Open-label Phase 1/2 Study with a Single Arm of Interferon Gamma-1b Treatment of Osteopetrosis

Short title: ACTIMMUNE in OP

Regulatory Sponsor: Lynda Polgreen, MD, MS
Assistant Professor
Pediatric Endocrinology
LA BioMed at Harbor-UCLA
1124 West Carson Street, RB-1, Box 446
Torrance, CA 90502
Telephone: 310-222-1961
Facsimile: 310-222-3887
lpolgreen@labiomed.org

Funding Sponsor: Horizon Pharma

Study Product: Interferon gamma-1b **(**ACTIMMUNE®)

IND Number: 127052

Trial Registration: NCT02666768

IRB Number: 21549-01

Version / Date: version 1.8 / 13-Dec-2017

Version History

Version #	Approval Date	Significant Changes from Previous Version
1.1		1. Added anti-drug antibodies (see Sections 6.2.4 and 6.3.2, and Tables 1 and 2). 2. Added specific genotypes that will be tested if an osteopetrosis related mutation has not been previously identified (see Section 6.3.2 and Tables 1 and 2). 3. Added justification for maximum dose and criteria to de-escalate dose (see Sections 3 and 6.3.6) 4. Added the following withdrawal criteria: subject contracts an intercurrent illness that requires treatment not consistent with the protocol requirements. (see Section 5.4.8) 5. Added specification that the method used to calculate body surface area (BSA) is the Mosteller method (see Section 6.3.6). 6. Added exclusion criteria of history of hematopoietic cell transplantation (HCT) 7. Additional details on ex vivo assays were added to Section 6.3.5.5.
1.2	22Mar2016	Study subject compensation was clarified in section 6.9.
1.3	12Apr2016	1. Added that screening will be within 3 months of baseline visit 2. Added 25-hydroxy vitamin D level at 6 week lab visit 3. Added additional instructions for processing blood and urine specimens (see Table 2 and sections 6.2.2, 6.2.3, and 6.2.4) 4. Safety monitor was changed due to conflict of interest for Dr. Peter Liu (see Appendix A)
1.4	7Sept2016	Added collection of a PAXgene tube for RNA extraction and buffy coat to be stored for future DNA studies (see Table 2, and Sections 6.3.2 and 9.6).
1.5	22Nov2016	Added “thalassemia or other hemoglobinopathy” to the exclusion criteria.
1.6	8Mar2017	1. Updated PI contact info 2. Changed UMN site PI due to Dr. Petryk leaving UMN. 3. Section 5.4.4: Language was clarified to indicate that HCT cannot be expected to occur within 12 months of staring this study. 4. Section 5.4.6: Added exclusion criteria of a left ventricular ejection fraction of <30% to ensure consistency in using the screening ECHO results in determining enrollment in study. 5. Section 6.3.2: Corrected labs to be collected at weeks 4 and 6 and month 9. This paragraph is now consistent with informed consent document and other areas in this protocol. 6. Section 6.3.2: Added adults ≥18 years old to the vitamin D and calcium treatment plans.

1.7	10Aug2017	1. Section 6.3.4.2: Language was clarified to add 3% and 38% sites of the non-dominant radius. 2. Clarification of timing of RNA and DNA sample collection was added to Table 1. 3. Section 6.9: Clarification of subject compensation between LA Biomed and University of Minnesota sites.
1.8	13Dec2017	1. Section 6.2.2: Clarification of DNA, RNA and Urine collection, processing, shipping and Storage. 2. Section 6.9: Correction of subject compensation which will be the same at each site. 3. Section 6.3.6: Extended duration for study drug titration to maximum dose and added recommendations for medications that may be used to treat adverse effects of the study drug. 4. Changed site PI and clinical coordinator at UMN, and clinical coordinator at LA BioMed. 5. Improved language in sections 5.4.4, 6.2.2, 6.2.3, and 8.2 to improve clarity. 6. Section 6.2.3: Changed the shipping contact and address for samples sent to UMN.

Table of Contents

Study Summary

Title	Open-label Phase 1/2 Study with a Single Arm of Interferon Gamma-1b Treatment of Osteopetrosis
Study Sponsor	Lynda Polgreen, MD, MS Assistant Professor, Pediatric Endocrinology David Geffen School of Medicine Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center
Principal Investigator (PI)	Lynda Polgreen, MD, MS Assistant Professor, Pediatric Endocrinology David Geffen School of Medicine Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center
Study Design	multi-center open-label phase 1/2 clinical trial
Study Duration	Subjects will participate in the study for 1 year. It is expected that enrollment will be completed in 18 months and the last subject will complete the protocol 2.5 years after study initiation.
Study Center(s)	1. LABioMed at Harbor-UCLA 1124 W. Carson St. Torrance, CA 90502 2. University of Minnesota 2450 Riverside Avenue Minneapolis, MN 55454
Study Objectives	1. To determine the feasibility and tolerability of interferon gamma-1b treatment in patients with intermediate osteopetrosis. 2. To determine the safety and efficacy of interferon gamma-1b in patients with intermediate osteopetrosis.
Study Endpoints	Primary Endpoint: The primary objective of this pilot study is to determine the feasibility and tolerability of interferon gamma-1b treatment for 1 year in patients with intermediate osteopetrosis. Specifically, i) the ability to enroll patients, and ii) continued treatment throughout the 1-year observational period. Secondary Endpoints: The secondary objectives of this study will focus on change in immunologic and hematologic function, bone mineral density and osteoclast function, physical function and quality of life.
Number of Subjects	10
Main Inclusion Criteria	• Intermediate Osteopetrosis, defined as: o Anemia, or o Neutropenia, or o ≥ 1 serious infection over prior year defined as requiring hospitalization and/or IV antibiotics, or o History of impaired bone healing • Age > 1 year
Duration of Observation	1 year
Statistical Methods	Descriptive analyses of baseline characteristics and outcomes will include means and standard deviations for continuous variables and frequencies for categorical variables. Confidence intervals and P-values will be based on a t-distribution. Statistical significance will be considered as $p<0.05$. The primary endpoint and safety analyses will be primarily descriptive reporting the number and percentage of compliance and AEs. The secondary endpoints for hematologic function, immune function, bone mineral density, physical function and quality of life will be evaluated by paired t-tests or chi-squared to evaluate change from baseline to 12 months. Secondary endpoints related to in vitro osteoclast assays will be descriptive.

List of Abbreviations

AE	Adverse Event
BMI	Body Mass Index
CAII	Carbonic Anhydrase II
CBCD	Complete Blood Count with Differential
CFR	Code of Federal Regulations
CHQ-PF50	Children's Health Questionnaire-Parent Form 50
CLCN7	Chloride Channel 7
CNS	Central Nervous System
CRF	Case Report Form
CRP	C-Reactive Protein
CTSI	Clinical and Translational Science Institute
DNA	Deoxyribonucleic Acid
DSMB	Data and Safety Monitoring Board
EDTA	Ethylenediaminetetraacetic Acid
ELISA	Enzyme Linked Immunosorbent Assay
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HCT	Hematopoietic Cell Transplantation
HIPAA	Health Insurance Portability and Accountability Act of 1996
IATA	International Air Transport Association
ICH	International Conference on Harmonization
IM	Intramuscular
IV	Intravenous
IDS	Investigational Drug Services
Ig	Immunoglobulin
IL	Interleukin
IND	Investigational New Drug
IRB	Institutional Review Board
ITT	Intent-To-Treat
LFT	Liver Function Tests
OSTM1	Osteopetrosis-associated Transmembrane Protein 1
PHI	Protected Health Information
PI	Principal Investigator
PP	Per Protocol
pQCT	Peripheral Quantitative Computed Tomography
RANKL	Receptor Activator of Nuclear Factor-κ B Ligand
REPA	Report of External Professional Activities
ROM	Range of Motion
SAE	Serious Adverse Event
SDS	Standard Deviation Score
SF-36	Medical Outcomes Study Short Form-36
SST	Serum Separation Tube
SC	Subcutaneous
TBD	To Be Determined
TCIRG1	T-cell Immune Regulator 1
UMN	University of Minnesota
UPIRTSO	Unanticipated Problems Involving Risk To Subjects or Others

USP	United States Pharmacopeia
vBMD	Volumetric Bone Mineral Density

1 Study Contact Information

1.1 Principal Investigator (PI) / Sponsor

Lynda Polgreen, MD, MS
Assistant Professor
Pediatric Endocrinology
LA BioMed at Harbor-UCLA
1124 W. Carson St.
Liu Research Bldg (RB3)
Torrance, CA 90502
Telephone: 310-222-1961
Facsimile: 310-972-2962
Email: lpolgreen@labiomed.org

