNAP mRNA expression both at baseline and upon exposure to medium promoting osteogenic differentiation. Genetic rescue or addition of adenosine reduced TNAP expression in CD73-deficient cells to levels found in control cells, suggesting that adenosine elicits its effects via binding to cognate cell surface receptors and not by acting directly on the TNAP enzyme. Adenosine receptor agonists are not approved for use in human patients, mitigating against their potential therapeutic use. However, bisphosphonates, a class of drugs known to inhibit the activity of the enzyme TNAP, are widely used to treat disorders of bone metabolism.

3.2 Treatment Options

Due to their profound and powerful effects in altering bone physiology, bisphosphonates represent a major class of drugs used for the treatment of bone diseases. Bisphosphonates inhibit bone resorption by selectively adhering to mineral surfaces and interfering with bone-resorbing osteoclasts.[2] They are chemically stable analogs of a naturally occurring pyrophosphate characterized by the presence of a P-C- P bond. The P-C-P structure facilitates conformational variation, either by changing the two lateral chains (R1 or R2) or by esterifying the phosphate groups. The ability of bisphosphonates to bind hydroxyapatite crystals and prevent crystal growth and dissolution is enhanced when the R1 side change comprises a hydroxyl group,[3] thereby increasing its affinity for calcium and, therefore, bone mineral.[4] For maximal potency, the nitrogen atom in the R2 side chain must be a critical distance away from the P-C-P group and in a specific configuration. Bisphosphonates can be classified into 2 major groups: non-nitrogen- containing and nitrogen-containing. The former group, such as clodronate and etidronate (Didronel), can be metabolically incorporated into nonhydrolyzable forms of ATP by inhibiting ATP-dependent intracellular enzymes. The more potent, nitrogen-containing type of bisphosphonates, such as pamidronate (Aredia), alendronate (Fosamax), risedronate (Actonel), ibandronate (Boniva), and zolendronate (Zometa), inhibit farnesyl pyrophosphate synthase, thereby preventing the biosynthesis of isoprenoid compounds essential for the posttranslational modification of small guanosine trisphosphate (GTP)-binding proteins such as Rabs, Rhos, and Racs.[5]

In animals, bisphosphonates, administered orally or parenterally, efficiently inhibit ectopic calcification in vivo by preventing experimentally induced calcification of soft tissues. They decrease not only mineral deposits but also the accumulation of cholesterol, elastin, and collagen in the arteries. Etidronate administered subcutaneously inhibits calcification of aortic valves when implanted subcutaneously in rats.[6] The vascular calcification produced in rats with adenine-induced renal failure by feeding high phosphate chow was prevented by daily treatment with either etidronate or pamidronate, the latter being 100-fold more potent. In addition, aortic calcification and bone formation were reduced, and the calcification of pre-calcified vessels was arrested, but not reversed.[7]

Bisphosphonates are used to treat several bone disorders, including osteoporosis in adults, Paget's disease, bone metastases, postmenopausal women with vertebral compression fractures, elderly men with non-traumatic fractures, and pediatric bone diseases, such as osteogenesis imperfecta. In humans, etidronate was the initial bisphosphonate to be used for fibrodysplasia ossificans progressiva and Paget's disease, the first clinical disorder in which a dose-dependent inhibition of bone resorption could be demonstrated. In pediatrics, pamidronate has proven remarkably effective in increasing bone in children with the inherited brittle bone disorder, osteogenesis imperfecta. The continued improvement in bisphosphonate formulations fosters enhanced suppression of disease progression. Bisphosphonates accumulate in bone, making it important to evaluate the effects of long-term treatment. From a clinical standpoint, it is promising that the inhibition of bone resorption reaches a steady-state level and is then progressively lowered when bisphosphonates are given continuously. There seems to be no progression of antiresorptive effects with time, suggesting that a bisphosphonate remains active as long as it stays buried in bone.[8] Within the therapeutic dose range, there appears little risk of a continuous and progressive decrease in bone turnover that would lead to an increase in bone fragility. In vitro, bisphosphonates can, in a dose-dependent fashion, inhibit the formation of osteoclast-like cells in long-term cultures of human bone marrow cells, suggesting that long-term use of bisphosphonates could reduce the production of bone-resorbing cells.[2]

In a large longitudinal cohort study (MESA; the Multi-Ethnic Study of Atherosclerosis, 3,710 women assessed), investigators evaluated the relationship between bisphosphonates and cardiovascular calcifications.[9] This study reported a decreased prevalence of cardiovascular calcification in older subjects (>65 years of age) and more prevalent cardiovascular calcification in younger ones (<65 years of age). However, results from younger patients are surprising. It is still unclear the extent to which bisphosphonate treatment in younger patients correlates with more advanced osteoporosis, which is linked to an increased risk of developing cardiovascular diseases. Although the vascular calcifications in ACDC are clearly distinct, there may be some degree of commonality between the two.

In a recent study the effect of a combination therapy with atorvastatin and etidronate on aortic atherosclerotic plaque formation and composition was evaluated by MRI.[10] A total of 87 patients with hypercholesterolemia were treated either with atorvastatin alone or in combination with etidronate. The combination treatment significantly reduces both thoracic and abdominal aortic plaques whereas treatment with atorvastatin alone was less effective.

There are several smaller clinical studies evaluating the impact of bisphosphonates (mostly etidronate) on vascular calcifications in long-term hemodialysis patients. Vascular calcifications in these patients are more complex than calcifications in atherosclerotic lesions and involve intimal calcification

(atherosclerotic type) and medial calcifications (Monkeberg sclerosis type). In these trials on uremic patients, bisphosphonate treatment is associated with a decrease in vascular calcifications.[11,12]

Calciphylaxis is a rare and often lethal complication in patients with end-stage kidney disease. It is characterized by rapid progressive systemic medial calcification in arteries, and leads to vascular thrombosis and skin necrosis. There are several case reports indicating that bisphosphonates were successful in treating calciphylaxis.[13]

Fahr's syndrome or Idiopathic Basal Ganglia Calcification is a rare inherited neurological disorder characterized by calcifications of the basal ganglia, and cerebral cortex. This progressive neurological disorder results in disability and death. Histological evaluations showed severe medial calcifications of midsize to small arteries. There have been case reports where bisphosphonate treatment improved neurological dysfunctions.[14]

Generalized Arterial Calcification of Infancy (GACI) is a severe form of vascular calcification localized within the medial vessel wall in all major arteries throughout the body.[15] GACI is caused by inactivating mutations in the ENPP1 gene that is directly upstream of CD73 in the extracellular ATP metabolism pathway (Fig. 1). GACI is associated with a high mortality rate owing to the development of arterial calcification with subsequent detrimental complications of the cardiovascular system in early infancy.
Inactivating mutations of ENPP1 lead to decreased levels of inorganic pyrophosphate that can be hydrolyzed by tissue non-specific alkaline phosphatase (TNAP), a key enzyme whose activity is dysregulated in ACDC. Bisphosphonates decrease mortality in GACI. One infant was started on bisphosphonates on the seventh day of life, and the arterial calcifications completely resolved by 3 months of age. The mechanisms by which bisphosphonates influence GACI are uncertain but they might act at various levels to alter calcium balance[16,17]. The mechanistic relationship and histopathological appearance suggest that GACI and ACDC involve overlapping signaling pathways.

