Phase 2a Study of Interferon Gamma-1b for the Treatment of Autosomal Dominant Type 2 Osteopetrosis

Michael Econs, MD
Indiana University School of Medicine Department of Endocrinology and Metabolism
1120 West Michigan Street Room 459
Indianapolis, IN 46202

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Protocol Physician (Sponsor-investigator)

Michael J. Econs, MD
Glenn W. Irwin, Jr., Professor of Endocrinology and Metabolism
Director: Division of Endocrinology and Metabolism
Professor of Medicine and Medical and Molecular Genetics
Indiana University School of Medicine
Endocrinology Service Line Leaders, Indiana University Health Physicians
1120 W. Michigan Street
Gatch Clinical Building CL 459
Indianapolis, IN 46202
Tel: 317-274-1339
Fax: 317-278-0658
Email: mecons@iu.edu

PROTOCOL SIGNATURE PAGE

The signature below constitutes the approval of this protocol and the attachments, and provides the necessary assurances that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable U.S. federal regulations and ICH guidelines.

The Lead Principal Investigator should sign below.

Principal Investigator: Michael J Econs, M.D.

_____ Date _____

Name / Title

The original signature pages should be filed with Indiana University. A copy of this signature page should be maintained at each site in the regulatory binder.

Contents

1.0 – OVERVIEW AND BACKGROUND

1.1 BACKGROUND

Autosomal dominant Osteopetrosis type 2 (ADO2) is a heritable osteosclerotic disorder that usually results from heterozygous missense mutations in the *CLCN7* gene [1, 2], and is characterized by a wide range of symptoms and severity including multiple fractures, impaired vision and hearing, pancytopenia and osteomyelitis [3-5]. ADO2 is presently incurable, although anecdotal evidence and recent studies in a murine model suggest that interferon gamma-1b may have beneficial effects [6].

ADO2 (also known as Albers-Schönberg disease and "marble bone disease") is an autosomal dominant disorder with an incidence of 5.5 in 100,000 people and penetrance of approximately 66% [2, 4]. We have kept the designation ADO2 for the disease we are studying because the disease caused by activating mutations in the LRP5 gene, while not technically an osteopetrosis, is inappropriately still referred to as ADO1 in the literature. Because of the incomplete penetrance and a mild phenotype in some individuals with *CLCN7* mutations, ADO2 is sometimes referred to as the "benign" form of osteopetrosis to distinguish it from the severe "malignant" recessive form, which is life-threatening in infancy. However, referring to ADO2 as "benign" is a misnomer because most ADO2 patients suffer complications from their disease. In a previous study of 62 ADO2 patients, hip or femur fractures occurred in 16% of ADO2 children and 49% of ADO2 adults, *and all 4 of the most severely affected patients have died from complications of osteopetrosis* [4]. In the ADO2 population at Indiana University, there was a high rate (19%) of severe visual loss (thought to be due to lack of bone resorption to widen the optic canal during growth). Additionally, 16% of the patients had osteonecrosis or osteomyelitis, and bone marrow failure occurred in 3% of patients [4]. Similar to these study results, Benichou et al [5] studied 42 ADO2 patients and found that 78% of patients had fractures and over half had orthopedic procedures with half of those having at least 3 procedures. They also documented a high rate of mandibular osteomyelitis (12.9%). In contrast to our study [4], only 5% had visual loss due to optic nerve compression [5]. Thus, in the two largest studies to date, ADO2 patients manifest significant, and in some cases, severe disease.

Although very high dose calcitriol (up to 18 mcg per day) has been used anecdotally (L. Key, personal communication), this approach has not been shown to be efficacious in ADO2. The only reference [7] cited on the clinical management of ADO2 in OMIM (http://www.omim.org/entry/166600, accessed May 31, 2015) is a case report of a child with severe recessive osteopetrosis, who had a partial response to calcitriol. Indeed, Dr. Econs' experience treating ADO2 patients with high dose calcitriol has not resulted in improvements in bone mineral density or biochemical markers of bone turnover, while having risk of nephrolithiasis and nephrocalcinosis. Moreover, the calcitriol studies in the ADO2 mouse model indicated no benefit and a possible worsening of the bone phenotype (see preliminary data). As a result, Dr. Econs has abandoned the practice of treating ADO2 patients with calcitriol and is currently following these patients off therapy, essentially observing the natural progression of the disease.

1.2 PRECLINICAL AND CLINICAL EXPERIENCE

The first evidence of a potentially effective therapy comes from studies of interferon gamma in a novel ADO2 "knock-in" mouse with a G213R missense mutation in the *Clcn7* gene [8], which is syntenic to the human G215R mutation identified in several ADO2 patient families [2]. Mice homozygous for the G213R mutation have severe osteopetrosis, absent tooth eruption, and die by 4 weeks of age, which is similar to the human recessive disease caused by mutations in *CLCN7* and several other genes [8]. Mice heterozygous for the G213R mutation have high whole body aBMD and bone volume per total volume (BV/TV) at distal femur and increased numbers of poorly resorbing osteoclasts, resembling human ADO2 [8]. Although the ADO2 mouse does not display spontaneous fractures as seen in the adult human disease, the fact that these mice have a 2-fold increase in BV/TV at 12 weeks of age and have been successfully used to test two potential therapies, one of which results in a partial correction of the phenotype (see below), indicates that this model is an excellent model of osteoclast dysfunction. In concert with human disease, the phenotype of our ADO2 mice gets more pronounced with age.

1.3 SCIENTIFIC RATIONALE

Interferon gamma-1b increases bone resorption and returns the bone phenotype towards normal in the ADO2 mouse:

Interferon gamma-1b (IFN-G) is used in patients with recessive forms of osteopetrosis to control disease until a donor can be found for BMT, which has been shown beneficial in this very severe disorder [9]. IFN-G is not approved for use in ADO2, as it has never been tested in this disease. Moreover, cell culture data is inconclusive regarding whether IFN-G increases or decreases normal osteoclast development, number, and/or activity [10-17]. Therefore, we sought to use our newly developed ADO2 mouse model to test whether murine interferon gamma and/or high dose calcitriol would decrease bone mass in ADO2 mice.