1.2 UMN Site PI

Paul Orchard, MD
Professor
Pediatric Blood and Marrow Transplantation
University of Minnesota
420 Delaware St., MMC 366
Minneapolis, MN 55455
Telephone: 612-626-2313
Facsimile: 612-626-4074
Email: orcha001@umn.edu

1.3 Co-Investigators

Kim Mansky, PhD
Associate Professor
Develomental/Surgical Sci-Orthodont
Room 16-146 MoosT
515 Delaware St SE
Minneapolis, MN 55455
Telephone: 612-626-5582
Facsimile: 612-301-5293
Email: kmansky@umn.edu

1.4 Study Personnel

Clinical Coordinator (LA BioMed):

Allison Barnwell
Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center
1124 West Carson Street
Torrance, CA 90502
Office: (310) 7803-5623
allison.barnwell@labiomed.org

Angel "Jun" Zozobrado
Los Angeles Biomedical Research Institute at Harbor-UCLA Medical Center
1124 West Carson Street
Torrance, CA 90502
Office: (310) 781-3652
azozobrado@labiomed.org

Clinical Coordinator (UMN):

Ka Xiong
University of Minnesota
Masonic Cancer Center
Clinical Trials Office
Minneapolis, MN 55455
Office: (612) 626-5331
herx0088@umn.edu

2 Introduction / Background and Rationale

2.1 **Description of the disease**.

Osteopetrosis is a rare inherited metabolic bone disease characterized by impaired osteoclast function resulting in defective bone resorption and generalized high bone mass and mineral density (**BMD**). In patients with severe disease, this high bone mass compromises bone marrow space leading to marrow failure and frequent infections, along with hepatosplenomegaly from extramedullary hematopoiesis (1). Visual defects may result due to impingement of cranial nerves. Other sequelae include hypocalcemia and hypocalcemic seizures and "brittle bones" or osteopetrotic rickets with an increased risk of fracture (2).

Most patients with severe, autosomal recessive osteopetrosis have a defect in the osteoclast H^+-ATPase proton pump; mutations affecting the production of carbonic acid and the chloride channel have been reported as well (1). The mutations result in varying degrees of osteoclast dysfunction and a wide spectrum of disease. Osteopetrosis is generally divided into three categories based on clinical aspects of disease: 1) infantile or "malignant," inherited in an autosomal recessive fashion; 2) "intermediate," associated with non-lethal recessive mutations, or a more severe phenotype of dominant disease; this is the patient population that will be included in this study; and 3) adult ("benign") autosomal dominant osteopetrosis with generally milder clinical course (1). Currently, the only treatment for individuals with severe forms of osteopetrosis is hematopoietic cell transplantation (**HCT**), however survival in patients with autosomal recessive osteopetrosis treated with HCT is only around 55% (3). Therefore, this treatment is only indicated in select individuals with life-threatening complications of their disease. Clearly, additional treatments for osteopetrosis are needed both for individuals who are not candidates for HCT and to prolong the time until HCT is needed.

2.2 **Interferon gamma-1b in osteopetrosis**.

Interferon gamma is a naturally occurring cytokine that has been shown to have anti-microbial and anti-viral immunomodulatory effects (4), and is a potent stimulator of superoxide anion production which in turn promotes the formation and activation of osteoclasts (5). Interferon gamma-1b (**IFN-γ1b**) has been shown to increase osteoclast size, tartrate acid phosphatase staining, and superoxide anion production in cell culture from patients with osteopetrosis (6). In addition, osteopetrotic mice had increased marrow space following treatment with IFN-γ1b (7). Two previous studies of IFN-γ1b in a small group of individuals with osteopetrosis found a decrease in trabecular bone area, an increase in marrow space, a decrease in the number of severe infections requiring antibiotic therapy, and an increase in superoxide generation by granulocyte-macrophage colonies (8,9).

Despite these encouraging findings and FDA approval of interferon gamma-1b for the treatment of severe, infantile "malignant" osteopetrosis, IFN-γ1b is not routinely prescribed for the treatment of intermediate osteopetrosis because the FDA approval does not include this more milder form of the disease; however, many of these patients have disease progression requiring HCT just at a later age than that for the severe infantile form. Due to these issues, and the small number of patients previously treated (8,9) there is a need to further delineate who will benefit from treatment, as some osteoclast defects may be more responsive than others.

2.3 Significance of research

At the conclusion of this study, we will have obtained data on the tolerability and efficacy of IFN-γ1b that, if encouraging, will be used to increase awareness about this treatment option among clinicians taking care of patients with intermediate osteopetrosis. We expect a decrease in disease burden, and improvement in health and quality-of-life for participants in our study. Finally, the proposed project should provide further insight into possible variability in response to treatment based on genetic abnormality. These contributions are significant because there is currently no effective treatment for osteopetrosis except HCT, which carries a high mortality rate (3,10).

3 Interferon gamma-1b (ACTIMMUNE) Description/Indications

ACTIMMUNE® (IFN-γ1b), a biologic response modifier, is a single-chain polypeptide containing 140 amino acids. ACTIMMUNE is a synthetic version of IFN-γ1b, a naturally occurring biologic response modifier.

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, 0.05 mg polysorbate 20 and Sterile Water for Injection. Note that the above activity is expressed in International Units (1 million IU/50 mcg). This is equivalent to what was previously expressed as units (1.5 million U/50 mcg).

ACTIMMUNE was first approved by the FDA in 1999 (Biologics License Application # 103836) and is currently FDA approved for the treatment of chronic granulomatous disease and severe malignant osteopetrosis. FDA has approved ACTIMMUNE to decrease the number and severity of infections in patients with chronic granulomatous disease and to delay the progression of severe, malignant osteopetrosis.

The original Phase 1 clinical development of ACTIMMUNE was conducted in oncology/AIDS patients and maximum tolerated doses based on intramuscular (IM) or intravenous (IV) administration were established for doses ranging from 10-1000 mcg/m^2/day. Subsequently, daily doses of 100 mcg/m^2 ACTIMMUNE by the subcutaneous (SC) route of administration was chosen based on an NCI study comparing immunological activity (including enhanced superoxide/hydrogen peroxide production) of different doses and routes of administration in melanoma patients (11). SC

administration was compared with IM administration using the 100 mcg/m^2 dose. SC administration resulted in enhanced hydrogen peroxide production and Fc receptor expression by monocytes. A comparison of two schedules, daily and three times weekly, suggested that monocyte activation may persist for up to 72 hours after ACTIMMUNE administration. Of the doses tested, 100 mcg/m^2 administered daily or three times weekly appeared to be the most effective biological response modifier regimen, and due to ease of administration, the SC route was preferred. Since then, many studies have found similar tolerability and toxicity for 100 mcg/day and 200 mcg/day (12–17). Thus, based on these previous studies of dosing safety and efficacy, we chose a maximum dose of 100 mcg/m^2 SC three times weekly for this Phase 1/2 trial.

This is an investigator-initiated, investigator-held IND, multi-institutional research study. ACTIMMUNE is FDA-approved are described above, but not for the treatment of intermediate osteopetrosis.

4 Study Objectives / Hypotheses

4.1 Primary Hypothesis

The primary hypothesis to be tested is:

That treatment with IFN-γ1b will be feasible and tolerable to patients with intermediate osteopetrosis.