4. Investigational Plan

4.1 Subject Population Selection and Eligibility

Study subjects must meet diagnostic criteria for ACDC. Enrolled subjects must be able to travel to the NIH for visits. Although only 9 patients have been diagnosed with ACDC so far, the accrual ceiling is set at 20 participants to allow for the enrollment of newly diagnosed ACDC patients. Additional subjects may be recruited with the help of referring physicians who report newly diagnosed cases of ACDC. Information about this study will be posted on ClinicalTrials.gov and the NHLBI recruitment website.
Currently, six subjects from the U.S. and one subject from Italy are available for screening and enrollment in the trial, which will take place in the United States at the NIH Clinical Center in Bethesda, Maryland for all subjects. This is proposed as a ten-year study but may be extended with IRB approval due to recruitment of newly diagnosed ACDC patients.

4.2 Inclusion and Exclusion Criteria

Inclusion Criteria

Inclusion and exclusion criteria are to be assessed at Screening and Baseline prior to starting study drug. Each subject must meet the following criteria to be enrolled in this study: Subjects must be diagnosed with ACDC based on genetic tests confirming mutation(s) in NT5E and evidence of lower extremity arterial

calcifications.

- Either gender and any ethnic background or race
- Age 18-80 years
- Willingness and legal ability to give and sign informed study consent
- Willingness to travel to NIH and local sites for scheduled protocol studies and treatment

Exclusion Criteria

Subjects who meet any of the following criteria will be excluded from the study:

- Subjects not diagnosed with ACDC
- Subjects <18 or >80
- Subjects who are unable or unwilling to sign an informed consent
- Severe renal impairment [estimated creatinine clearance/eGFR of < 30ml/min (calculated using CKD-EPI equation)]
- Longstanding diabetes mellitus (more than 10 years)
- Known abnormality of the esophagus that would interfere with the passage of the drug
- Known sensitivity to etidronate
- Pregnancy
- Any other medical or social condition that, in the opinion of the Principal Investigator, might put the subject at risk of harm during the study or might adversely affect the interpretation of the study data.

4.3 Study Design and Methods

A total of nine patients have been diagnosed with the newly described, rare disease, ACDC.[1] The small patient population precludes us from conducting a larger scale clinical trial. There are currently no treatment options available for ACDC patients. However, our preliminary in vitro studies using patient fibroblasts suggest that bisphosphonate administration is a potential therapeutic approach for stopping the progression of lower extremity vascular calcification, because of its potent effects in inhibiting TNAP activity.

This is an open-label, non-randomized, single-arm pilot study to evaluate the effectiveness of etidronate treatment in attenuating the progression of lower extremity arterial calcification in patients diagnosed with ACDC. Currently, there are seven patients identified (five patients from one family; two patients from two separate families). Patients newly diagnosed with ACDC may also be enrolled if all study criteria are met. Subjects living in the U.S. will be followed and evaluated over a 3-year treatment period, with scheduled visits to the clinic approximately every 6 months (see table 2. Schedule of Events).

Subjects living abroad will be followed and evaluated with scheduled visits every year, to mitigate the travel burden in this sick population. Please see marked sections of the schedule of events that applies for those subjects being seen yearly. The study will be conducted in three phases described in Section 4.4 through 4.6.

With Amendment T, the last and only study subject still on treatment will only receive 11 out of 12 cycles of etidronate due to drug discontinuation by the manufacturer, Mylan/Teva Pharmaceuticals and last lot of study drug expiring on December 31, 2019. Follow-up and testing will be performed otherwise as per protocol schedule of events.

At this time, as we will not have access to additional drug, this study is now in follow-up only.

Potential and enrolled participants may have had evaluation procedures conducted for clinical purposes outside of the NIH or at NIH under the following NHLBI and NHGRI protocols:

- 10-H-0126 *Cardiovascular Disease Discovery Protocol*
- 02-H-0050 *Technical Development of Cardiovascular Magnetic Resonance Imaging*
- 18-H-0108 *Vascular Disease Discovery*
- 95-H-0047 *Diagnosis and Treatment of Patients with Heart and Vascular Disease (Training Protocol)*
- 76-HG-0238 *Undiagnosed Disease Protocol*

Prior, concurrent and subsequent results from procedures performed under these protocols or outside the NIH for clinical purposes may be used to supplement the clinical evaluations and research data analysis performed under this protocol to address primary and secondary objectives.

All tests will follow NIH Clinical Center policies. Clinical consent forms for individual tests will be obtained in addition to the study protocol consent. All the tests are performed to monitor disease progression and drug effects.

4.4 Pre-Treatment Phase

Note: Due to the nature of the disease being studied, and the potentially lengthy pre-treatment phase of this study, it is possible that patients already enrolled in the pre-treatment phase of the study may need to undergo clinically indicated interventions related to their disease course. In these situations, these clinical procedures and laboratory tests may be used in the data analysis for this study. Any cost resulting from clinically indicated procedures or preventative action taken prior to beginning the study treatment will be the responsibility of the subject and/or the subject's insurance. The pre-treatment phase can take between one and five years to complete.

4.4.1 Establishing Baseline Rate of Disease Progression

Rate of disease progression will be established using two CT calcium score studies and two ABI studies. CT calcium scores will be observed at least 1 year (but no more than 5 years) apart, and ABI studies will be observed at least 6 months (but no more than 5 years) apart. Potential participants may have had CT calcium score and ABI conducted at NIH under the protocols listed in section 4.3 above or, for clinical purposes outside of the NIH. If these CT calcium scores and/ or ABI are comparable to those conducted for this protocol, these tests can be used to establish rate of disease progression. The quality of this CT scans will be established by Marcus Y. Chen, M.D. In these cases, the first studies for the CT calcium score and ABI to establish rate of disease progression may not need to be repeated during the pre-treatment phase. If these studies have not been done on other NIH or NHLBI protocols, the first studies for both the CT and the ABI may be collected during a separate pre-treatment evaluation visit to occur within one year prior to the initiation of treatment. To ensure consistent measurement of calcification for this population, all CT calcium scoring for ACDC patients and potential ACDC patients is done by Marcus Chen, M.D., an associate investigator on this protocol.

4.4.2 Pre-Treatment Evaluations

- Any available Hand X-rays

Within 2 years of start of treatment:

- Coronary CT

Within 1 year of start of treatment:

- DEXA scan

Within 3 months of start of treatment:

- Treadmill test
- MRI
- CAVI (optional)

Within 1 month of start of treatment:

- Complete history and physical examination
- Laboratory tests listed in Table 1 (except for the pregnancy test)
- Screening 12-lead EKG
- Dental Consult
- Cardiovascular Consult
- Questionnaires (Appendices 1 and 2)

No more than 48 hours prior to the start of treatment:

- Women of childbearing potential will have a urine or serum hCG pregnancy test

In the event of a treatment delay for clinically indicated interventions resulting in testing falling out of the specified window, the tests will be repeated. As a consequence, the pre-treatment phase may result in multiple visits and will only be completed with the initiation of drug. The first day the patient receives drug will be considered Day 0 of the Treatment Phase (see Section 4.5)

4.5 Treatment Phase (Clinical Monitoring and Intervention)

The Treatment Phase consists of twelve Drug Cycles [approximately 3 months each, ± 2 weeks (with exception for acceptable drug interruptions described below)]. Participants living in the U.S will have one scheduled visit to the NIH Clinical Center every 6 months. Participants living abroad will have one scheduled visit to the NIH once a year.