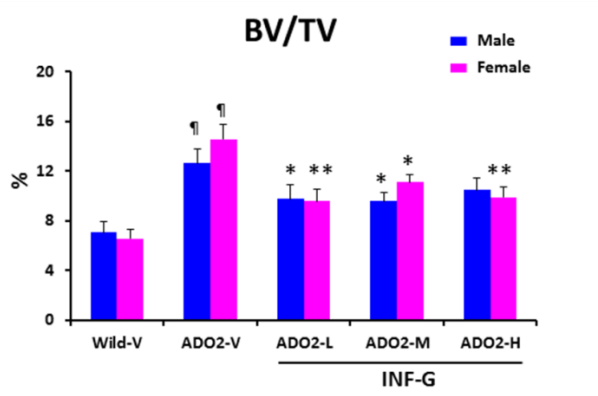

Figure 2A: Interferon gamma significantly attenuated the BV/TV gain in both male and female ADO2 mice

Six-week-old male and female ADO2 mice (N=10/group) were treated with vehicle, or calcitriol (low 0.1 µg/kg, medium 0.5 µg/kg, or high 1 µg/kg doses) or interferon gamma (low 22.5 µg/kg, medium 37.5 µg/kg, or high 100 µg/kg dose) 5 times per week for 8 weeks. Bone was phenotyped by DXA and micro-computed tomography (µCT). Serum and urine biochemistries and bone resorption markers were also analyzed. Mice treated with low and medium doses of calcitriol showed a trend of higher aBMD and BV/TV whereas high dose calcitriol significantly ($p<0.05$) increased bone

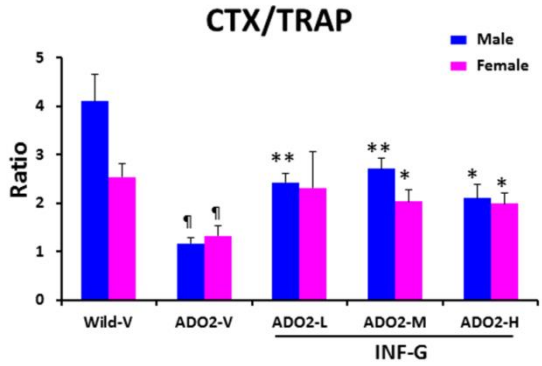

Figure 2B: Serum CTX/TRAP ratio was increased due to interferon gamma treatment in ADO2 mice in both male and female

mass compared to vehicle, indicating that calcitriol actually worsened the bone phenotype of ADO2 mice [6]. ADO2 mice treated with all doses of IFN-G significantly ($p<0.05$) attenuated the increase of whole body aBMD [6] and BV/TV at the distal femur in both male and female mice as compared to those treated with vehicle (Figure 2A). Importantly, the reduction in BV/TV due to IFN-G treatment was to a level approximately half way between WT mice and the ADO2 mutant mice (the therapeutic midpoint), indicating that there was an incomplete, but meaningful reduction in the abnormal bone phenotype with only 8 weeks of therapy. Additionally, serum CTX and the CTX/TRAP ratio were increased in the IFN-G groups, but not in the calcitriol treated groups, indicating that there was an increase in bone resorption with IFN-G treatment ([6] and Figure 2B). This study demonstrates that IFN-G has potential to treat human ADO2 and these results have led us to propose a short-term human trial with human Interferon gamma-1b (Actimmune®).

2.0 – OBJECTIVES

2.1 PRIMARY OBJECTIVE

- To evaluate the effect of treatment with ACTIMMUNE at week 14 on bone resorption markers (i.e. C-telopeptide [CTX], and N-telopeptide (NTX)/creatinine ratio) in subjects, age 3-65, diagnosed with Autosomal Dominant Osteopetrosis Type 2 (ADO2) due to heterozygous mutations in the chloride channel 7 (*CLCN7*) gene.

2.2 SECONDARY OBJECTIVES

- To evaluate the effect of ACTIMMUNE at a stable dose over a 6 to 12 week treatment period on bone turnover markers (tartrate-resistant acid phosphatase type 5b [TRAP5b], N-telopeptide (NTX), and CTX/TRAP5b ratio).
    - To evaluate the change in CTX level from the end of treatment (Week 14 visit) to 1 month after discontinuation of ACTIMMUNE treatment (Week 18).
    - To evaluate changes in bone formation markers (P1NP and bone specific alkaline phosphatase) at Weeks 8 and 14.

2.3 SAFETY OBJECTIVE

- To evaluate the safety and tolerability of ACTIMMUNE in ADO2 adults.

3.0 – STUDY DESIGN

3.1 STUDY DESCRIPTION

This is a single center, open-label, dose-escalation study evaluating the efficacy, as defined by biochemical endpoints, and safety profiles of ACTIMMUNE in ADO2 subject.

We will treat 12 ADO2 subjects (children or adults age 3-65) with Actimmune® via a dose escalation protocol to a dose of 50 $\mu g/m^2$ subcutaneously three times per week (TIW) for 8 weeks. If serum CTX does not increase by more than 25% by week 8, the dose will be increased to 100 $\mu g/m^2$ subcutaneously TIW.

Individual subjects in whom ACTIMMUNE administration increases bone resorption markers during the 14 weeks of this trial will be eligible for a 1 year extension trial.

3.2 STUDY DURATION

Subject involvement from screening to completion of study will be approximately 157 days or approximately 5 months. Screening procedures can be done over a 30 day period prior to baseline (day 0). Study drug will be started on Day 0 and continued through 14 weeks. Subjects will be seen at the completion of the 14 weeks for end of treatment and then 4 weeks later for a safety follow-up visit. There will be 8 visits during the trial. Six of these visits will occur at Indiana University Hospital. Visit 3 and 5 can occur by phone with the coordinator and have labs collected at a local lab collection site.

3.3 ENROLLMENT

There will be 12 subjects enrolled into the treatment phase of this study. Additional subjects may be required to allow for screen failures and if a subject drops out before visit 5 they will be replaced with a new subject. If a subject drops out after visit 5 they will not be replaced. Enrollment is not expected to be difficult due to the extensive experience of the investigator with this patient population and the strong Osteopetrosis Support group.

Screening will focus on patients from known ADO2 kindreds. Individuals should have either been diagnosed with Osteopetrosis and have a clinical phenotype and/or family history that is consistent with ADO2, have been told that they have an abnormally high bone density (>3SD about mean for age and sex), or a clinical presentation consistent with ADO2.

3.4 STUDY ENDPOINTS

Primary Efficacy Endpoint

The primary efficacy endpoint is the difference in the subject's observed change in bone markers (serum CTX and fasting urine NTX) over the treatment period (from Baseline to Week 14).

Secondary Efficacy Endpoints

- Secondary endpoints will include analysis of CTX and NTX over time while on a stable dose of Actimmune® and analysis of these markers after discontinuing drug to determine if patients rebound off drug.
- In addition, we will measure markers of bone formation (P1NP and bone specific alkaline phosphatase) to test whether increased bone resorption from interferon gamma-1b therapy is coupled to increased bone formation.
- We will also obtain baseline measurements of bone density via DXA, spine QCT, and pQCT of the distal radius and tibia, as well as retrospectively assessing the fracture rate in the 5 years prior to initiation of therapy (via a questionnaire with confirmation by review of radiographs films and/or reports) to facilitate a future longer term extension trial that would test whether long term treatment with Actimmune® decreases BMD and reduces fractures in ADO2 patients.

Please see statistical section (section 8.0) for the statistical analysis plan.

Safety Endpoints

Adverse event (AE) data will be presented by subject. Clinical laboratory safety data and vital sign data will be summarized by subject with descriptive statistics for Baseline, post-dose, and change from Baseline to post-dose values. Physical examination findings will be listed by subject. AEs will be summarized by categories and grades. Counts and 95%

Agresti-Coull confidence intervals will be calculated.