4.2 Secondary Hypotheses

The secondary hypotheses are:

1. Compared to baseline, treatment with IFN-γ1b will result in:

    a. Decreased tibial volumetric bone mineral density (**vBMD**) and estimated bone strength measured by peripheral quantitative computed tomography (**pQCT**);

    b. Decreased total body and lumbar spine areal BMD (**aBMD**) by dual-energy x-ray absorptiometry (**DXA**);

    c. Increased levels of markers of bone remodeling (see Table 1);

    d. Increased osteoclast activity by *in vitro* testing;

    e. Increased white blood cell (**WBC**) and platelet counts in patients that have lower than expected blood counts at baseline.

    f. Increased height standard deviation score (**SDS**) for participants <18 years with open growth plates;

    g. Decreased inflammation measured by erythrocyte sedimentation rate (**ESR**);

h. Decreased pain score (based on prior 4 weeks);

i. Increased physical function score (based on prior 4 weeks); and

2. Compared to the 12 months prior to study entry (based on medical record review or subject recollection), treatment with IFN-γ1b over 12 months will result in:

a. Decreased number of hospitalizations for infection;

b. Decreased number of days treated with antibiotics;

c. Beneficial change in WBC and platelet counts in patients who have lower than expected blood counts at baseline;

d. Beneficial change in ESR;

e. Increased height SDS for participants <18 years with open growth plates; and

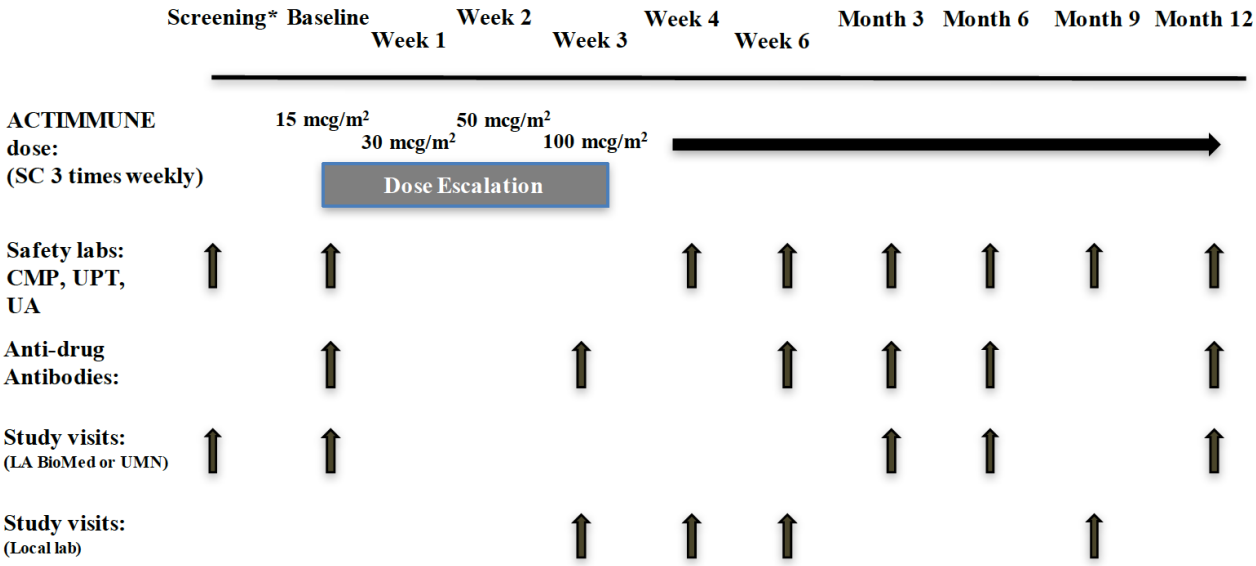
3. Patients will not develop neutralizing antibodies.

5 Study Design

5.1 Overview of Study Design

This is a multi-center, open-label, 12-month clinical trial of ACTIMMUNE treatment of patients with intermediate osteopetrosis. Study design is summarized in Figure 1. Routine clinical safety and efficacy data (see Table 1) will be obtained at baseline, weeks 4 and 6, and months 3, 6, 9, and 12

following the initiation of therapy. Additional outcomes (see Table 1) will be obtained at baseline, 3, 6 and 12 months.



*Includes medical history, ECHO, and EKG.

Fig. 1. Study Design Schematic

5.2 Anticipated Duration of the Clinical Investigation

Each subject will be enrolled in the study for 12 months. It is expected that 15 patients will be screened over 18 months to enroll 10 participants, with the final subject completing study participation 2.5 years after study initiation. The final study report will be completed approximately 3 years after study initiation. If the study is terminated prior to completion, a closeout report will be filed with the Institutional Review Board (**IRB**) within 3 months of study termination.

5.3 Evaluations and study timeline

Table 1. Evaluations and study timeline

	Screening[a]	Baseline	# of months from 1st IFN-γ 1b treatment (± 1 week)		
			3	6	12
General					
• Medical history	X	X	X	X	X
• Physical exam		X	X	X	X
• Genotype[b]		X			
• Anthropometric measurements	X	X[f]	X	X	X
• RNA and DNA assays		X	X	X	X
Safety Measures[c]					
• Basic metabolic panel (BMP)	X	X	X	X	X
• Liver function tests (LFT)	X	X	X	X	X
• Urine pregnancy test (UPT)[e]	X	X	X	X	X
• Urinalysis (UA)	X	X	X	X	X
• ECHO with interpretation	X				
• EKG with interpretation	X				

• Anti-drug antibodies (ADA)[g]		X	X	X	X
Bone Biomarker Testing[d]					
• Formation Testing (bone specific alkaline phosphatase [BAP], type 1 procollagen [P1NP])	-	X	X	X	X
• Resorption testing (C-telopeptide [CTX], Tartrate resistant acid phosphatase type5b [TRAP5b])	-	X	X	X	X
• RANKL, OPG, CK-BB	-	X	X	X	X
Endocrine Assessment					
• Total 25-OH Vitamin D (25OHD)[h]	-	X	X	X	X
• Serum ionized calcium	-	X	X	X	X
• Urine calcium: creatinine	-	X	X	X	X
• Intact parathyroid hormone (iPTH)	-	X	X	X	X
Inflammatory/Immunology					
• CBC with differential and platelets	-	X[f]	X	X	X
• Erythrocyte sedimentation rate (ESR)	-	X[f]	X	X	X
• Immunologic phenotyping (CD3, 4, 8, 14, 15, 16, 19, 34, 56)	-	X	X	X	X
Neuropsychological testing					
• Pain assessment	-	X	X	X	X
• Quality of life survey	-	X	X	X	X
Orthopedic assessment					
• Dual energy X-ray absorptiometry (DXA) lumbar spine, total body, total hip and femoral neck (adults ≥18yrs only)	-	X	-	-	X
• Peripheral Quantitative CT (pQCT) left tibia	-	X	-	X	X
In vitro osteoclast function assays – Mansky Lab					
• Gene expression – osteoclast marker genes	-	X	X	X	X
• Osteoclast activity – ability to release calcium phos from coated plate	-	X	X	X	X
Infectious outcomes					
• Number of hospitalizations in prior 3 months	-	X[f]	X	X	X
• Number of days treated with antibiotics in last 3 months	-	X[f]	X	X	X
• Type of infectious organism	-	X[f]	X	X	X

RANKL=Receptor activator of nuclear factor kappa-B ligand; OPG=osteoprotegerin; CK-BB=creatinine kinase isoenzyme BB.

[a] Screening visit evaluations will count for baseline evaluations where applicable. Screening visit will be within 3 months of baseline visit

[b] Genetic testing for CLCN7, CAII, TCIRG1, OSTM1, and RANKL mutations will be done at the University of Minnesota if not already performed clinically

[c] Safety labs will also be obtained at weeks 4 and 6 ± 2 days, and month 9 ± 1 week; subjects will be contacted on a weekly basis either by email or telephone to monitor safety during the initial 4 week dose escalation period.

[d] Sample collection will be postponed if needed to ensure collection is more than 2 weeks since last fever (>100.4°F [38°C] axillary) or fracture.

[e] Post-menarchal females.

[f] Height (for subjects <18 years), CBC, and ESR will be obtain from the patient's medical record when available and number of hospitalizations and infectious data from the patient's medical record or if not available then the patient's recollection at the baseline visit.

[g] ADA testing will also be performed at weeks 3 and 6 ± 2 days after the first dose, and 2-3 weeks after last dose, and then every 4-6 weeks until values return to baseline.

[h] 25OHD level will be checked at 6 weeks as well.

5.4 Study Population

This study will include patients diagnosed with osteopetrosis who are ≥1 year of age, have anemia, neutropenia, frequent serious infections, or impaired bone healing.

5.4.1 Sample Size

This is a pilot study; therefore, the sample size was determined based on clinician expert opinion and prevalence of the disease (estimated at 1/100,000-500,000) to estimate the number of potential participants who could be enrolled over 18 months.

5.4.2 Subject Recruitment

Subjects will be recruited from the Osteopetrosis Society website, clinicaltrials.gov, and provider referral.