Etidronate will be administered at 20mg/kg daily x 14 days, followed by 10 weeks off study drug (12 weeks = one cycle). Participants living in the U.S will return to the NIH Clinical Center for evaluation every 6 months. Participants living abroad will return for evaluation every 12 months. Subjects may receive up to 12 cycles of etidronate.

In instances where subjects experience gastrointestinal discomfort (i.e. diarrhea, nausea), etidronate may be administered as a divided daily dose (10mg/kg twice daily x 14 days). Dividing the daily dose often alleviates gastrointestinal complaints that occur in 2 to 3 out of 10 patients taking a 10 to 20mg/kg/day dose of Etidronate.[21]

Drug interruptions: Unscheduled, clinically indicated treatment interruptions will be allowed for up to 1 year. Subjects will continue to be monitored during the drug interruption. All visits for the treatment phase will be completed regardless of drug interruption; therefore, the treatment phase may take longer than 3 years to complete if drug interruption occurs.

Participants who have a drug interruption longer than 1 year will require re-establishment of baseline rate of progression to begin the treatment phase again.

If a subject misses a dose of the study drug, the subject will be instructed to add a day to the 14- d a y drug cycle for each missed day to take the missed dose(s) of study drug. At least a 7-week drug-free interval must be maintained between the drug cycles. If a subject is not able to take all missed doses 7 weeks prior to the next drug cycle, he/she will be instructed not to take the missed doses past the 7-week cutoff and to resume the drug schedule at the next schedule drug cycle.

Unscheduled visits may occur during the treatment phase. During these unscheduled visits, other appropriate clinical tests and procedures will be utilized as indicated to manage complications related to the primary disease and to stabilize the clinical condition. Additional visits or extended course visits to the NIH Clinical Center may be necessary to manage disease related complications.
If a subject undergoes lower extremity bypass, amputation, or other clinical intervention, during the study, the bypass/intervention limb, or remaining limb in the case of an amputation, will still be monitored.
However, only the data collected on the remaining/non-intervention limb will be used to assess the effectiveness of the study drug.

Continued communication will be maintained with subjects' primary physician throughout their participation in the study.

4.6 Medical and Surgical History and Physical Examination

Medical history (including medication history involving over-the-counter and herbal medications), physical examination, and laboratory values will be completed on each subject for every admission while on the study. Vital signs and biophysical data (including blood pressure, heart rate, respiratory rate, temperature, height, and weight) will also be collected at each visit. At each study visit participants will complete a Rheumatoid Arthritis questionnaire (Appendix 1) to assess hand pain; and, the Duke Activity Index (Appendix 2) to measure functional capacity. These questionnaires will not be collected at follow-up visits.

Research test results and clinical data including, but not limited to, medical history, physical examination, ABI data, and MRI data will be evaluated (i.e. Cardiovascular Consultation) to determine if any clinically indicated interventions are necessary prior to the start of the treatment phase) and throughout the study.
The principal investigator and medical advisory investigator will review the results of the cardiovascular consultations to determine if there are any significant issues that will need to be closely monitored throughout treatment.

Subjects will also be sent for a dental consult where an oral exam will be performed and a panoramic x- ray will be taken to document his/her oral health prior to the first dose of the study drug, at Visit 3 and at the end of the treatment phase (Visit 6). The purpose of this consult is to monitor the participants' oral health, as etidronate has been associated with jaw osteonecrosis. The incidence of jaw osteonecrosis is associated with long-term (i.e. greater than 3 years) use of oral bisphosphonates[18]. It is not anticipated that participants will development jaw osteonecrosis within the treatment period of 3 years for this study.
However, the principal investigator and medical advisory investigator will review the results of the dental exam to determine if there are any significant dental issues that will need to be closely monitored throughout treatment. Prior to treatment with the study drug, incidental findings from the dental consultation requiring intervention will be sent to the subject's designated dentist for treatment prior to beginning the study drug. Any cost resulting from clinically indicated procedures or preventative action taken prior to beginning the study treatment, will be the responsibility of the subject and/or the subjects insurance. Any dental problems that arise during the study treatment phase that are considered likely attributable to the study drug and will be paid for by NIH. We may request that participants provide NIH with a record of dental examinations or treatments from the subjects' designated dentist during the course of this protocol.

4.7 Laboratory Tests and Procedures

Blood and urine specimens for the measurement and evaluation of serum chemistries will be collected at each site

visit. Clinical laboratory procedures may include those presented in Table 1.

We may also perform 24-hour urine collection to determine calcium and phosphate content of urine. Any laboratory test result that the Investigator considers clinically significant will be repeated at the discretion of the investigator to rule out laboratory error. For tests where a persistent abnormality is considered to be drug related, repeat analyses will be performed until the cause is determined and either a return to normality occurs or the investigator deems the abnormality to be of no clinical significance. Any values judged by the investigator to represent a clinically significant, abnormal change from baseline during the treatment phase will be recorded as an adverse event. Per Clinical Center policy, no more than 550 mL of blood will be collected over an 8-week period.

Table 1. List of Laboratory Tests

Hematology: • Complete CBC and Differential **Urine for:** • Phosphorus - Inorganic • Calcium • Urinalysis	**Serum Chemistry:** Chem 14 • Acute Care Panel • Mineral Panel • Hepatic Panel • Total Protein • CK • Uric Acid • LD • Alkaline phosphatase (bone specific/heat specific) • Lipid panel • C-reactive protein (CRP) • Fibrinogen • Human chorionic gonadotropin (hCG) (for females of childbearing potential) • activated Partial thromboplastin time (aPTT) • PT/INR • Research blood, for storage for studies related to the subject's disorder • Parathyroid hormone (PTH) • 25-hydroxyvitamin D levels (Vitamin D) level in serum

Research Blood: Serum and plasma will be collected per visit (keeping blood draw volume limits as per NIH CC policy), stored at - 80C and analyzed when sufficient samples are accumulated. We will screen research blood for known marker associated with vascular calcification like, MGP, Osteopontin, RankL, BMPs, Fetuin among others.

4.8 Study Procedures

Note: Study procedures will be performed following the Schedule of Events Table 2 at the end of this section.

Surface Electrocardiogram (EKG): A surface EKG will be recorded to help exclude cardiac abnormalities in subjects with vascular calcifications. The EKG will take approximately 5 to 10 minutes.