The percentage of subjects with baseline nephrocalcinosis will be summarized using descriptive statistics along with degree of nephrocalcinosis. Individual subjects development of new nephrocalcinosis or worsening of existing nephrocalcinosis (identified by increasing grade) will be listed as an AE.

3.5 ONGOING SAFETY REVIEW

All collected safety data including adverse events, safety test results and changes in concomitant medications will be reviewed by the principal investigator or designated physician sub investigator in a timely fashion. Study nurses will review the results for abnormal results as soon as received and if necessary contact the physician immediately.

A data and safety monitoring board (DSMB) will be established to monitor the study. This DSMB will be approved by the IRB prior to enrollment in the study is started. The DSMB will review data twice a year. The DSMB will have authority to stop the trial for safety reason or to modify the trial if necessary for safety issues. Due to the small study population and short study duration the DSMB will not be doing interim analysis for effectiveness.

3.6 STUDY RISKS

Risks of Taking Actimmune

The following data on adverse reactions are based on the subcutaneous administration of *ACTIMMUNE* at a dose of 50 mcg/m2, three times weekly, in patients with Chronic Granulomatous Disease (CGD) during an investigational trial in the United States and Europe.

The most common adverse events observed in patients with CGD are shown in the following table (from product insert):

Percent of Patients

Clinical Toxicity	ACTIMMUNE	Placebo
Fever	52	28
Headache	33	9
Rash	17	6
Chills	14	0
Injection site erythema or tenderness	14	2
Fatigue	14	11
Diarrhea	14	12
Vomiting	13	5
Nausea	10	2
Myalgia	6	0
Arthralgia	2	0
Injection site pain	0	2

Miscellaneous adverse events, which occurred infrequently in patients with CGD and may have been related to underlying disease included back pain (2 percent versus 0 percent), abdominal pain (8 percent versus 3 percent) and depression (3 percent versus 0 percent)

for *ACTIMMUNE* and placebo treated patients, respectively (product label).

Similar safety data were observed in 34 patients with severe malignant Osteopetrosis.

Radiation

Subjects will be exposed to radiation as part of the protocol testing. The protocol will be approved by Indiana University Radiation Safety Committee prior to enrollment. The informed consent will provide subjects information about the risks of additional radiation exposure above what would be considered standard of care for ADO2 subjects.

Risk of Blood Draws

The risks that can occur with venipuncture include discomfort, bruising, feeling faint or passing out, and infection. To reduce the likelihood of complications from blood draws, only trained phlebotomists or nurses will draw blood at the study visits using clean technique.

Loss of Confidentiality

There is the unlikely chance that health information is viewed by someone outside the research team who is not authorized to see health information. However, standard procedures to ensure that this does not happen will be followed.

3.7 STUDY BENEFITS

The major potential benefit to the subject is that the treatment may result in increased bone resorption, which may reduce disease burden by reducing bone fragility and, thereby, fracture risk. Additionally, treatment may reduce the risk of infection, particularly osteomyelitis, and may improve hematopoietic marrow space, resulting in less anemia, pancytopenia, and/or extra medullary hematopoiesis.

ADO2 is an osteosclerotic disorder due to defective bone resorption. There is wide variation in disease severity, but most affected individuals experience fractures with almost half of affected adults experiencing hip or femur fractures. Other manifestations include visual loss, osteonecrosis and/or osteomyelitis, and bone marrow failure. Currently, there is no therapy for the disease; however, studies in our ADO2 mouse model indicate that interferon gamma-1b may be effective in treating the human disease. Successful completion of the proposed study will provide evidence that administration of Actimmune® induces osteoclastic bone resorption in ADO2 patients, a critical first step to determining whether the drug is a useful treatment for the disorder. This study will provide critical data on feasibility and dosing and determine if a long-term study is indicated. Interferon gamma-1b could be the first successful therapy for ADO2.

3.8 INCLUSION OF CHILDREN

ADO2 affects children as well as adults. The study medication has been previously tested in children for other indications. It is felt that the earlier the intervention can occur in disease process the increased chance of improved outcomes.

4.0 – STUDY MEDICATION

4.1 STRUCTURE AND NOMENCLATURE

ACTIMMUNE® (Interferon gamma-1b) is a single-chain polypeptide containing 140 amino acids. Production of *ACTIMMUNE* is achieved by fermentation of a genetically engineered *Escherichia coli* containing the DNA, which encodes for the human protein. *ACTIMMUNE* is a highly purified sterile solution consisting of non-covalent dimers of two identical 16,465 dalton monomers; with a specific activity of 20 million International Units (IU)/mg ($2x10^6$ IU per 0.5 mL) which is equivalent to 30 million units/mg (product label).

4.2 PHYSICAL PROPERTIES

ACTIMMUNE is a sterile, clear, colorless solution filled in a single-use vial for subcutaneous injection. Each 0.5 mL of ACTIMMUNE contains: 100 mcg (2 million IU) of Interferon gamma-1b formulated in 20 mg mannitol, 0.37 mg disodium succinate hexahydrate, 0.14 mg succinic acid, and 0.05 mg polysorbate 20 and Sterile Water for Injection. *Note that the above activity is expressed in International Units (1 million IU/50mcg). This is equivalent to what was previously expressed as units (1.5 million U/50mcg)* (product label).

4.3 MECHANISM OF ACTION

Interferons bind to specific cell surface receptors and initiate a sequence of intracellular events that lead to the transcription of interferon-stimulated genes. The three major groups of interferons (alpha, beta, and gamma) have partially overlapping biological activities that include immunoregulation such as increased resistance to microbial pathogens and inhibition of cell proliferation. Type 1 interferons (alpha and beta) bind to the alpha/beta receptor. Interferon-gamma binds to a different cell surface receptor and is classified as Type 2 interferon. Specific effects of interferon-gamma include the enhancement of the oxidative metabolism of macrophages, antibody dependent cellular cytotoxicity (ADCC), activation of natural killer (NK) cells, and the expression of Fc receptors and major histocompatibility antigens (product label).

In recessive osteopetrosis, the exact mechanism(s) by which *ACTIMMUNE* has a treatment effect has not been established. Changes in superoxide levels during ACTIMMUNE therapy do not predict efficacy.

Pharmacokinetics

The intravenous, intramuscular, and subcutaneous pharmacokinetics of *ACTIMMUNE* have been investigated in 24 healthy male subjects following single-dose administration of 100 mcg/m^2. *ACTIMMUNE* is rapidly cleared after intravenous administration (1.4 liters/minute) and slowly absorbed after intramuscular or subcutaneous injection. After intramuscular or subcutaneous injection, the apparent fraction of dose absorbed was greater than 89%. The mean elimination half-life after intravenous administration of 100 mcg/m^2 in healthy male subjects was 38 minutes. The mean elimination half-lives for subcutaneous dosing with 100 mcg/m^2 was 5.9 hours. Peak plasma concentrations, determined by ELISA, occurred approximately 7 hours (0.6 ng/mL) after subcutaneous dosing. Multiple dose subcutaneous pharmacokinetic studies were conducted in 38 healthy male subjects. There was no accumulation of ACTIMMUNE after 12 consecutive daily injections of 100

mcg/m^2 (package insert).