5.4.3 Subject Screening

Due to the rarity of this disease patients will be traveling from all parts of the country to participate and therefore the screening may occur prior to the subject traveling to the study center to avoid unnecessary travel and therefore consent will be obtained verbally by phone when necessary (by the PI or study coordinator). At the Baseline visit, if not signed previously, consent and assent forms will be again reviewed in detail and the patient and/or their parents/legal guardians will have the opportunity to ask any questions they may have. They will also be asked to describe the protocol prior to signing these forms to ensure that all details are clearly understood. Medical records will then be reviewed to confirm eligibility.

5.4.4 Prior and Concomitant Therapy

Potential participants will not be enrolled if they expect to be treated with HCT within 12 months of entering the study. All other therapies are permitted.

5.4.5 Inclusion Criteria

Patients will be eligible to participate in the study if all of the following conditions exist:

1. Diagnosis of osteopetrosis based on radiographic findings and
2. Anemia (Hemoglobin <12 g/dL) not related to iron deficiency, or
3. Neutropenia (Neutrophil count <1000 neutrophils/ul unsupported with cytokines), or
4. Thrombocytopenia (Platelet count <50,000 cells x 10^9/L), or
5. History of impaired bone healing, or
6. ≥ 1 serious infection over prior year defined as requiring hospitalization and/or IV antibiotics, and
7. Age > 1 year; and
8. Ability to travel to a study center for every 3-6 month study visits; and

9. Patient or parent/legal guardian is able and willing to provide informed consent. For patients 7 to 17 years of age, assent must also be provided.

5.4.6 Exclusion Criteria

Patients will be excluded from participation in the study if any of the following conditions exist:

1. History of HCT;
2. Pregnancy or breastfeeding;
3. Known or suspected allergy to interferon gamma-1b or related products;
4. Participation in simultaneous therapeutic study that involves an investigational study drug or agent within 4 weeks of study enrollment;
5. ALT greater than 3 fold higher than normal;
6. Thalassemia or other hemoglobinopathy due to potential for inhibition of erythropoiesis and/or increased red blood cell destruction (18–21); or
7. Left ventricular ejection fraction measured by ECHO of <30%; or
8. Any other social or medical condition that the Investigator believes would pose a significant hazard to the subject if the investigational therapy were initiated or be detrimental to the study.

5.4.7 Participation of Females

The fetal risks associated with IFN-γ1b are not known, but pre-clinical animal data demonstrate some risk (Pregnancy category C). Females of childbearing potential must agree not to become pregnant during the study and must have a pregnancy test at each study visit (baseline, month 3, month 6, and month 12). Because of the risk involved, sexually active subjects and their partners must use two methods of birth control. They must continue to use both methods until 1 month after stopping study drug. Two of the birth control methods listed below may be chosen:

- Hormonal contraception

- Male or female condoms with or without a spermicidal
- Diaphragm or cervical cap with a spermicidal
- Intrauterine device (IUD)

5.4.8 Study subject Exit/Discontinuation Criteria

National Cancer Institute (**NCI**) Common Terminology Criteria for Adverse Events (**CTCAE**), Version 4 will be used to define an Adverse Event (**AE**). The CTCAE Grades 1 through 5 with unique clinical descriptions of severity for each AE will be used and defined as follows:

Grade 1 Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated.

Grade 2 Moderate; minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental Activities of Daily Living (**ADL**)*.

Grade 3 Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self care ADL**.

Grade 4 Life-threatening consequences; urgent intervention indicated.

Grade 5 Death related to AE.

A Semi-colon indicates 'or' within the description of the grade.

*Instrumental ADL refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.

**Self care ADL refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

Subjects will discontinue participation in study for any unexplained CTCAE Grade 3 or higher that is not reasonably related to the underlying disease process or treatments. In addition, subjects will discontinue study participation if any of the following conditions exist:

1. Subject voluntarily withdraws from the study.
2. Subject's death.
3. Subject completes the protocol.
4. Pregnancy.

5. Subject acquires any of the listed exclusion criteria.

6. Subject is non-compliant with the protocol. For example, missing the Month 3 visit (no visit by 120 days after beginning therapy).

7. Subject's well-being, in the opinion of the Investigator would be compromised by continuation in the study.

8. Subject contracts an intercurrent illness that requires treatment not consistent with the protocol requirements.

6 Study Procedures

6.1 Informed Consent and Assent

Each subject or the subject's parent/legal guardian will be provided a consent form describing this study and providing sufficient information to make an informed decision about their participation in this study. The consent form will be submitted with the protocol for review and approval by the IRB for the study. The formal consent of an adult subject or minor subject's parent/legal guardian, using the IRB-approved consent form, must be obtained before that subject is submitted to any study procedure. This consent form must be signed by the subject and/or parent/legal guardian, and the Investigator-designated research professional obtaining the consent. A blank copy of the IRB-approved form must be kept on-site by the Investigator.

Consent process (in person) will take place in a private room. We will ensure that there is an ample waiting period available between informing the prospective subject and obtaining the consent. There will be dialogue during each contact with the subject to ensure ongoing consent is voluntary.

Non-English Speaking Subjects

- Spanish speaking subjects will be included.
- Telephone or interpreter will be used.

Subjects who are not yet adults (infants, children, teenagers)

- Subjects in the United States are considered minors if they are 17 years or younger, with the exception of Alabama (18 years), Nebraska (18 years or upon marriage), and Mississippi (20 years)
- Parental permission will be obtained from:
  - One parent even if the other parent is alive, known, competent, reasonably available, and shares legal responsibility for the care and custody of the child.

- Parental or guardian (as defined by DHHS and FDA regulations) consent will be obtained. A guardian must provide written documentation of the legal ability to consent to for the child's participation in research. A copy of this documentation will be kept with the consent document.
- Assent will be obtained from all minor subjects aged 7 to 17 years.
- Assent will be documented with their signature on the assent form.

Adults Unable to Consent

The individuals from whom permission will be obtained are the following in descending order of priority:

- The person's agent pursuant to an advance health care directive.
- The conservator or guardian of the person having the authority to make health care decisions for the person
- The spouse of the person
- An individual as defined in Section 297 of the Family Code
- An adult son or daughter of the person
- A custodial parent of the person
- Any adult brother or sister of the person
- Any adult grandchild of the person
- An available adult relative with the closest degree of kinship to the person

The process for assent of the subjects is as follows:

- Assent will be required of all subjects age 7-17 years [except for Alabama (age 7-18 years), Nebraska (age 7-18 years or upon marriage), and Mississippi (age 7-20 years)] and for cognitively impaired adults.
- The process for assent will include reviewing the assent document with the subject and their parent/guardian in a verbal face-to-face discussion, asking them for questions and discussing these, then asking the subject to sign and date the assent document if they would like to participate in the study.
- Assent will be documented in a progress note.

6.2 Laboratory Testing Procedures

6.2.1 Specimen Preparation

Specimens for analysis will be collected as described in Table 2 at the time points indicated in Table 1. Venipuncture will be performed by trained certified nursing assistants, nurses, nurse practitioners, or physicians and collection and handling will be per the manufacturer's recommendations. A central venous catheter may be utilized for all blood sampling if the patient currently has one. Serum, plasma, and urine will be collected and stored at -80°F.