Treadmill Test: A symptom-limited treadmill progressive test (Gardner Protocol) with gradual increase in grade will be performed in accordance with standard practice to evaluation exercise capacity and pain on exertion in subjects with decreased peripheral arterial perfusion. Briefly, the test will use a constant

speed of 2 mph and gradual increase in grade of 2% every 2 minutes beginning at 0% grade. Distance to onset of pain and to maximal pain will be recorded. Lower extremity arterial Doppler pressure will be measured immediately post exercise.

Ankle-brachial index measurements: The ABI is performed to investigate the functional consequences of abnormalities of vascular anatomy. Systolic blood pressure is measured using Doppler ultrasound on both brachial, posterior tibial, and dorsalis pedis arteries using a blood pressure cuff inflated over the brachial or gastrocnemius muscles. Measurements are obtained during resting steady-state conditions in both limbs, and immediately post exercise for 26 minutes or until lower extremity arterial pressure returns to baseline. The ABI for each limb represents the ratio of the highest arterial pulse pressure for each limb to the highest brachial pressure of the two limbs.

Cardiovascular and Lower Extremity CT: Calcium scores will be obtained from non-contrast computed tomography (CT) scans to evaluate peripheral arterial calcifications. Imaging procedures may include scout images of the body in the posterior-anterior and lateral directions, non-contrast images of the heart, and non-contrast images of the lower extremity.

After the CT data have been acquired and stored, FDA-approved software will 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. The reconstructed images may be further processed to extract quantitative data with respect to the pathophysiology involved and/or to evaluate the images themselves. Data and images may be stored for future evaluation and additional processing. Coronary and lower extremity calcium scores will be determined according to Agatston[19].

MRI: Exploratory MRI studies may be employed to evaluate the lumen of the artery and degree of collateral vessels, given that the high degree of calcification is precluding that evaluation by CT. Study- specific MRI scans will be performed using an FDA-approved MRI scanner. Imaging procedures will include localizer scans, two-dimensional studies of cardiovascular anatomy and function, contrast- enhanced cardiovascular MRI studies, and three-dimensional angiograms. The exact examination performed depends on the individual patient requirements or clinical findings.

Gadolinium Contrast (optional): Subjects may receive an intravenous injection of gadolinium (Gd) chelate not to exceed 0.2 mmol/kg of Gd per bolus injection and 0.4 mmol/kg of Gd per examination. The injection will be made by hand or using an MRI compatible power injector. Gadolinium contrast is an extra-cellular agent, so the distribution volume is a measure of the fractional volume occupied by the vascular and interstitial space.

DEXA Scan: A dual x-ray absorptiometry (DEXA) scan will be performed to evaluate mineral bone density before initiation of bisphosphonates. The standard sites for DEXA for bone density determination are PA and lateral lumbar spine, Proximal Femur (Hip), and Forearm (1/3 radius). DEXA is a low radiation x-ray procedure. The radiation exposure is less than 1 mRem for the 3 tests combined, which is much less than daily background radiation.

Hand X-Ray: Standard x-rays of the hands for assessment of metacarpal joint calcification will be performed. The estimated radiation exposure is less than 1 mRem.

Cardio-Ankle Vascular Index (CAVI) (optional): CAVI is a novel indicator of arterial stiffness. This measurement will be obtained to assess initial stiffness of the arteries and changes during the treatment phase. The test is not invasive and consists of measurement of BP in the four extremities while monitoring ECG by

single lead and the heart sound.

Tissue otherwise discarded at the time of medically indicated surgery: Subjects who enter the protocol may require medically indicated surgery where diseased or redundant tissue at the surgical site would be extracted. Portions of the tissue/s may be submitted to the pathology laboratory at the request of the surgeon. If residual tissue is available that would otherwise be discarded as 'surgical-waste', the investigator, with fully informed consent from the subject, may request the tissue/s to study to enhance the understanding of the disease pathophysiology. Participants who undergo medically indicated surgical procedures will be asked to sign a separate Tissue Collection informed consent document.

Table 2. Schedule of Events

Evaluation	Pre-Treatment Evaluations* /Time 0	Treatment Evaluations						Follow-up Evaluations[8,#] (non-mandatory)
		Visit 1/6mo (±1mo)	Visit 2/12mo (±1mo)*	Visit 3/18mo (±1mo)	Visit 4/24mo (±1mo)*	Visit 5/30mo (±1mo)	Visit 6/36mo (±1mo)*	
Informed Consent	X							
I/E Criteria	X							
Demographics	X							
Dental Consult	X			X#			X	
Cardiovascular Consult	X	X	X	X	X	X	X	X
Medical History[2]	X	X	X	X	X	X	X	X
Questionnaires	X	X	X	X	X	X	X	
Vital Signs[3]	X	X	X	X	X	X	X	X
Physical Exam	X	X	X	X	X	X	X	X
Laboratory Tests	X	X#	X	X#	X	X#	X	X
Research Blood	X	X	X	X	X	X	X	X
Lipid Panel	X		X		X		X	X
CRP	X		X		X		X	X
Urine Test[7]	X	X#	X	X#	X	X#	X	X
Pregnancy Test[3]	X	X#	X	X#	X	X#	X	X
EKG	X	X	X	X	X	X	X	X
ABI	X[1]	X#	X	X#	X	X#	X	X
CAVI (optional)	X	X	X	X	X	X	X	X
Treadmill Test	X	X	X	X	X	X	X	X
MRI	X						X	X
CT lower extremities	X[1]		X		X		X	X
CT coronaries	X[4]						X	X
Hand X-Ray	X[9]		X		X		X	X
DEXA scan	X		X		X		X	X
Drug Accountability	X[6]	X	X	X	X	X	X	
AE Assessment[5]	X[6]	X	X	X	X	X	X	

1. Subjects' must have two CT scans of the lower extremities (at least 1 year apart, but no more than 5 years apart) and two ABI tests (at least 6 months apart, but no more than 5 years apart) to establish baseline rate of disease progression (see Section 4.4.1)
2. Include a review of previous/ongoing medications
3. Only females who are not diagnosed as postmenopausal or not of childbearing potential
4. If not performed within two years prior to involvement
5. Adverse Events will also be assessed during follow-up contact.
6. Drug Accountability and AE Assessment will only be necessary for the visit at which the subject in administered the study drug (Day 0).
7. Urine test may include 24-hour urine collection
8. Optional follow-up evaluations may take periodically as needed. All follow-up visits and procedure are optional.
9. Any available during life time Hand X-rays may be collected for comparison

Follow-up Evaluations/Procedures/Visits for subjects living abroad may be conducted in their county of residence

* Scheduled visits to the NIH Clinical Center for subjects living abroad.

4.9 Pharmaceuticals

Study Treatment

Product Name: Didronel (etidronate disodium)

Supply: Dispense in a tight, light-resistant container as defined in the USP using a child-resistant closure. Commercial supply will be received through the NIH Pharmacy. Drug will be supplied to subjects by the NIH Pharmacy every 3 to 6 months.

Product Description: Didronel tablets contain either 200 mg or 400 mg of etidronate disodium, the disodium salt of (1-hydroxyethylidene) diphosphonic acid, for oral administration. This compound, also known as EHDP, regulates bone metabolism. It is a white powder, highly soluble in water. The 400 mg tablets are white capsule-shaped tablets with ED 400 on one side and G on the other side.