4.4 STUDY RELATED INFORMATION

Contraindications

ACTIMMUNE is contraindicated in patients who develop or have known hypersensitivity to interferon-gamma, *E. coli* derived products, or any component of the product.

Precautions

Isolated cases of acute serious hypersensitivity reactions have been observed in patients receiving *ACTIMMUNE*. If such an acute reaction develops the drug should be discontinued immediately and appropriate medical therapy instituted. Transient cutaneous rashes have occurred in some patients following injection but have rarely necessitated treatment interruption (product label).

Actimmune is a category C drug in pregnancy, therefore, female subjects who are pregnant or anticipating pregnancy will not be enrolled and pregnancy tests will be done for females of reproductive potential.

5.0 – ELIGIBILITY

5.1 INCLUSION CRITERIA

Subjects must meet all of the following criteria:

1. Subject is diagnosed with clinically significant ADO2 as determined by the investigator.

Individuals will be screened who have either been diagnosed with osteopetrosis and have a clinical phenotype and/or family history that is consistent with ADO2, have been told that they have an abnormally high bone density (>3SD above mean for age and sex), or a clinical presentation consistent with ADO2. Initial contact will be with members of ADO2 kindreds who have known disease.

2. Provide written informed consent for competent adults and for minors provide written assent (if appropriate) and written informed consent by a legally authorized representative after the nature of the study has been explained, and prior to any research-related procedures
3. Ages 3 to 65 years inclusive.
4. Willing to use reliable method of contraception [i.e. oral or patch hormonal contraceptives, intrauterine device, physical barrier methods, tubal ligation or hysterectomy, vasectomy (partner) or abstinence] throughout the study and for 30 days after the last dose of study drug.

5.2 EXCLUSION CRITERIA

Subjects will be ineligible for study participation if they meet any of the following criteria.

1. Any unstable illness that in the investigator's opinion precludes participation in the study.
2. Serum calcium >10.6 mg/dl at screening.

3. eGFR using the MDRD equation in adults (or the modified Schwartz equation for children) of < 35 ml/min/1.73m^2.
4. Nephrocalcinosis on screening ultrasound Grade 3 or higher [18]. Subjects with grade 3 or higher nephrocalcinosis will be excluded because we anticipate that use of study drug will increase bone resorption, resulting in increased urinary calcium excretion, which could, potentially, lead to worsening nephrocalcinosis. The grading scale is listed below:
   0 = Normal
   1 = Faint hyperechogenic rim around the sides and tip of the medullary pyramids
   2 = More intense echogenic rim with echoes faintly filling the entire medullary pyramid
   3 = Intense echoes throughout the medullary pyramid
   4 = Solitary focus of echoes at the tip of the medullary pyramid/nephrolithiasis
5. Use of any investigational product (drug or device) within 30 days prior to randomization.
6. Subject reported history of hepatitis C.
7. A recent (past 5 years) history of alcoholism or intravenous drug abuse.
8. History of hypersensitivity to IFN-ɣ or *E. coli*-derived products.
9. History of liver disease as evidenced by laboratory results at Screening (aspartate aminotransferase [AST] or alanine aminotransferase [ALT] >2x the upper limit of normal), except when in the opinion of the investigator the liver disease is caused by extra medullary hematopoiesis.
10. Pregnant or nursing women or those who plan on becoming pregnant during the study.
11. Preexisting cardiac conditions including ischemia, congestive heart failure, or arrhythmia.

5.3 EARLY TERMINATION OF A STUDY PARTICIPANT

Treatment will stop and the subject will return for an exit visit if any of the following conditions occur:

1. withdrawal of consent
2. pregnancy (female participants)
3. anaphylaxis requiring hemodynamic support (i.e. epinephrine and/or blood pressure medications) or mechanical ventilation
4. any medically important event such as a concurrent illness or complications judged to significantly increase the risk of study therapy.
5. any investigator judgment
6. If any of the following dose-limiting toxicities occur:
   a. Increase in liver transaminases more than 5 times upper end of normal, total bilirubin ≥2.5 mg/d.
   b. Grade 3 or higher AE except as mentioned below.
   c. Develops Grade 2 or higher nephrocalcinosis, or symptomatic nephrolithiasis.

<u>Special circumstance: Grade 3 and 4 toxicity of absolute neutrophil count (ANC, includes neutrophils and bands) or platelet count:</u>

1. Marrow failure due to crowding from lack of bone resorption is part of the underlying disease of ADO, and there is no current effective approved therapy for ADO to prevent or treat this. Because of this, some subjects may have b*aseline* values of ANC or platelets that are near the Grade 3 toxicity level, *prior to* study drug. However, since there is no

effective therapy for ADO, and there is potential for interferon gamma 1b to improve marrow space and function if it proves effective to increase osteoclastic activity, subjects will be allowed to continue in study under the following conditions:

a. If there is a decrease in absolute neutrophil count (ANC, includes neutrophils and bands) or platelet count to a Grade 4 toxicity (e.g. ANC < 500 or Platelets < 25k), we will hold study drug and monitor CBC with differential weekly until recovery to a grade 3 toxicity level (ANC 500 – 1,000; or platelets 25k-50k) and, after consultation with the patient and/or family (if a minor), restart at half the previous dose of interferon gamma 1b and continue to monitor CBC with differential weekly.
b. If the patient remains above the target ANC after 2 weeks the investigators may consider re-challenge with the previous dose of interferon gamma 1b, with weekly CBC with differential monitoring.
c. If toxicity reoccurs at the lowered dose we will again hold study drug and monitor CBC with differential weekly until recovery to a grade 3 toxicity level (ANC 500 – 1,000; or platelets 25k-50k), and then can restart again with further lowering the dose by half again, or discontinue therapy, in consultation with the patient and/or family.
d. If the patient remains above the target ANC after 4 weeks the investigators may consider re-challenge with the previous dose of interferon gamma 1b, with weekly CBC with differential monitoring.

Additional Considerations:

1. There are potential for SAE during the study r*elated to the underlying disease.* These may include hospitalization or surgery for a fracture, or complications of anemia, bone marrow failure or infection, or vision loss. Patients will discontinue study drug during hospitalizations or surgeries, but will not be eliminated from the study except as described below, or at the investigator discretion based on severity of the incident and likelihood of relationship to study drug.
2. Severe vision loss, which may possibly occur during the study period, *is a part of the underling disease, usually presenting in children,* for which there is not currently any effective medical therapy to prevent. In fact the severe vision loss that occurs in a percentage of patients with this disorder is only one of the reasons that this study is needed, because it may be possible with an effective treatment to prevent severe vision loss, but that cannot be determined except in future larger and longer trials, because the mechanism of vision loss is thought to be optic nerve compression during skull growth. Although unlikely to occur during the short time frame of the study, if severe vision loss is detected clinically during this study, the individual patient will be stopped in the trial for the purposes of facilitating potential surgical treatment if deemed necessary by the treating clinician.
3. A threshold of 25% of subjects having unexpected SAE's would result in an expedited review by the DSMB for whether the study should be modified or completely discontinued, except when the SAE is death or pregnancy having birth defect or for other SAE considered by the investigator (or DSMB) to represent a significant medical hazard. In the unlikely event of a subject death or the latter two events occur during the study, the study will be stopped and reviewed by the DSMB for whether the study should be discontinued. Because there is some

risk of death with the underlying disorder, this will need to be assessed by the DSMB on a case by case basis.