Table 2. Laboratory Analytes and Collection Information.					
Analyte	**Specimen**	**Tube Type**	**Notes**	**Batched at end of study?**	**Volume**
High sensitivity erythrocyte sedimentation rate (**ESR**)	Plasma	K_2EDTA (purple-top tube)	Quest/Fairview	no	8 mL blood (2 tubes)
Bone specific alkaline phosphatase (**BAP**)	Plasma	K_2EDTA (purple-top tube)	Mortari lab (store Orchard lab)	yes	
Type 1 procollagen (**P1NP**)	Plasma	K_2EDTA (purple-top tube)	Mortari lab (store Orchard lab)	yes	
Osteoprotegrin (**OPG**)	Plasma	K_2EDTA (purple-top tube)	Mortari lab (store Orchard lab)	yes	
C-telopeptide (**CTX**)	Plasma	K_2EDTA (purple-top tube)	Mortari lab (store Orchard lab)	yes	
Tartrate resistant acid phosphatase type 5b (**TRAP5b**)	Plasma	K_2EDTA (purple-top tube)	Mortari lab (store Orchard lab)	yes	
Receptor activator of nuclear factor-κ B ligand (**RANKL**)	Plasma	K_2EDTA (purple-top tube)	Mortari lab (store Orchard lab)	yes	
Complete blood count with differential (**CBCD**)	Whole blood	K_2EDTA (purple-top tube)	Quest/Fairview	no	4 mL (1 tube)
Immunologic phenotyping (CD3, 4, 8, 14, 15, 16, 19, 34, 56)	Whole blood	K_2EDTA (purple-top tube)	Fairview	no	
Genetic testing for Carbonic Anhydrase II (**CAII**), Chloride Channel 7 (**CLCLN7**,) Osteopetrosis-associated Transmembrane Protein 1 (**OSTM1**), RANKL, T-cell Immune Regulator 1 (**TCIRG1**) gene mutations	Whole blood	K_2EDTA (purple-top tube)	Fairview	no	4 mL (1 tube)
Liver function tests (**LFT**)	Serum	SST (red-top tube)	Quest/Fairview	no	10 ml blood (2 tubes)
25-hydroxy vitamin D (**25OHD**)	Serum	SST (red-top tube)	Quest/Fairview	no	
Calcium	Serum	SST (red-top tube)	Quest/Fairview	no	
Intact parathyroid hormone (**iPTH**)	Serum	SST (red-top tube)	Quest/Fairview	no	
Basic metabolic panel (**BMP**)	Serum	SST (red-top tube)	Quest/Fairview	no	
Creatinine kinase - BB isoenzyme (**CK-BB**)	Serum	SST (red-top tube)	Mortari lab (store Orchard lab)	yes	
Anti-drug antibodies (**ADA**)	Serum	SST (red-top tube)	Intertek (store Polgreen/Orchard labs)	yes- at month 12 visit	4 ml blood
Storage	Serum	SST (red-top tube)	Polgreen/Orchard labs	NA	5 ml blood
Storage	Plasma	K_2EDTA (purple-top tube)	Polgreen/Orchard labs	NA	4 ml blood
Storage	Urine	sterile container	Polgreen/Orchard labs	NA	8 ml urine
Urine pregnancy test, calcium to creatinine ratio (**ca:cr**) and urinalysis (**UA**)	Urine	sterile container	Quest/Fairview POC UPT	no	10 ml urine
Ex vivo assays (Mansky Lab)	Whole Blood	K_2EDTA (purple-top tube)	Manksy lab	no	10 ml (1 tube)
RNA expression	Whole Blood	PAXgene RNA tube	Chen lab	yes	2.5 ml
DNA	Buffy coat	K_2EDTA (purple-top tube)	Chen lab	NA	10 ml

6.2.2 Research Specimen Handling and Storage (See Manual of Operations for instructions for non-LA BioMed sites)

Samples for Bone Biomarkers and Storage

Plasma. Collect blood in 3 purple-top (ethylenediaminetetraacetic acid [EDTA]) tubes (4 mL each). After collection, thoroughly mix the tube by gentle inversion 8 times. Label the tube with the Subject's Study ID number, study visit, date of visit, and "plasma". **Do not write the subject's name or any other identifying information on the blood tube.** Place the tube upright in a rack in the refrigerator (2 to 8°C) until processed and stored at -80°C.

If need to transport to the lab for processing, wrap the tube in a paper towel and place it in a plastic bag with an absorbent pad. Place the plastic bag containing the tube in a Styrofoam mailer with a frozen gel pack or on ice. The gel pack will ensure refrigerator temperature (2 to 8°C) during transport to the laboratory.

2 tubes: Centrifuge for 15 minutes at 4000 x g within 30 minutes of collection. Aliquot 0.5 ml into each 2 ml cryovial (8 cryovials) and store samples at -80°C.

1 tube: Invert 8-10 times immediately after drawing. Keep tube cooled on ice, or with a cold pack, or in a 4C refrigerator. Transport to Translational Genomics & Population Sciences laboratory on ice for processing and storage of buffy coat.

Serum. Collect blood in 2 red-top (serum separator tube [SST]) tubes (5 mL each). After collection, allow blood to clot for 30 minutes. Label the tube with the Subject's Study ID number, study visit, date of visit, and "serum". **Do not write the subject's name or any other identifying information on the blood tube.** Place the tube upright in a rack in the refrigerator (2 to 8°C) until processed and stored at -80°C.

If need to transport to the lab for processing, wrap the tube in a paper towel and place it in a plastic bag with an absorbent pad. Place the plastic bag containing the tube in a Styrofoam mailer with a frozen gel pack or on ice. The gel pack will ensure refrigerator temperature (2 to 8°C) during transport to the laboratory.

Centrifuge for 15 minutes at 4000 x g within 30 minutes of collection. Aliquot 0.5 ml into each 2 ml cryovial (10 cryovials) and store samples at -80°C.

Urine

Collect urine in a sterile container (5 mL each). Label the tube with the Subject's Study ID number, study visit, date of visit, and "urine". **Do not write the subject's name or any other**

identifying information on the urine tube. Place the tube upright in a rack in the refrigerator (2 to 8°C).

If need to transport to the lab for storage prior to shipping, wrap the tube in a paper towel and place it in a plastic bag with an absorbent pad. Place the plastic bag containing the tube in Styrofoam mailer with a frozen gel pack or on ice. The gel pack will ensure refrigerator temperature (2 to 8°C) during transport to the laboratory.

Whole Blood

Ex vivo assays:

Collect 1 purple-top (EDTA) tube (10 mL). Label the tube with the Subject's Study ID number, study visit, date of visit, and "whole blood". **Do not write the subject's name or any other identifying information on the blood tube. Send to Dr. Kim Mansky per instructions below for arrival at Mansky lab within 36 hours of sample collection.**

Genetic Testing

Collect 1 purple-top (EDTA) tube (5 mL). Label the tube with the Subject's Study ID number, study visit, date of visit, and "whole blood". **Do not write the subject's name or any other identifying information on the blood tube. Send to Troy Lund/Kelly Miettunen per instructions below.**

Immunologic phenotyping

Collect 1 purple-top (EDTA) tube (5 mL). Label the tube with the Subject's Study ID number, study visit, date of visit, and "whole blood". **Do not write the subject's name or any other identifying information on the blood tube. Send to Troy Lund/Kelly Miettunen per instructions below.**

RNA expression

Collect 1 PAXgene RNA tube (2.5 ml blood in a 10 ml tube). Invert 8-10 times immediately after drawing. Maintain tubes in upright position at room temperature for a minimum of 2 hours. Store PAXgene tubes in a wireframe rack (Styrofoam racks may cause the tubes to crack). Transport to TGPS laboratory on ice.

*****For EDTA (1) and PAXgene RNA tube (1)** please email the following people the day prior to a subject's scheduled visit to expect samples from the visit: Dr. Ida Chen, ichen@labiomed.org, Vicki Liu, yehengliu@labiomed.org, Ubaydah Nasri unasri@labiomed.org, and Kaye Roll, kroll@labiomed.org.

All specimens must be labeled with a code. Unlabeled specimens will be destroyed.

All specimens must be accompanied by a completed lab sheet.

6.2.3 Specimen Shipment

Whole blood for immunologic phenotyping, genetic testing, and ex vivo assays by Dr. Mansky **collected at Harbor-UCLA** will be shipped at ambient temperature by Fed Express overnight, following International Air Transport Association (IATA) regulations (double package or bag all specimens; include an absorbent square in each bag; affix "Exempt Human Specimens" label to box). Ship for arrival within 36 hours of collection to:

Troy Lund/Kelly Miettunen
425 East River Parkway
MCRB 660
Minneapolis, MN (612) 626-5768

Contact Kelly Miettunen 48 hours in advance by Phone (above) or Email: landb018@umn.edu prior to shipping. Deliver before 10:30 am on Tuesday – Thursday.

Contact Dr. Kim Mansky by Phone: 612-626-5582 or email: kmansky@umn.edu 48 hours prior to shipping.

Bone Biomarkers

Samples for bone biomarkers will be stored at each site at -80°C until the end of the study at which time all samples will be shipped/transported on an adequate amount of dry ice to Dr. Angela Mortari, Cytokine Reference Lab. For shipping samples to Dr. Mortari, samples will be shipped on an adequate amount of dry ice to the study PI following International Air Transport Association (IATA) regulations (double package or bag all specimens; include an absorbent square in each bag; affix "Exempt Human Specimens" label to box). Ship for arrival within 36 hours:

Angela Mortari

Cytokine lab
13-127 PWB
516 Delaware St. SE
Minneapolis, MN 55455

Contact Dr. Mortari 48 hours in advance by Phone: (612) 626-7057 or email: panos001@umn.edu. Deliver before 10:30 on Tuesday – Thursday.