Storage: Store at 25°C (77°F), excursions permitted to 15-30°C (59-86°F), in a dry place.

Route of Administration: Oral

Dose: 20mg/kg daily x 14 days, or 10mg/kg twice daily x 14 days, followed by 10 weeks off study drug (rounded to the nearest tablet size)

Toxicities: see section 8.4, risks of etidronate

Drug interactions: There have been isolated reports of patients experiencing increases in their prothrombin times when etidronate was added to warfarin therapy. Increased INR monitoring will be highly recommended during the initiation and all subsequent re-initiations of study drug throughout the study. If a patient is on warfarin during the treatment phase of the study, INR should be monitored at least weekly during the initiation of etidronate treatment until a stable INR is achieved. While the patient is on etidronate and warfarin, INR should be monitored at least monthly. If etidronate therapy is discontinued, INR should continue to be monitored weekly until a stable value is reached. Continued communication will be maintained with subjects' primary physician throughout etidronate therapy to ensure that he/she is monitored between visits to the NIH Clinical Center.
Results for INR monitoring will be requested from the primary physician but will not be used for research analysis.

Stability: Stable under normal conditions

Packaging and Labeling: See attached package insert[20].

Selection and Timing of Dose: When all entry criteria have been met and scheduled tests have been completed, the first dose of study drug (20mg/kg) will be taken by the subject under supervision of the study personnel while at the NIH Clinical Center. Dose will be rounded to nearest tablet size. All subjects will be assigned a 6-month drug schedule prior to their departure. Participants living abroad will be assigned a 12-month drug schedule prior to their departure.

Etidronate is prescribed on a cyclical drug schedule. For the purposes of this study, participants will take etidronate daily for two weeks (14 days), followed by 10 weeks off study drug (with exception for acceptable drug interruption described in section 4.5).[10] This drug schedule will occur four times yearly during the 6-

course treatment phase.

Dosing Instructions: To maximize absorption and clinical benefit, etidronate should be taken at least one hour prior to first food or drink (other than water) of the day. To facilitate delivery to the stomach and thus reduce potential for esophageal irritation, etidronate tablets should be swallowed whole with a full glass of plain water (6-8 oz) while the subject is standing or sitting in an upright position. Subjects should not lie down for one hour after taking etidronate. To maximize absorption, patients should avoid taking the following items within two hours of dosing:

- Food, especially food high in calcium, such as milk or milk products.
- Vitamins with mineral supplements or antacids which are high in metals such as calcium, iron, magnesium, or aluminum.

In instances where subjects experience gastrointestinal discomfort (i.e. diarrhea, nausea), etidronate may be administered as a divided daily dose (10mg/kg twice daily x 14 days). Dividing the daily dose often alleviates gastrointestinal complaints that occur in 2 to 3 out of 10 patients taking a 10 to 20mg/kg/day dose of etidronate.[21]

Once the study has ended, subjects who wish to continue taking etidronate will be responsible for the cost of the medication, and treatment will be the responsibility of their primary physician.

Treatment Compliance: Investigators carefully selected the study drug for optimal safety, compliance, and efficacy. Subjects will be prescribed and given the drug initially at the NIH Clinical Center. Subjects will be presented with a pre-determined dosing schedule and will be called to remind and/or verify that the drug has been taken. Follow-up phone contact may be made the week before the first dose day, again after the first week of the study drug, and the week following the end for the drug cycle to ensure subject compliance and to assess any adverse reactions to the study drug. At least two phone calls will be made per drug cycle to assess compliance with the drug schedule and monitor for adverse reactions. Records of follow-up contact to verify subject compliance with drug intake will also be documented in the individual research chart.

If a subject misses a dose of the study drug, the subject will be instructed to add a day to the 14-day drug cycle for each missed day to take the missed dose(s) of study drug. At least a 7 week drug-free interval must be maintained between the drug cycles. If a subject is not able to take all missed doses 7 weeks prior to the next drug cycle, he/she will be instructed not to take the missed doses past the 7-week cutoff and to resume the drug schedule at the next schedule drug cycle.

Follow-up phone calls may be placed as often as daily while the subject is on study drug if a subject needs assistance to remain compliant with the drug schedule. If subjects become unable or unwilling to continue treatment they will be removed from the study. The subjects will be instructed to return remaining drug, in original drug containers, to NIH at each applicable study visit for a drug count. Any missed doses of the study drug will be disposed of in a medical waste box. The study pharmacist or designee will be responsible for drug accountability and record of drug interruptions.

4.10 Off-label Use of Drugs

Medications will be used beyond the indications specified in the Prescriber Information. In addition to the study drug, the off-label uses apply to beta-blockers and calcium-channel blockers for slowing the resting

heart rate, and gadolinium-based contrast agent for imaging the heart. These off-label uses will comply with prevailing community standards. An IND is not required since the objective of this study is not to develop information about the product's safety or efficacy and these uses meet the requirements for exemption.

The use of these drugs for this protocol meets the requirements for exemption from the Investigational New Drug regulations, 21 CFR 312, specifically:

1. The investigational drug is lawfully marketed in the United States
2. The investigation is not intended to be reported to the FDA as a well-controlled study in support of a new indication for use of the drug product
3. The investigation is not intended to support a significant change in advertising to an existing lawfully marketed prescription drug product
4. The investigation does not involve a route of administration or dosage level or other factor that significantly increases the risks (or decreases the acceptability of the risks) associated with the use of the drug product.
5. The investigation will be conducted in compliance with the requirements for institutional review set forth in FDA regulations 21 CFR 56, and requirements for informed consent as set forth in FDA regulations 21 CFR 50
6. The investigation will be conducted in compliance with FDA regulations 21 CFR 312.7: Promotion and charging for investigational drugs.

4.11 Off-Treatment

After completing all 6 courses of a treatment subjects will be considered off treatment. Off-treatment date is the last day of the last dose of the study medication taken.

4.12 Follow-up Visits

Once subjects complete the full course of treatment, they may be asked to return to NIH periodically for non-mandatory follow-up visits based on patient availability During these visits, patients may receive any of the tests listed on the schedule of events, tailored on the basis of their symptoms and disease progression.

5. Biostatistical Considerations

5.1 Primary Endpoint and Analysis

This trial is designed to evaluate the effectiveness of etidronate in subjects diagnosed with ACDC. The primary endpoints will be CT calcium score and ankle-brachial Index (ABI). The planned analyses will include descriptive statistics and longitudinal trends in the CT calcium score and ABI. Percentage changes in CT calcium score and ABI will be compared between the pre-treatment phase baseline and treatment phase. The significance of the changes will be reported with the use of t-test or rank-based test as appropriate. Secondary endpoints include, treadmill test results (section 4.7), functional capacity measured by the Duke Activity Index (Appendix 2), and changes in hand pain based on Rheumatoid Arthritis questionnaire (Appendix 1).

Data analysis will include all data collected on 12 drug cycles and follow-up for the first 6 patients accrued, as well as the 11 drug cycles and follow-up for patient #7.