Subjects who stop study will be asked to return for a final early termination visit.

6.0 – STUDY PROCEDURES

6.1 INFORMED CONSENT

Consent/assents will be completed prior to any study related procedure. Consents/assents can be obtained by designated staff as long as there is a physician investigator available for questions.

The procedures planned will be fully explained to the subject and/or family. All consent/assent discussions will occur in a private room. The investigational nature and objectives of the trial, the procedures involved, and their attendant risks and discomforts will be carefully explained to the potential subject and their questions answered. Potential subjects (or parents/guardians) will be informed that they have the right to decline participation in the study and that this decision will not impact their medical care. Subjects will also be notified of their right to withdraw from the study at their (or their parent/guardian's) choice, though recommendations for monitoring for subject safety will also be provided when appropriate.

Assents will be obtained from all children age 7 to 18 if appropriate. If a child is not appropriate for assenting then justification of this must be documented by a physician investigator. If a child turns 7 during the conduct of the study then assent must be obtained at next visit. It is also expected if a child turns 18 while on trial they will be asked to sign a full consent at next visit.

6.2 PHYSICAL EXAM

Physical exam will be completed by a physician investigator. Complete physicals will be done at screening and visit 1 (unless within 10 days of screening) 4 and 6. A brief physical exam can occur at visit 2 and 7.

6.3 VITAL SIGNS

Measured at all clinic visits after subject has had a chance to rest for approx. 5 mins. Vital signs should be taken with subject sitting with legs uncrossed. Attempt should be made to use same arm and cuff size for each visit. Abnormal results should be rechecked and physician notified if vitals remain abnormal.

6.4 ADVERSE EVENTS

Subjects will be asked about any changes in his/her health status at each site visit and phone call. Adverse events that occur within four weeks prior to Day 0 and prior to dosing on Day 0 will be considered baseline signs/symptoms. Adverse events or a worsening of a condition listed as medical history that occur after the dose on Day 0 through the end of the

study will be considered treatment-emergent adverse events (TEAEs). All SAEs that occur from the signing of informed consent through two weeks after study discontinuation will be recorded.

All AE and SAE information will be reviewed by physician investigator for assessment.

6.5 CONCOMITANT MEDICATIONS

Concomitant medication data will be collected from the subject, for all medication taken in the last 30 days. This will include prescription, over the counter medications and vitamins/supplements. Subjects will be asked about any medication changes at each site visit and phone call, with a particular focus on pain medications. Changes in the use of pain medicines will be analyzed to determine if subjects experience change in pain with therapy. This analysis may be complicated by the use of some pain medications as antipyretics (NSAIDs, acetaminophen). Efforts should be made to determine the exact indication for use of pain medications such as NSAID and Acetaminophen.

6.6 RENAL ULTRASOUND

Standard renal ultrasound will be completed according to visit schedule. Ultrasounds should be read locally and nephrocalcinosis graded according to the following grading scale:

0 = Normal

1 = Faint hyperechogenic rim around the sides and tip of the medullary pyramids

2 = More intense echogenic rim with echoes faintly filling the entire medullary pyramid

3 = Intense echoes throughout the medullary pyramid

4 = Solitary focus of echoes at the tip of the medullary pyramid/nephrolithiasis

6.7 HEIGHT, WEIGHT AND BSA

Height will be measured using a stadiometer that has routine calibration. Weight will be measured on scales that are routinely calibrated. All measurements should occur without shoes, pockets emptied and minimal clothing. (i.e. coats, heavy sweaters removed) BSA will be calculated using the equation below

$$Body\ Surface\ Area = \sqrt{\text{height (cm)x weight (kg)} \div 3600}$$

6.8 STUDY DRUG

Subjects will receive subcutaneous doses of ACTIMMUNE TIW for a total of 14 weeks. The dose of study drug will be determined using the subject's body surface area (BSA) calculated at each clinic visit as:

$$Body\ Surface\ Area = \sqrt{\text{height (cm)x weight (kg)} \div 3600}$$

To minimize side effects of the medication and improve tolerance we will start with a low dose and increase the dose according to a protocol developed by Devane et al [19]. Dose escalation will be titrated as follows:

- Day 0 to day 6: Subjects will receive 15 µg/m^2 SC TIW
- Day 7 to 14 : Subjects will receive 30 µg/m^2 SC TIW
- Day 15-55: Subjects will receive 50 µg/m^2 SC TIW
- Day 56 (end of week 8): Serum CTX will be assessed. Titration will occur by week 9 based upon serum CTX

- Week 9 (approx. day 63):
    - If CTX has increased by ≥25% above the mean of screening and baseline visits CTX values, the ACTIMMUNE dose stays at 50 μg/m^2 TIW through week 14.
    - If CTX is <25% above the mean of screening and baseline visits CTX values, then the ACTIMMUNE dose is increased to 100 μg/m^2 TIW through week 14.

These doses are based on the current approved dose of Actimmune® for severe, malignant recessive osteopetrosis and animal studies [6] demonstrating that relatively high doses of mouse interferon gamma 1b increased bone resorption in the ADO2 mouse. Data in melanoma patients showed that doses of 100 μg /m^2 TIW subcutaneously (SC) were well tolerated (N=5), however daily dosing of either 100 μg /m^2 SC (N=5), 100 μg /m^2 IM (N=10), or 250 μg /m^2 IM (N=5) resulted in suppressed absolute neutrophil count, and at the highest dose increased liver enzymes [20]. Their data also suggested that monocyte activation returned to normal after 72 hours of dosing, supporting the need for TIW dosing.

Dose reductions will be allowed on a case-by-case basis to manage subsequent drug-related adverse events (AEs). If, in the opinion of the investigator, severe reactions occur during dose escalation, the dose will be reduced to the previous dose level or will be interrupted until the adverse event resolves and then restarted at the previous or a lower dose level. Additionally, the dose can be reduced based on laboratory values. For example, we anticipate that the calcium/creatinine ratio will increase on drug due to increased bone resorption (the goal of therapy). Subjects will be encouraged to have high fluid consumption (>2 L/day for adults, >30ml/kg/day for children) and those subjects with urine calcium/creatinine ratios above 0.25 will be encouraged to consume a low calcium diet of less than half the age appropriate RDA. However, if either fasting random or 24 hour urine calcium/creatinine ratio increases above 0.5 the investigator can reduce the dose of study drug and/or put the subjects on a lower calcium diet.