Storage

Samples for storage will be kept at each study site at -80°C until the end of the study at which time all samples will be shipped on an adequate amount of dry ice to the study PI following International Air Transport Association (IATA) regulations (double package or bag all specimens; include an absorbent square in each bag; affix "Exempt Human Specimens" label to box). Ship for arrival within 36 hours:

Lynda Polgreen
1124 W. Carson St.
RB-3, Rm 221
Torrance, CA 90502

Contact Dr. Polgreen 48 hours in advance by Phone: (310) 222-1961 or email: lpolgreen@labiomed.org prior to shipping. Deliver before 10:30 on Tuesday – Thursday.

6.2.4 Serum Sample Procedure for Immunogenicity assays:

Collect blood samples to analyze Anti-Drug Antibodies (ADA) to IFN-γ and Neutralizing antibodies (Nab) to IFN-γ levels using 4.0 mL blood collection tube tubes (BD Vacutainer® SST™ Serum Separation Tubes).

Collect a minimum of 2mL serum per time point described in Table 1.

After obtaining the blood sample, inverting the collection tube 5 times allow the blood to clot for 30 minutes at ambient temperature (19-24˚C).

Centrifuge sample at approximately 1000-1300 RCF for 10 minutes at room temperature in a swinging bucket centrifuge.

Transfer duplicate serum aliquots of approximately equal volume (minimum of 2 x 100 µL for ADA and 500 µl for Nab with backup of 500 µl per aliquot), using standard laboratory technique, into 2 polypropylene cryovials.

Secure a label to each storage tube. Labels should contain the following information:
Sponsor: Dr. Lynda Polgreen

Sample Type: Human serum
Sample date: DD-MMM-YYYY
Sample time: XX:XX
Protocol: 21549-01
Subject Number: TBD
Time point: TBD
Serum Aliquot: TBD

Within 90 minutes of collection, store both aliquot samples upright at -20°C +/-5°C or -70°C ± 10˚C.

Store at -70°C ± 10˚C until collection of month 12 sample, then ship all study subject samples as follows. If samples are collected after month 12, then ship immediately to Intertek as follows.

Ship as separate shipments Serum Aliquot 1 and Serum Aliquot 2 samples for potential analysis of ADA and Nab with an adequate supply of dry ice via Fedex Monday through Wednesday to:

Attn: Beth Cecil (ADA samples) or Lynn Jiang (Nab samples)
Intertek Pharmaceutical Services
10420 Wateridge Circle
San Diego, CA 92121

Provide an inventory log listing of all samples shipped with each shipment. Include a paper copy with the shipment and an electronic version via email to Beth Cecil (beth.cecil@interteck.com) and Lynn Jian (lynn.jiang@intertek.com).

Send a shipment notification including a confirmation of the shipment date, courier name, and tracking information via email to Beth Cecil (beth.cecil@interteck.com) and Lynn Jian (lynn.jiang@intertek.com).

6.3 Clinical Procedures

6.3.1 Medical History

A study physician and/or study coordinator will review the following with the subject/parent or legal guardian at the screening baseline visit:

- Current and past 1 year prescription medications and doses,
- current and past medical history,
- review of systems,
- family history,
- number of hospitalizations over the last 12 months, and

- number of days treated with antibiotics over the last 12 months.

For patients <18 years of age, height data will be collected from 6 and/or 12 months prior to the baseline visit.

Changes in the medical history and a complete review of systems will be reviewed at each subsequent visit.

If a subject is unable to return to the clinical site for the Month 3 visit they will be contacted via telephone to discuss any changes in health since their last study visit and then withdrawn from the study. Three attempts will be made to contact the subject by phone before terminating the patient on the study.

6.3.2 Laboratory Evaluations

Blood and urine will be collected at the time points described in Table 1.

CBCD, LFT, ESR, 25OHD, Calcium, iPTH, UA, urine ca:cr will be performed in real time by the Fairview Diagnostics Laboratory at UMN and at Quest for subjects seen at Harbor-UCLA at baseline, 3, 6, and 12 months. BMP, LFT, and UA will be performed at UMN, Quest, or the subject's local clinical lab at weeks 4 and 6, and month 9. Anti-drug antibody testing will be performed by Intertek Pharmaceutical Services at Baseline, at weeks 3 and 6, and months 3, 6 and 12, after the first dose, and 2-3 weeks after last dose, and then every 4-6 weeks until values return to baseline. Genetic testing for mutations in TCIRG1, CLCN7, CAII, OSTM1 and RANKL genes will be performed at the Baseline visit at the University of Minnesota for patients who have not had an osteopetrosis related mutation previously identified.

25OHD will be measured by liquid chromatography tandem mass spectrometry, and an attempt will be made to maintain levels between 30 ng/ml and 75 ng/ml. Treatment of vitamin D insufficiency/deficiency will be as follows:

Vitamin D insufficiency: total 25-OH vitamin D 20-29 ng/ml

Age	Vitamin D	Calcium (elemental)*
0-1 years	1,000 IU/d x 8 weeks	NA
1-3 years	1,000 IU/d x 8 weeks	500 mg/d
4-8 years	1,000 IU/d x 8 weeks	800 mg/d
>8 years	2,000 IU/d x 8 weeks	1,300 mg/d

*Add calcium supplements if oral intake is inadequate or serum calcium is low.

Vitamin D deficiency: total 25-OH vitamin D 10-20 ng/ml: obtain PTH and serum calcium levels.

Age	Vitamin D	Adequate Intake Calcium (elemental)*	If PTH>65 pg/ml or serum calcium is low, add additional calcium as follows: Calcium (elemental) max dose 2000 mg/day Start at higher dose and taper over 4 weeks to AI.
0-1 years	2,000 IU/d x 8 weeks	NA	30-75 mg/kg/day divided 2-4 times daily
1-3 years	2,000 IU/d x 8 weeks	500 mg/d	30-75 mg/kg/day divided 2-4 times daily
4-8 years	4,000 IU/d x 8 weeks	800 mg/d	30-75 mg/kg/day divided 2-4 times daily
>8 years	50,000 IU/wk x 8 weeks (Rx)	1,300 mg/d	30-75 mg/kg/day divided 2-4 times daily

*Ensure oral intake of calcium is adequate.

Severe Vitamin D deficiency: total 25-OH vitamin D <10 ng/ml: obtain PTH and serum calcium levels.

Age	Vitamin D	For all patients with severe vitamin D deficiency: Calcium (elemental) max dose 2000 mg/day Start at higher dose and taper over 4 weeks to AI.
0-1 years	2,000 IU/d x 8 weeks	30-75 mg/kg/day divided 2-4 times daily
1-3 years	2,000 IU/d x 8 weeks	30-75 mg/kg/day divided 2-4 times daily
4-8 years	4,000 IU/d x 8 weeks	30-75 mg/kg/day divided 2-4 times daily
>8 years	50,000 IU/wk x 8 weeks (Rx)	30-75 mg/kg/day divided 2-4 times daily

For post-menarchal female patients, a urine pregnancy test will be performed at Screening and before the Baseline, Month 3, and Month 6 visits. Plasma and serum will be collected as described in Table 1. Immunologic phenotyping will be performed by University of Minnesota. BAP, P1NP, OPG, CTX, TRAP5b, RANKL, and CK-BB will be performed by the Fairview Cytokine Reference Laboratory at the University of Minnesota. Expression, methylation, and genotype data will be generated at LABioMed in the laboratories of Drs. Ida Chen and Kent Taylor.

6.3.3 Medical examination and Anthropometrics

A trained pediatric provider will perform a physical examination at each visit. Tanner staging of pubertal development, including breast and pubic hair development in girls, and testicular size and pubic hair development in boys will be performed at baseline, 6, and 12 months for participants < 18 years of age or < Tanner stage 5. Height will be measured 3 times standing without shoes. The average of 3 measurements by the same observer using identical technique with a wall-mounted stadiometer will be recorded. Each subject will be repositioned between each measurement. Height standard deviation score (SDS) will be calculated based on the

National Center for Health Statistics 2000 data as provided by the Center for Disease Control. Weight to be measured standing, once with subject in light clothing or dressing gown with shoes removed. These measurements will be used to calculate body mass index (BMI) as weight (kg) divided by height (m^2).

6.3.4 Radiographic assessments

6.3.4.1 Dual energy x-ray absorptiometry (DXA)

DXA (Hologic [Harbor] and GE [Minnesota]) measurements will be obtained for total body less head, L1-L4 lumbar spine, and for subjects ≥18 years of age femoral neck and total hip as well. Standardized procedures for participant positioning and data processing will be used for all scans. Standardization of data from different machines is done by standardized phantom scanning protocols.