5.2 Sample Size

There are no CT calcium score data or ABI data on patients with ACDC in the published literature on which to base sample size calculations. The study will accrue up to 20 subjects (9 patients have been diagnosed so far). Per subject off study criteria has been discussed on section 7.4. No formal study level stopping rule will be imposed due to lack of effectiveness profile of the treatment and limited sample size.

6. Sample Collection, Storage, and Data Management

Samples will be de-identified prior to storage on the 5th floor of building 10 in laboratory for the principal investigator following current NIH sample storage guidelines. Access to research samples will be limited, using a locked freezer. Samples and data will be stored, using codes assigned by the investigators or their designee(s). Data will be kept on the NHLBI P:drive, accessible through password-protected computers. Only the members of the research team will have access to the samples and data.

All human subjects' personally identifiable information (PII), eligibility and consent verification will be recorded. Primary data obtained during the conduct of the protocol will be kept in secure network drives that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual participant.

At the completion of the protocol (termination), samples and data will either be destroyed, or after IRB approval, transferred to another existing protocol or a repository. Participants are provided the option (Standard informed consent document, section 14) to be contacted in the future should information be obtained about their diagnosis, treatment, related symptoms, or associated risks of their disorder from future research using their samples. Participants who agree to be contacted in the future will be asked to maintain communication with the research team to provide up-to-date contact information following the completion of this study.

End of study procedures: Data will be stored in locked cabinets and in password protected network servers until no longer of scientific value.

Loss or destruction of data: Should we become aware that a major breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

Data will not be sent outside NIH without IRB notification and an executed agreement. However, incidental findings requiring intervention from the dental consultation will be sent to the subject's designated dentist.

7. Data and Safety Monitoring

7.1 Monitoring

Accrual and safety data will be monitored by the Sponsor/Principal Investigator. The protocol will be continuously evaluated for any unusual or unpredicted complications with the aim of detecting and preventing unacceptable increase in morbidity and mortality over and above that anticipated from etidronate. Literature searches will be conducted at least annually to seek safety information (21 CFR 312.32(b)).

Prior to implementation of this study, the protocol and the proposed subject informed consent document will be reviewed and approved by a properly constituted IRB operating according to the 21 CFR 56. This

committee must approve all amendments to the protocol or informed consent document, and conduct continuing annual review so long as the protocol is open to accrual or follow up of subjects.

Quality assurance and control monitoring will be consistent with the NHLBI Division of Intramural Research Clinical Research Quality Assurance and Quality Control Policy.

Please refer to policy 801 for definitions.

7.2 Adverse Event Management

The principal investigator or designee will be responsible for assessing adverse events. Information on adverse events will be solicited from subjects through questions from study personnel and information volunteered by the subject. All AEs, regardless of severity, will be recorded, graded, assigned an attribution, verified, and followed until satisfactory resolution. Adverse events will be captured from the start of the first etidronate administration (day 0) until 30 days following the last dose of treatment.
Following this period, any observed or volunteered adverse events will NOT be reported or recorded unless they are greater than Grade 1 and deemed possibly, probably or definitely related to a procedure, test or screening examination that was administered as part of this protocol.

Adverse events will be attributed (unrelated, unlikely, possibly, probably or definitively) to study medication and/ or disease and/or procedure/testing. AEs will be graded by severity utilizing CTCAE version 4.0. A copy of the criteria can be downloaded at http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

7.3 NIH IRB, FDA, and CD reporting

Reports to the IRB: The PI or designee will refer to HRPP Policy 801 "Reporting Research Events" to determine IRB reporting requirements and timelines.

Reports to the CD: The PI or designee will refer to NHLBI DIR guidelines to determine CD reporting requirements and timelines.

Expedited Reporting: events requiring expedite reporting will be submitted to the IRB per Policy 801, "Reporting Research Events."

Reports to the IRB at the time of Continuing Review: The PI or designee will refer to HRPP Policy 801 " Reporting Research Events 'to determine IRB reporting requirements.

Reports to the FDA: The sponsor-representative or designee will refer to abbreviated IDE requirements of 21 CFR 812.2(b), to determine FDA reporting requirements and timelines.

7.4 Removal of Subjects from the Study

The investigator may withdraw a subject from the study for any of the following reasons:

- A serious or intolerable adverse event related to etidronate occurs

- A clinically significant change in a laboratory parameter related to etidronate occurs requiring drug discontinuation
- The sponsor investigator terminates the study
- The subject requests to be discontinued from the study
- Subject non-compliance
- A clinically significant change that requires a drug interruption longer than 1 year (2 courses).

8. Human Subjects Protection

8.1 Rationale for Subject Selection

Subjects of both genders will be considered for inclusion in this study. There will be no racial, ethnic, or gender discrimination. Cognitively impaired and institutionalized persons will not participate in this study. Criteria for exclusion or withdrawal from the study are based on the presence of other disease processes that may be harmful to the health of subjects.

8.2 Rationale for the Exclusion of Children

Subjects under 18 years of age will not be considered for inclusion in this protocol because the disease has not been described in children, and the safety and effectiveness of etidronate has not been established in pediatric subjects.

8.3 Rationale for the Exclusion of Pregnant Women

Pregnant women will not be eligible for this study. There are no adequate and well controlled studies of etidronate in pregnant women. Females of childbearing potential must not be pregnant or actively seeking pregnancy in order to participate in this study. Some form of contraception must be used by female subjects of childbearing potential while enrolled. Contraception use will be confirmed at time of enrollment and at subsequent visits.

8.4 Evaluation of Benefits and Risks/Discomforts

The research involves greater than minimal risk to subjects, with the prospect of direct benefit (45 CFR 46.102).

Subjects will receive close monitoring for disease progression. There is potential benefit on disease progression and reversal through drug treatment, and benefit secondary to treatment with decrease in lower extremity pain and increased mobility and exercise tolerance.

Etidronate: Etidronate may cause local irritation of the upper gastrointestinal mucosa. Subjects will be monitored after the first dose given at CC and at every course visit for dysphagia, odynophagia, retrosternal pain, new or worsening heartburn, new thigh or groin pain for atypical femoral fracture risk, and hypersensitivity reactions. Hypocalcemia, musculoskeletal pain, and osteonecrosis of the jaw have been reported. Most common adverse reactions reported include back pain, dyspepsia, pain in extremity, diarrhea, headache, alopecia, nausea, and myalgia. Subjects will be instructed to contact the research team to report problems with the listed side effects of etidronate.

CT Scans: Non-contrast CT scans of the heart and lower extremities to assess calcification are safe procedures; however, it is associated with a small amount of ionizing radiation. The radiation exposure for

the coronary CT calcium score is estimated to be less than 0.5 rem (5.0 mSv) and the exposure for the lower extremity CT calcium score is estimated to be less than 1.4 rem (14 mSv). Risks of radiation exposure are detailed in the consent section using wording provided by the NIH Radiation Safety Committee. In some subjects, lying on the table may result in some discomfort due to back pain or claustrophobia.