Attempts should be made to keep dosing on a Monday, Wednesday, Friday dosing schedule. Missed doses can be taken the next day but two doses should not be taken in the same day. If a dose is missed and it is time for the next dose do not give 2 injections in one day. If two days have passed since the last dose the dose will be skipped.

Subjects should be instructed on how to give subcutaneous injections including drawing up the medication. The optimal sites for injection are right and left posterior arm and upper leg.

Study medication and supplies will be provided to subjects at clinic visits. Vials of *ACTIMMUNE* must be placed in a 2–8°C (36–46°F) refrigerator immediately upon receipt to ensure optimal retention of physical and biochemical integrity. DO NOT FREEZE. Avoid excessive or vigorous agitation. DO NOT SHAKE. An unentered vial of *ACTIMMUNE* should not be left at room temperature for a total time exceeding 12 hours prior to use. Vials exceeding this time period should not be returned to the refrigerator; such vials should be marked do not use. Vials are single dose vials and should be marked used after one use.

Vials should be provided to subject with cooler and gel packs sufficient to keep refrigerated during transit home. Subjects should also be provided with syringes, needles, alcohol wipes and sharps containers.

Study medication diary

Subjects will be provided a dosing diary. This should be used to note all doses given

including the dose, date and time. This should also be used to record missing doses.

Compliance

All vials used and unused should be returned with dosing diary at each study site visit. Subject compliance will be assessed based on dosing diary. Compliance will be calculated:

(Actual doses taken ÷ expected dose)*100= percent compliant

6.9 LAB COLLECTION

Venous blood samples will be collected by the experienced study staff from each subject using clean technique. Aliquots of blood will be sent for laboratory tests described in schedule of events.

Blood should be collected with the subjects in a fasting state with nothing but water 8 hrs prior to the draw.

Urine NTX should be from the second morning urine. Subject should void upon rising from bed and discard. The sample should then be collected from the next void.

6.10 RADIOGRAPHS

Skeletal survey radiographs

This should include anterior/posterior (A/P) and lateral cervical, thoracic and lumbar spines, swimmers view, A/P upper and lower ribs, pelvis, A/P femurs, A/P humeri, lateral skull.

Duel Energy absorptiometry (DXA)

This will measure lumbar spine, total body (total body minus head for children <18), total hip and femoral neck. Bone density testing is being done to document skeletal phenotype and to provide baseline if subjects continue in possible extension.

Spine QCT with Volumetric Spine BMD

This will be done per standard procedure for the radiology department. This will only be done in subjects 10 and older.

pQCT of Radius and Tibia

This will be done according to standard procedure. This will only be done on subjects 5y/o and over. pQCT will be used to assess cortical and trabecular bone sites at the radius and tibia to provide baseline evidence of the disease phenotype and to follow into possible extension study. Tomographic images of the distal (4% of bone length from distal end) and diaphyseal (66% of bone length from distal end) radius and tibia will be acquired with a Stratec XCT-3000 pQCT machine [21-24]. Standardized segmenting and thresholding algorithms will be used to acquire cortical and trabecular bone properties at the diaphyseal and distal sites, respectively. Measures will include vBMD, BMC, total area, cortical area, average cortical thickness, and periosteal and endosteal perimeters. The polar strength-strain index (SSI) and bone strength index (BSI) will be determined at the diaphyseal and distal sites, respectively [25].

Osteopetrosis is a disease of incredibly dense and thickened cortical bone, thus the quanititative assessment of trabecular bone density by QCT is a more accurate method for identifying the subtle or early changes of treatment which is paramount for this study.

6.11 STUDY VISIT SCHEMATIC

Study Phase	**Screening**	**Treatment Period**						**Follow-up**
Visit #	**SV[1]**	**1[2]**	**2**	**3**	**4**	**5**	**6**	**7[3]**
Week		**Baseline**	**2**	**4**	**8**	**11**	**14/ET**	**18**
Study Days (± visit window)	**-30 to -1 days**	**Day 0**	**14 (± 3) days**	**28 (± 3) days**	**56 (± 5) days**	**77 (± 5) days**	**98 (± 5) days**	**126 (± 3) days**
In person visit to IU[4]	X	X	X		X		X	X
Informed consent	X							
Review of inclusion/exclusion criteria	X	X						
Demographics and medical history	X							
SF-36		X					X	
Safety Assessments								
Physical examination	X	X	X		X		X	X[10]
Vital Signs: blood pressure, pulse, temperature	X	X	X		X		X	X
Baseline sign/symptom[9]	X	X						
Adverse Events[9]		X	X	X	X	X	X	X
Prior/concomitant medications	X	X	X	X	X	X	X	X
Renal ultrasound	X							X[11]
Study Medication								
Weight/height, BSA[5]	X	X	X		X		X	
Educate/review of dosing technique and dose level		X	X		X			
Study medication dose given at study visit[6]		X	X		X			
Dispense study drug[7]		X	X		X			
Drug compliance assessed by diary			X	X	X	X	X	
Dose escalation decision					X			
Laboratory Testing								
Serum pregnancy test [14]	X							
Urine pregnancy test [14]		X	X	X	X	X	X	X
Genotype (not required for enrollment)		X						
Efficacy assessments: Resorption marker testing -C-telopeptide [CTX], NTX, Tartrate resistant acid phosphatase type5b [TRAP5b])	X	X	X	X	X	X	X	X
Formation marker Testing (bone specific alkaline phosphatase [BAP], type 1 procollagen [P1NP])	X	X	X	X	X	X	X	X
RANKL, OPG, CK-BB (all batched and measured at the end of the study)		X			X		X	
Clinical laboratory evaluation (hematology, chemistry, PTH, Vit D $1,25(OH)_2$, urinalysis)	X	X	X	X	X	X	X	X
Fasting Urine: Calcium & Creatinine	X	X	X	X	X	X	X	X
IFN γ level prior to dose Day1; 6 hours post dose V1, V2 and V4		X	X		X		X	
IFN γ immunogenicity testing (prior to dosing except safety visit)[15]		X	X	X	X		X	X

Radiology Testing								
Skeletal survey (radiographs)	X							
Dual energy X-ray absorptiometry (DXA) lumbar spine, total body, total hip and femoral neck[8]	X							
Spine QCT[13]	X							
pQCT of radius and tibia [12]	X							
spine volumetric BMD by QCT[13]	x							