6.3.4.2 Peripheral quantitative computed tomography (pQCT)

Detailed measures of bone mass and strength will be assessed using pQCT (XCT 2000L [Harbor-UCLA] and XCT-3000 [UMN], Orthometrix Inc., White Plains, NY). This is a non-invasive technique that allows for 3-dimensional assessment of bone volumetric density, bone geometry and estimates of bone strength. Images will be taken at the 3%, 38%, and 66% sites of the left tibia and the 3% and 38% sites of the non-dominant radius. The reference line will be the proximal end of the distal growth plate. Standardized procedures for participant positioning and data analysis will be used for all scans. Image processing and calculation of bone parameters will be completed using the manufacturer's software (version 5.5) and thresholds established in the literature.

6.3.5 Pain and physical function testing

6.3.5.1 Child Health Questionnaire - Parent Form 50 (CHQ-PF50)

The CHQ-PF50 is a 50-item, parent-completed questionnaire designed to measure the physical and psychosocial well-being of children between the ages of 5 and 18. The CHQ-PF50 has been widely used in studies of chronic illness in childhood. Twelve components of quality of life are measured including: physical functioning, role/social limitations due to physical health, bodily pain/discomfort, general health, mental health, roe/social limitations due to emotional-behavioral difficulty, behavior, and self-esteem. The impact of the child's disorder on parents and family functioning (i.e., time impact, emotional impact, family activities, family cohesion) are also measured. Items are measured on a likert scale, summed for each subscale and linearly transformed to a 0 to 100 scale, where higher scores reflect better functioning. Composite scores for physical functioning (Physical Summary Score) and psychosocial functioning (Psychosocial Summary Score) are calculated based on population norms. This questionnaire will be administered to all participants between the ages of 5 and 17 years.

6.3.5.2 Medical Outcomes Study Short Form-36 (SF-36)

The SF-36 is a widely used measure of patient-reported quality of life for participants 18 years of age and older. The SF-36 measures 8 health domains, including physical functioning, role limitations due to physical health, bodily pain, general health perceptions, vitality, role limitations due to emotional problems, and mental health. Items are rated on a likert scale. Summed scores of the scales are then transformed linearly to values from 0 (worst health) to 100 (best health). Two summary measures (Physical and Mental Component Summary scores) are derived from population-based normative data. The SF-36 has been used in studies of adult chronic illness, and as an important component of treatment outcome studies. This questionnaire will be administered to all participants aged 18 years and above.

6.3.5.3 **Patient and parent-reported pain intensity (acute and within the past week).**

Visual analogue scales from the Pediatric Pain Questionnaire (PPQ) (22) will be used to assess intensity of the child's pain from the child's and parent's perspective (for children ages 5-17). Visual analogue scales are widely used in both pediatric and adult pain studies due to their good reliability and validity, and they do not tend to result in the clustering of scores as can be seen with Likert-type scales (23,24). The visual analogue scales included in the PPQ consist of two 100 mm horizontal lines that measure both present pain and pain in the past week. Each line is anchored with developmentally appropriate pain descriptions (i.e., "No pain, Not hurting" to "Hurting a whole lot, Severe pain") and accompanying happy and sad faces. Scores on the PPQ range from 0 to 100, with higher scores representing more pain. For adults, the Visual Analogue Scale (VAS) for Pain (25,26), a 100 mm horizontal line with anchors appropriate for adults, ranging from "no pain" to "pain as bad as it could be; worst possible pain," will be used to assess pain intensity in the present moment and over the past week. As in the PPQ visual analogue scales, the VAS score is determined by measuring the distance (mm) on the 100 mm horizontal line between "no pain" and the patient's mark, providing a range of scores from 0-100, with higher scores representing more pain intensity.

For participants aged 18 years and older, pain intensity and its impact on daily functioning will be captured as part of the SF-36 questionnaire.

6.3.5.4 Infectious Outcomes

The frequency of infectious outcomes will be quantified by asking participants for the number of hospitalizations in the last 3 months and the number of days treated with antibiotics over the last 3 months (IV and PO will be counted separately). The type of infectious organism will be documented as well.

6.3.5.5 In vitro osteoclast function assays

Osteoclasts will be isolated from whole blood by Ficoll gradient and CD14+ selection. The CD14+ cells will be cultured in macrophage colony stimulating factor (M-CSF) and

RANKL until multinuclear, TRAP positive cells are present in the culture. Assays will be performed to determine the direct and indirect effects of IFNγ-1b as follows:

- normal osteoclasts
- normal osteoclasts + IFN-γ1b (0.25 ng/ml, 0.5 ng/ml, and 1.0 ng/ml),
- diseased osteoclasts (baseline)
- diseased osteoclasts (baseline)+ IFN-γ1b (0.25 ng/ml, 0.5 ng/ml, and 1.0 ng/ml)
- diseased osteoclasts (month 6)
- diseased osteoclasts (month 6) + IFN-γ1b (0.25 ng/ml, 0.5 ng/ml, and 1.0 ng/ml)
- normal osteoclasts + 10% serum from treated patients
- diseased osteoclasts (baseline) + 10% serum from treated patients
- diseased osteoclasts (month 6) + 10% serum from treated patients

Outcome measures will be as follows:

For phenotypic analysis, gene expression (osteoclast marker genes such as but not limited to *c-fos*, *Nfatc1*, *DC-STAMP*, *cathepsin K*) will be measured by real time RT-PCR. Osteoclast cultures will be stained for TRAP, photographed and osteoclast size and number will be measured. Osteoclast cultures will also be plated on calcium phosphate coated dishes and activity of the osteoclasts (ability to release calcium phosphate from coated plate) will be evaluated. Calcium phosphate dishes will be treated, photographed and percent area of the well absorbed, number of resorption pits and average size of the pit will be measured. All of the data collected will be used to determine if changes in differentiation and/or activity are occurring in the osteoclasts in response to treatment.

6.3.6 Administration of ACTIMMUNE

The first SC injection of ACTIMMUNE will be administered by the patient or their parent/guardian under the guidance of a medical assistant, nurse or physician. Subsequent injections will be administered independently by the patients or their parent/guardian.

Titration dosing will be used to reduce the severity of ACTIMMUNE related flu-like symptoms as described by Devane et al (27). Body surface area (BSA) will be calculated by the Mosteller method (28).

For subjects with BSA >0.5 m^2:

- 10 mcg/m^2 SC three times weekly during week 1
- 20 mcg/m^2 SC three times weekly during week 2

- 30 mcg/m^2 SC three times weekly during week 3
- 40 mcg/m^2 SC three times weekly during week 4
- 50 mcg/m^2 SC three times weekly during week 5
- 60 mcg/m^2 SC three times weekly during week 6
- 70 mcg/m^2 SC three times weekly during week 7
- 80 mcg/m^2 SC three times weekly during week 8
- 90 mcg/m^2 SC three times weekly during week 9
- 100 mcg/m^2 SC three times weekly during week 10 and the remainder of the study if tolerated. ACTIMMUNE may be reduced on a case-by-case basis to manage subsequent drug-related AEs (e.g. flu-like reactions, elevated liver function tests or any drug-related Grade 2 or higher AE).

For subjects with BSA $\leq$ 0.5 m^2:

- 0.3 mcg/kg SC three times weekly during week 1
- 0.6 mcg/kg SC three times weekly during week 2
- 0.9 mcg/kg SC three times weekly during week 3
- 1.2 mcg/kg SC three times weekly during week 4
- 1.5 mcg/kg SC three times weekly during week 5
- 1.8 mcg/kg SC three times weekly during week 6
- 2.1 mcg/kg SC three times weekly during week 7
- 2.4 mcg/kg SC three times weekly during week 8
- 2.7 mcg/kg SC three times weekly during week 9
- 3.0 mcg/kg SC three times weekly during week 10 and the remainder of the study if tolerated. ACTIMMUNE may be reduced on a case-by-case basis to manage subsequent drug-related AEs (e.g. flu-like reactions, elevated liver function tests or any drug-related Grade 2 or higher AE).