Although uncommon, if a subject has resting tachycardia, beta-blocker may be used to achieve adequate image quality. Heart rates of less than 80 beats per minute are desired for coronary calcium scoring CT exam. If a contraindication to metoprolol use is present (asthma with regular use of inhaler, chronic severe lung disease, or active decompensated heart failure, Mobitz type 2 second degree or 3rd degree atrio-ventricular heart block), metoprolol will not be administered. Diltiazem may be administered in subjects with asthma or chronic severe lung disease to help slow the resting heart rate.

Prior to administering metoprolol or diltiazem, a baseline heart rate (HR) and blood pressure (BP) will be obtained. Since the approach to pre-scan resting heart rate reduction is rapidly changing, we will generally adhere to the following guidelines but recognize that the supervising physician may customize the doses on an individual patient:

- A net dose of metoprolol 50-200 mg or diltiazem 90-360 mg will be administered orally over a period of approximately 1-2 hours titrated to achieve a heart rate less than 60 beats per minute. In addition, metoprolol 5-30 mg intravenously or diltiazem 5-30 mg intravenously may be administered at the judgment of the supervising physician if heart rate is not adequately controlled and the patient appears capable of tolerating the additional dose.
- If baseline HR is <60 bpm, generally no additional metoprolol or diltiazem is administered; however, the supervising physician may elect to use a small dose to blunt heart rate increases during the scan.
- The physician covering the cardiac CT may adjust the dose of metoprolol or diltiazem on an individual basis.

Cardiac vital signs will be obtained and documented again approximately thirty minutes following metoprolol or diltiazem administration. If at any time the heart rate is <50 bpm and/or the systolic BP is <100, the supervising physician will be notified.

MRI: MRI uses no ionizing radiation and is quite safe when performed on a properly screened population. Potential risks relate to the magnetic field's effect on subjects with implanted metal objects (i.e. cerebral aneurysm clips, cochlear implants, etc.). The magnetic field can cause twisting or movement of these objects, thus causing harm to the subject. Also, the radiofrequency deposition of MRI can potentially cause burns in subjects with pacemakers or other implanted coiled wires. Subjects will be screened for these objects and excluded as indicated.

Although unlikely with our imaging parameters, peripheral nerve stimulation may cause discomfort. Peripheral nerve stimulation occurs when magnetic field gradient switching occurs fast enough to cause peripheral nerve depolarization. Typically, the patient may feel a twitch such as in the buttock, leg, or across the bridge of the nose. There is no physical danger from peripheral nerve stimulation. However, as gradient switching rates increase, smaller peripheral nerves (such as those that cause pain) can be excited. The switched gradients also generate noise in the scanner. Peak sound power produced in the magnet will be less than 140 dB and 119 dBA. The FDA limits constant noise exposure to 140 dB and 99 dBA for two hours. To stay within FDA guidelines on dBA limits, all subjects will wear hearing protection in the form of

earplugs and/or headphones.

All our scans are within FDA guidelines for radiofrequency power levels. However, in cases of a damaged or dysfunctional surface coil, there is a small chance of local warming in the body. For safety purposes, we ask patients to report this sensation so we can modify scans or verify integrity of hardware.

There is no data that shows any significant adverse effects of exposure to static magnetic fields. There are well known minor adverse effects associated with high magnetic fields. These include nausea, metallic taste, and detection of flashes of light. All of these are associated with moving too rapidly in the magnetic field.

Non-significant Risk IDE: The use of the MRI scanner constitutes a non-significant risk (NSR) device (IDE). With regard to radiofrequency power levels (SAR limits), we operate the MRI scanner at the FDA limitsestablished for MRI in general applications. We utilize the following limits for the body coil: SAR 4 watts/kg average over 15 minutes. The limits for the head transmit/receive coil are: SAR 8 watts/kg average over 5 minutes. The scanner gradient performance (dB/dt limit) is set on an individual basis to avoid painful peripheral nerve stimulation.

Investigational or research MRI coils may be used in the protocol. The coils are noninvasive devices external to the body. The coils act as antennae to receive small radiofrequency signals out of the body. Coils of the type we use are used daily in clinical MRI practice.

The use of the MRI research coils, research pulse sequencing, and research image processing constitute the research component of this device which are not FDA approved. All research coils undergo safety testing at the NIH which is supervised by the NMR Safety Committee. There is no potential for serious risk to the health, safety, or welfare of the subjects using these research modalities.

Currently, none of these devices are undergoing FDA Premarket Notification (510(k)) submission. Thus, there is no IDE for the protocol. Research image acquisition programs, research analysis software, and research radiofrequency (RF) receive coils may be used in these MRI studies. Participants will not receive sedation or anesthesia for the MRI scan.

Gadolinium-based contrast agents (GBCAs) are injected medications used to improve MRI images. Most patients experience a metallic taste when gadolinium contrast is injected. Some (2%) report mild symptoms such as headache, nausea or vomiting, or a rash near the injection site. Rarely (<0.1%) patients experience severe symptoms such as wheezing, shortness of breath, and low blood pressure as part of an allergic (anaphylactoid) reaction that may require emergency medical treatment.

In a few cases per million, usually in patients with severe kidney disease, gadolinium contrast can cause a rare, debilitating or even fatal, skin disease called Nephrogenic Systemic Fibrosis (NSF) that cause thickening of the skin and other organs. Since physicians became aware of the disease, began screening patients at risk of kidney disease, and switched to safer ("macrocyclic") forms of gadolinium contrast, new reports of NSF are much rarer.

There are also reports of gadolinium retained in the brain, bone, and skin. It is not known whether this is important to health. We use "macrocyclic" forms of gadolinium contrast, such as gadolinium contrast, such as gadobutrol, that are thought to reduce this risk.

DEXA Scan: Although DEXA is a safe procedure it is associated with a small amount of ionizing radiation. The radiation exposure is less than 1 mRem per study. In some subjects, lying on the table may result in some discomfort due to back pain or claustrophobia.

Hand X-Ray: Hand X-ray is a safe procedure but is associated with a very small amount of ionizing radiation exposure less than 1 mRem per study.

Treadmill Test: Shortness of breath and fatigue are often felt at the end of the exercise, and occasionally subjects may experience chest discomfort. Abnormal heart rhythms can be triggered by exercise, but these are rarely persistent or severe.

Ankle-brachial index (ABI): Inflation of blood pressure cuffs may cause transient discomfort. Subjects with fragile skin may suffer minor trauma. There is felt to be no other risk.

Surface EKG: There are no clinically significant discomforts associated with a routine EKG. Shaving local body hair with a sterile hair razor may be necessary to improve the quality of the EKG recording. Skin irritation may occur at the site of application for the adhesive electrode patches; however, the EKG will take approximately 5 to 10 minutes only and any skin irritation should be minimal.

Cardio-Ankle Vascular Index (CAVI) (optional): Inflation of blood pressure cuffs may cause transient discomfort. Subjects with fragile skin may suffer minor trauma (as per ABIs, related to usual BP measurements). This procedure involves the use of a VaSera VS-1500N system; an FDA approved vascular screening system.

Phlebotomy: Standard precautions for obtaining human blood samples will be taken. Transient discomfort and minor bruising may occur at the phlebotomy site. Vasovagal symptoms can occur during blood drawing. Intravenous catheters will be inserted by a nurse/technician who is well trained in that procedure.