1 Screening procedures can take place over more than one day/clinic visit provided consent/assent is obtained first and all assessments are completed within the designated window.
2 On Day 0 (Baseline), subjects will receive the first dose of study drug while in the clinic; however, Baseline assessments will be performed prior to dosing.
3 Eligible subjects completing 14 weeks of treatment may enroll in a long-term extension study at week 14 visit.
4 Subjects and/or caregivers will be contacted by email or phone to monitor safety and dosing logistics on a weekly basis through Week 14.
5 Height and weight will be measured at Screening and then at all treatment visits to determine BSA and dose.
6 Dosing on Day 0 and Weeks 2, 8 and 14 will be performed at the clinic. Starting dose on Day 0 will be 15 mcg/m2 for 7 days (Week 1), then escalated to 30 mcg/m2 for 7 days (Week 2). At the Week 2 visit, subjects will be dosed at 50 mcg/m2 in the clinic. If tolerated, subjects will continue on this dose until Week 8. At the Week 8 visit the dose may be escalated to 100 mcg/m2 until Week 14. All other doses will be administered at home before bedtime on a three day a week (TIW) schedule.
7 Subjects will be given a 2 week supply of study drug on Day 0. At Week 2 visit, subjects will be given six week supply. At Week 8 visit, subjects will be given a 6 week supply of study drug.
8 Baseline values will be used to measure efficacy in subjects rolling over into the long-term extension study.
9 Any change in health status including any new signs and symptoms that occur after consent but prior to Day 0 and prior to dosing on Day 0 will be considered baseline signs/symptoms. Adverse events occurring or worsening after the dose on Day 0 through the end of the study will be considered treatment-emergent adverse events (TEAEs). All SAEs that occur from the signing of informed consent through two weeks after study discontinuation will be recorded.
10 Brief physical examinations will be performed at the Week 2 and Follow-Up Safety Visits; all other examinations will be complete physical examinations.
11 If a subject undergoes early termination from the study after week 6, then a renal ultrasound will be included during an early termination visit.
12 competed only if subject ≥5 years old
13 completed only if subject is ≥ 10 years old.
14 for women of child bearing potential.
15. If a subject tests positive for anti-drug antibodies (ADA), he/she will be followed until ADA levels revert to baseline.

6.12 VISIT DETAILS

Screening (-30 to -1)

Screening can occur over more than one day as long as the consent processes is completed first.

Screening will include all of the following:

Consent
Review of inclusion/exclusion
Collection of demographics, medical history, prior and current medications and baseline signs and symptoms.
Physical exam, vitals, height and weight
Renal ultrasound
Skeletal survey
DXA
Spine QCT
pQCT
Blood testing will include: pregnancy, hematology, Chemistry, PTH, Vit D 1,25, CTX, TRAP5b, Bone specific alk phos (BAP), P1NP
Urine tests (fasting) will include: NTX, UA, calcium, creatinine

Visit 1 Baseline (day 0)

Review of inclusion/exclusions
Physical exam, vitals, height and weight
Review of baseline symptoms and concomitant medication
Educate on dosing technique and dose level
Study medication dose given (1st dose)
Review of adverse events (acute events as first dose will be given on this day)
SF-36
Dispensing of study medication for 2 weeks
Dispense dosing diary
Blood testing will include: Genotype, CTX, TRAP5b, BAP, P1NP, RANKL, OPG, CK-BB, hematology, Chemistry, PTH, Vit D 1,25$(OH)_2$, IFN γ pre dose immunogenicity
IFN γ level predose and 6 hours post dose.
Urine test will include: pregnancy, NTX, UA, calcium, creatinine

Visit 2 week 2/ day 14 (±3 day)

Vitals, height and weight
Review of adverse events and concomitant medication
Review of dosing and injection procedures
Study medication dose given
Dispensing of study medication for 6 weeks
Dispense dosing diary
Collect all used study medication
Assess study medication compliance after diary review
Blood testing will include: CTX, TRAP5b, BAP, P1NP, hematology, Chemistry, PTH, Vit D 1,25$(OH)_2$, IFN γ immunogenicity
IFN γ level predose and 6 hours post dose.
Urine test will include: pregnancy, NTX, UA, calcium, creatinine

Visits 3 week 4 (±3 day) and visit 5 week 11 (±5 day)

These visits may be completed over the phone after blood work has been at a local central lab collection location.

Review of adverse events and concomitant medication
Assess study medication compliance after diary review
Labs to be collected at local collection site: Blood testing will include: CTX, TRAP5b, BAP, P1NP, hematology, Chemistry, PTH, Vit D $1,25(OH)_2$
Urine test will include: pregnancy, NTX, UA, calcium, creatinine
IFN γ immunogenicity at Week 4

Visit 4 week 8 (±5 day)

Physical exam, vitals, height and weight
Review of adverse events and concomitant medication
Review of dosing and injection procedures
Dose escalation decision
Study medication dose given
Collect all used study medication
Dispensing of study medication for 6 weeks
Dispense dosing diary
Assess study medication compliance after diary review
Blood testing will include: CTX, TRAP5b, BAP, P1NP, RANKL, OPG, CK-BB, hematology, Chemistry, PTH, Vit D $1,25(OH)_2$, IFN γ immunogenicity
IFN γ level predose and 6 hours post dose.
Urine test will include: pregnancy, NTX, UA, calcium, creatinine

Visit 6 week 14 or early termination (±5 day)

Physical exam, vitals, height and weight
Review of adverse events and concomitant medication
SF 36
Assess study medication compliance after diary review
Collect all study medication supplies
Blood testing will include: CTX, TRAP5b, BAP, P1NP, RANKL, OPG, CK-BB, hematology, Chemistry, PTH, Vit D $1,25(OH)_2$, IFN γ immunogenicity
IFN γ level predose.
Urine test will include: pregnancy, NTX, UA, calcium, creatinine
If early term after 6 weeks renal ultrasound should also be done.

Visit 7 week 18 Follow up (±3 day)

Physical exam, vitals, height and weight
Review of adverse events and concomitant medication
Renal ultrasound
Blood testing will include: CTX, TRAP5b, BAP, P1NP, RANKL, OPG, CK-BB, hematology, Chemistry, PTH, Vit D $1,25(OH)_2$, IFN γ immunogenicity
IFN γ level predose.
Urine test will include: pregnancy, NTX, UA, calcium, creatinine

6.13 RETENTION PLAN

Participating subjects will be seen or contacted by phone (or email) at least weekly to query regarding any concerns the subject may have regarding participation or adverse events, and

to improve retention and compliance. If a subject is having problems with flu like symptoms, which may affect participation in the study, dosage adjustments may be made.

In addition to aid in retention, two study visits may be done over the phone with a local collection of blood and urine for laboratory assessments, in order to be more convenient for the subject. However, the Screening, Day 0, week 2, week 8, week 14 and week 18 visits must occur at the Indiana University School of Medicine

7. 0 - REPORTING OF ADVERSE EVENTS OR UNANTICIPATED PROBLEMS INVOLVING RISK TO PARTICIPANTS OR OTHERS

7.1 UNANTICIPATED PROBLEMS

In the event of physical injury resulting from the participation in this research, necessary medical treatment will be provided and billed as part of the subjects medical expenses. Costs not covered by the subject's health care insurer will be the subject's responsibility. Also, it is the subject's responsibility to determine the extent of health care coverage. There is no program in place for other monetary compensation for such injuries. However, the subject has not given up any legal rights or benefits to which he/she is otherwise entitled.

7.2 DATA SAFETY MONITORING BOARD

An external and independent Data Safety Monitoring Board (DSMB) from the MARCH (Midwest Area Research Consortium for Health) will monitor this study and be provided with information about subjects' outcomes and adverse events every six months.

The study team will review the data for safety and benefits on a continual basis. The team will then generate a summary report based upon that internal review including a decision to stop dose escalation. This report will be provided to our primary MARCH DSMB contact, who will review at their next full DSMB meeting. All adverse events related to dose limiting toxicity will be reported, as they occur, to the MARCH DSMB.

A threshold of 25% of subjects having unexpected SAE's would result in an expedited review by the DSMB for whether the study should be modified or completely discontinued, except when the SAE is death or pregnancy having birth defect or for other SAE considered by the investigator (or DSMB) to represent a significant medical hazard. In the unlikely event of a subject death or the latter two events occur during the study, the study will be stopped and reviewed by the DSMB for whether the study should be discontinued. Because there is some risk of death with the underlying disorder, this will need to be assessed by the DSMB on a case by case basis.

7.3 ADVERSE EVENTS

An adverse event is defined as any unintended or abnormal clinical observation that is not of benefit to the subject. Either the condition was not present prior to exposure to test medication, or it has worsened in intensity or frequency following exposure to test medication. Adverse events will be graded according to the National Cancer Institute (NCI) Common Terminology Criteria v4.0 (CTCAE):http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm

The Sponsor-Investigator will be responsible for fulfillment of safety reporting obligations to authorities, investigators, and Ethic Committees according to applicable regulations, including reporting to applicable competent authority.

Principal investigators (PI) must report to the IRB as soon as possible, but in all cases within 5 working days from notification any event that appears on the List of Events that Require Prompt Reporting to the IUPUI IRB.

List of Events that Require Prompt Reporting to the IUPUI IRB: Any of the following:

- Event (including adverse events, injuries, side effects during the research study), which in the opinion of the PI
  - caused harm to one or more subjects or others, or placed one or more subjects or others at increased risk of harm; AND
  - was unexpected; AND
  - was related to the research procedures or study medications

Note: After the study is closed with the IRB, these events should only be reported if they are profound or they demonstrate long-term risks that would necessitate notifying subjects.

- Protocol deviation/violation (as defined under this policy and on-site only)
- Change to the protocol taken without prior IRB review to eliminate apparent immediate hazard to a research subject (e.g. purposeful and for subject safety) (on-site only)
- Complaint of a subject that indicates unexpected risks, or complaint that cannot be resolved by the research team (on-site only)
- Interim findings and safety monitoring reports that indicate an unexpected change to the risks or potential benefits of the research, in terms of severity or frequency
- Publication in the literature that indicates an unexpected change to the risks or potential benefits of the research
- Change in FDA labeling or withdrawal from marketing of a drug, device, or biologic used in a research study
- Noncompliance (as defined in this policy and on-site only)

Unanticipated problems involving risk to volunteers or others, serious adverse events related to participation in the study, and all volunteer deaths related to participation in the study should be promptly reported to study staff by phone (317-948-8346), by email (marihart@iu.edu), or by facsimile (317-948-2254).

The principal investigator must complete the FDA MedWatch 3500a form and assess the relationship to study treatment and send the initial completed MedWatch form within 24 hours. The investigator must then ensure that the form is accurately and fully completed with follow-up information within 2 to 3 calendar days for deaths or life-threatening events and 5 calendar days for other serious adverse events. The original and the duplicate copies of the FDA MedWatch form and the fax confirmation sheet must be kept with the case report forms at the study site.

Follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not, and whether the subject continued or discontinued study participation. The MedWatch form sheet must be retained at Indiana University. Pregnancy follow-up should describe the outcome of the pregnancy, including any voluntary or spontaneous termination, details of the birth, and the presence or absence of any congenital abnormalities or birth defects.

8. 0 – STATISTICAL CONSIDERATIONS

The sample size N=12 is justified based on the following hypothesis testing: H0: ΔCTX(%)=0 versus Ha: ΔCTX(%)≥25% using one-sample Student's t-test. Consequently, we will have a power of 80% at type I error level 0.05 if the corresponding standard deviation is no bigger than 28.1%, which is considered as obtainable from our previous experiences.

Percent changes in bone markers (serum CTX and fasting NTX) from baseline, denoted as Δ's, will be summarized as means and standard deviations (SDs) and be tested as H0: Δ=0 versus Ha: Δ≠0. This endpoint will be assessed on all subjects tolerating at least 30 ug/m^2 TIW.

Longitudinal profiles of bone turnover markers will be evaluated by linear mixed effects models. Time will be included as both a continuous variable and a categorical variable to account for potentially nonlinear effects. Graphs of individual subject curves of CTX and NTX/creatinine ratios and other bone turnover markers over time will also be generated.

CTX and NTX/creatinine concentrations during dose escalation, at peak dose, and at study end will be analyzed by repeated measure analysis of variance to evaluate dose response and to determine whether bone resorption markers are stable once final dose therapy is achieved.

Fasting urine calcium/creatinine ratio between baseline and while on drug, which are anticipated to increase with therapy, will be analyzed by linear or nonlinear mixed effects models, depending the distribution of the ratio.

Baseline fracture rates (fractures per year in the 2 years prior to the start of the study) and the fracture rates in the year while on therapy for subjects enrolled in the long-term extension study will be summarized by descriptive statistics. We do not expect that there will be enough statistical power to conduct a formal hypothesis test for these 12 subjects alone.

Change from baseline in DXA in subjects enrolling in the long-term extension study will be measured at the end of one year of therapy in the extension study (primary endpoint for the extension study). DXA changes will be summarized by mean (SD) and 95% confidence interval.

The proportions of subjects obtaining ≥25% bone marker changes from baseline will be estimated with their 95% Agresti-Coull confidence intervals.

Baseline characteristics will be summarized by descriptive statistics.

Subjects may drop out for a variety of reasons including expected or unexpected AEs of the therapy. If a subject drops out before visit 5 they will be replaced with a new subject. This decision is based on having adequate data on the primary outcome of bone turnover

markers to interpret the study results. If a subject drops out after visit 5, they will not be replaced.

9. 0 – PRIVACY/CONFIDENTIALITY ISSUES

Data sources will include paper and electronic medical records and test results. Data will be recorded on paper, desktop computers, and laptop computers. The Principal Investigators, Research Coordinators, Co- Investigators, Biostatistics, and Governmental Agencies will have access to the individually identifiable data. This data will be safeguarded by locking storage cabinets and office doors, keeping information in areas with limited public access, password protecting computers and files, and regular back-ups of electronic data. Data will be retained indefinitely. When confidential data needs to be discarded, paper records will be shredded, CD's and diskettes will be destroyed, and data will be permanently deleted from computers. When possible, only de-identified data will be shared. If identifiable data will be shared, it will be done by fax in a secured area, on a shared drive with password protection, or by personal delivery by authorized research personnel.

10.0 FOLLOW-UP AND RECORD RETENTION

10.1 SUBJECT COMPENSATION

Subjects will be reimbursed for travel (driving) and parking to study visits and will receive an honoraria for each on site visit as follows: $100 for the first visit; $40 for subsequent visits; and $25 for local sample collection.

10.2 RECORD RETENTION

All records will be kept for a minimum of 15 years.

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