8.5 Informed Consent Process and Documentation

Each participant will receive an oral and written explanation of the goals, procedures, and risks of this study. The Principal Investigator and those Associate Investigators who are listed on the Key Study Personnel page of the protocol with consenting rights may obtain informed consent from research participants.
Consent will be obtained at the NIH Clinical Center. The original, signed informed consent document will be placed in the medical record, and the subject will receive a signed copy of the informed consent document. The informed consent document is translated into Italian.

In instances where a subject has medically indicated surgery outside of the NIH Clinical Center, informed consent will be obtained via technology and/or electronic processes rather than in person per the Clinical Center Policy and Communication Bulletin M77-2, using the Tissue Collection informed consent document. Subjects will be contacted by telephone and the investigational nature and research objectives of the tissue collection for this study. The subject will sign and date the informed consent. A witness will sign to confirm the consent process. The informed consent document will be faxed or mailed back to the consenting investigator, who will sign and date and send back a fully executed copy for the subject's records. The informed consent process will be documented on a progress note and the signed informed consent document will be placed in the medical record. Subjects will be provided a pre-paid envelop to send the signed consent document back to the investigator.

Should a potential non-English speaking subject be referred for participation in this study, the full informed consent documents will be translated into the subject's language. The use of short forms will not be requested, as this is a longitudinal study.

8.6 Subject Advocate

A subject's rights representative is available to subjects on this protocol. The representative can be reached at 301-496-2626 and is located in Building 10. Subjects may ask any questions about the study and may withdraw their consent at any time.

9. Conflict of Interest

The National Institutes of Health reviews NIH staff researchers at least yearly for conflicts of interest. The following link contains details on this process http://ethics.od.nih.gov/forms/Protocol-Review- Guide.pdf.

None of the members of the research team reported a potential conflict of interest.

10. Reimbursement for Travel

Reimbursement for local travel, U.S. travel, food, and lodging will be in accordance with NIH and NHLBI guidelines.

11. Monetary Compensation

Subjects will not be offered compensation for time and inconvenience while participating in this study.

12. Agreements

FWA: Dr. Hilaire is now in University of Pittsburg and no longer a volunteer in NIH, will be analyzing identifiable data as a Non-NIH engaged non-enrolling Investigator in this protocol. Dr. Hilaire's role in the research will be limited to data analysis. An FWA coverage agreement to cover this activity will be executed.

MTA: between NHLBI and Ann Rosenthal, MD and Jeffrey Wesson, MD, PhD of Medical College of Wisconsin. These recipients will receive de-identified patient and/or control tissues and will help us identify the composition of calcification and minerals found in the samples we send. We may link analyzed data to identifiers here in NIH.

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Appendix

Simplified Disease Activity Index (SDAI)

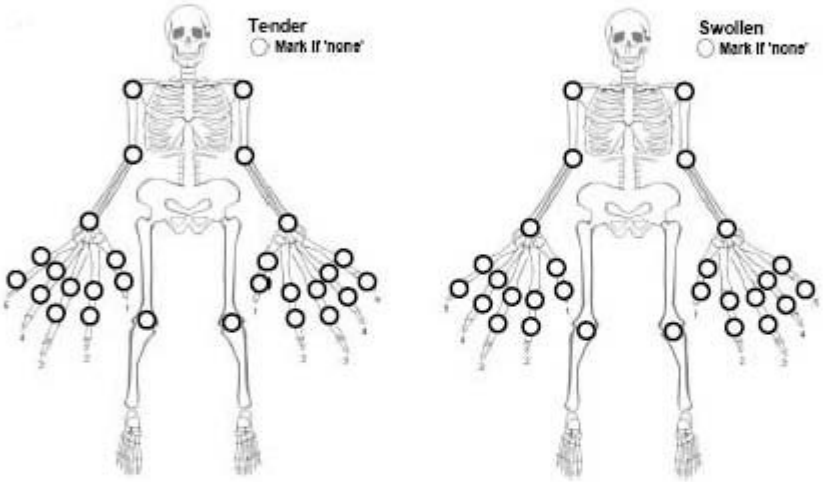
Patient assessment of global disease activity:
Considering ALL THE WAYS THAT YOUR ILLNESS AFFECTS YOU, rate how you are doing on the following scale. Place an X in the box below that best describes how you are doing.

VERY WELL 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 **VERY POOR**
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0

FOR PROVIDER USE ONLY

Provider assessment of global disease activity:

VERY WELL 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 **VERY POOR**
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0



PtGA ☐

+PrGA ☐

+28TJC ☐

+28SJC ☐

+CRP (mg/dl) ☐

Total SDAI ☐

Interpretation:

- Remission: SDAI ≤3.3
- Low Disease activity: 3.3 < SDAI ≤ 11
- Moderate Disease Activity: 11 < SDAI ≤ 26
- High Disease Activity: SDAI >26
- An SDAI reduction by 7 represents a moderate improvement. A reduction more than 17 represents a major improvement.

Adapted from

1. Smolen JS, Breedveld FC, Schiff MH, Kalden JR, Emery P, Eberl G, et al. A simplified disease activity index for rheumatoid arthritis for use in clinical practice. Rheumatology (Oxford). 2003 Feb;42(2):244-57.

2. Aletaha D, Smolen J, Ward MM. Measuring function in rheumatoid arthritis: Identifying reversible and irreversible components. Arthritis Rheum. 2006 Sep;54(9):2784-92.

Appendix

Duke Activity Status Index

These questions are about any physical limitations you might have. For each question, please rate whether, at the **present time,** you can do one or more of the activities. Some questions mention more than one activity. Answer according to the one **activity** you can do **best.** Please chose the one answer that best describes you. If you have never done an activity, or don't **usually** do it, answer "Don't do this for other reasons."

	Yes, with no difficulty	Yes, but with some difficulty	No, I can't do this	Don't do this for other reasons
Can you…				
1. take care of yourself, that is, eating, dressing, bathing or using the toilet?	1	2	3	4
2. walk indoors, such as around the house?	1	2	3	4
3. walk a block or 2 on level ground?	1	2	3	4
4. climb a flight of stairs or walk up a hill?	1	2	3	4
5. run a short distance?	1	2	3	4
6. do light work around the house like dusting or washing dishes?	1	2	3	4
7. do moderate work around the house like vacuuming, sweeping floors, or carrying in groceries?	1	2	3	4
8. do heavy work around the house like scrubbing floors, or lifting or moving heavy furniture?	1	2	3	4
9. do yard work like raking leaves, weeding or pushing a power mower yet?	1	2	3	4
10. have sexual relations?	1	2	3	4
11. participate in moderate recreational activities like golf, bowling, dancing, doubles tennis, or throwing a baseball or football?	1	2	3	4
12. participate in strenuous sports like swimming, singles tennis, football, basketball or skiing?	1	2	3	4

Please complete this questionnaire and return to D. Tripodi, in the envelope provided, before you are discharged from the hospital. If you have any questions regarding completion of this document have your nurse contact D. Tripodi for clarification.

Name: **Date:**