Over-the-counter and prescription medications can be used to treat flu-like reaction side effects per the study physician's discretion. Possible treatment options for the flu-like reaction include

administering ACTIMMUNE 1-2 hours prior to bedtime, acetaminophen administration 30 minutes prior to injection, and/or prednisone 5 mg po in morning on day of injection (titrate up to a maximum of 60 mg po day of injection – based on dosing for inflammatory disorder of musculoskeletal system; adjunct).

6.4 Unscheduled Study Visits

Additional visits will be scheduled with study physicians or local physicians as needed throughout the study period to address concerning signs or symptoms reported by subjects.

Subjects may withdraw voluntarily from the study at any time. If a subject opts to discontinue, attempts will be made to schedule and conduct an Early Termination visit for medical assessment.

If a subject withdraws after an AE, they will be followed until resolution of the issue or until their condition is stable. In the event that a subject becomes pregnant, then discontinuation, reporting, and follow-up procedures outlined in Section 8.3.4 will be followed.

6.5 Study Protocol Compliance / Treatment Adherence

Adherence to treatment will be evaluated by querying subjects every month either at scheduled study visits or by phone.

6.6 Deviations from the Clinical Protocol

When a deviation from the protocol is necessary for an individual subject, the Investigator must contact the study PI prior to the deviation (unless the deviation is safety related). The subject may continue in the study by mutual agreement of the Sponsor and the Investigator. A description of the deviation from the protocol and justification must be recorded on the Protocol Deviation Form.

6.7 Protocol Amendments After Study Initiation

Should changes in the study plan or protocol become necessary in the course of the study, those specific changes will be discussed and agreed upon by the Investigators and appropriate IRB approval obtained before the changes are implemented. All changes must be documented as protocol amendments.

6.8 Subject Withdrawal

Subjects will be encouraged to complete the study; however, they may voluntarily withdraw at any time. If a subject is withdrawn prior to study completion, attempts will be made to schedule and conduct an Early Termination visit for medical assessment. If a subject cannot be contacted after 5 telephone calls and 3 letters, they will be considered lost to follow-up.

Subjects who discontinue study medication (e.g., due to non-compliance, side effects, etc.) will be asked to remain in the study for follow-up per the study schedule to preserve the ability to perform a

safety analysis. If a subject or their legal guardian (as applicable) withdraws consent to participate in the study, attempts will be made to obtain permission to record survival and adverse event (malignancy, hospitalization for infection) data at the 12-month study point.

As long as the link between the sample ID code and the subject name is maintained by the clinical study site, it will be possible for subjects to ask that their samples be withdrawn. However, we anticipate this link will be destroyed at some point in the future, after which withdrawal of samples will not be possible. In addition, once DNA samples have been provided to collaborators, we cannot guarantee that all samples will be able to be tracked down.

6.9 Subject Compensation

Travel reimbursement up to $1,500 will be provided for each of the Screening, Baseline, Month 3, Month 6, and Month 12 study visits. This reimbursement can be applied to flights for subject or subject and 1 parent/guardian, gas, hotel (to be chosen and reserved by study coordinator for the number of nights required to complete study visit[s]), and/or food (up to $150/visit). Receipts will need to be provided to study coordinator for reimbursement. Subjects will also receive $50 at Screening, Baseline, Month 3, Month 6, and Month 12 study visits as compensation for their time and inconvenience.

6.10 Subject Compensation for Research Related Injury

If a subject is injured as a result of taking part in this study they will receive medical treatment, however this care will be billed to their insurance in the usual manner and will not necessarily be free of charge to the subject.

6.11 Sharing Results with Subjects

All results obtained in this study are for research only. No results will be shared with the subjects other than those needed to care for the subject during study participation (e.g. abnormal lab test). When results are shared with the subject, they will be discussed in person or by telephone.

7 Data Collection and Analysis

7.1 Subject Population(s) for Analysis

- Treatment-compliant population: Any subject who received at least 85% of prescribed doses and attended all scheduled study visits.

- Safety population: All subjects who receive any amount of treatment.

7.2 Statistical Methods

7.2.1 Sample size Determination

This is a pilot study to obtain data for a larger, definitive clinical trial. The sample size of 10 was determined based on feasibility estimates including potential screening failures

7.2.2 Statistical Analysis

Descriptive analyses of baseline characteristics and outcomes will include means and standard deviations for continuous variables and frequencies for categorical variables. Confidence intervals and P-values will be based on a t-distribution. Statistical significance will be considered as $p<0.05$. The primary endpoint and safety analyses will be primarily descriptive reporting the number and percentage of compliance and AEs. The secondary endpoints for hematologic function, immune function, and bone mineral density will be evaluated by paired t-tests or chi-squared to evaluate change from baseline to 12 months. Secondary endpoints related to in vitro osteoclast assays will be descriptive.

8 Safety and Adverse Events

8.1 Definitions

Adverse Event

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

- Results in study withdrawal
- Is associated with a serious adverse event (SAE)
- Is associated with clinical signs or symptoms
- Leads to additional treatment or to further diagnostic tests
- Is considered by the Investigator to be of clinical significance.

*Serious Adverse Event (**SAE**)*

An SAE is any AE that is:

- Fatal

- Life-threatening
- Requires or prolongs a hospital stay
- Results in persistent or significant disability or incapacity
- A congenital anomaly or birth defect

Important medical events are events that may not be immediately life-threatening, but are clearly of major clinical significance and may be SAEs. They may jeopardize the subject, and may require intervention to prevent one or the other serious outcomes noted above.

Hospitalization

Hospitalization shall include any initial admission (even if less than 24 hours) to a healthcare facility as a result of a precipitating clinical adverse effect; to include transfer within the hospital to an intensive care unit. Hospitalization or prolongation of hospitalization in the absence of a precipitating, clinical adverse effect (e.g., for a preexisting condition not associated with a new adverse effect or with a worsening of the preexisting condition; admission for a protocol-specified procedure) is not, in itself, a serious adverse effect.

Expected Adverse Event

Expected AEs are those that are known to be associated with or have the potential to arise as a consequence of participation in the study. This will include any AE or SAE that are expected consequences of having osteopetrosis or receiving treatment with IFN-γ1b described in Section 8.4.4.

Unexpected Adverse Event

An AE or suspected adverse reaction is considered unexpected if it is not listed in the drug package insert or Protocol at the specificity or severity that has been observed.

Unanticipated Problems Involving Risk To Subjects or Others (UPIRTSO)

An AE that in the opinion of the PI is unexpected, related to the study procedures, and serious.

8.2 Recording of Adverse Events

At each contact with the subject, the Investigator/study coordinator must seek information on AEs by specific questioning and, as appropriate, by examination. Information on all AEs should be recorded immediately in the source document, and also in the appropriate AE module of the case report form (**CRF**). All clearly related signs, symptoms, and abnormal diagnostic procedures resulting from a single clinical condition should be recorded individually in the source document and listed under the

single unifying diagnosis. (For example, fever, rigors, sore throat, and high neutrophil count might all be listed under a diagnosis of pharyngitis.)

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

AEs will be classified according to the National Cancer Institute (**NCI**) Common Toxicity Criteria (**CTC**) V 4.1 and causality will be classified as:

Unrelated - The AE is clearly NOT related to study procedures

Unlikely - The AE is doubtfully related to study procedures

Possible - The AE may be related to study procedures

Probable - The AE is likely related to study procedures

Definite - The AE is clearly related to study procedures

8.3 Reporting of Serious Adverse Events

8.3.1 Study Sponsor Notification by Investigators

An SAE must be reported to the study PI by telephone, fax, or email (preferred) within 24 hours of the event. An SAE Form must be completed by the Investigator and faxed or emailed to the study Sponsor within 24 hours. The Investigator will keep a copy of this SAE form on file at the study site. Report SAEs by phone, fax or email to:

Lynda Polgreen, MD, MS 310-222-1961 (telephone)
310-222-3887 (facsimile)
lpolgreen@labiomed.org

At the time of the initial report the following information should be provided:

- Study Identifier
- Study Center

- Subject Number
- A description of the event
- Date of onset
- Current Status
- The reason why the event is classified as serious
- Investigator assessment of the association between the event and study procedures

Within the following 48 hours, the Investigator must provide further information on the SAE in the form of a written narrative. This should include a copy of the completed SAE Form, and any other diagnostic information that will assist the understanding of the event. Significant new information on ongoing SAEs should be provided promptly to the study PI.

8.3.2 IRB Notification by Investigators

Reports of all SAEs (including follow-up information) must be submitted to the IRB within 10 working days if it falls under the UPIRTSO guidelines (unexpected and related to study drug). All deaths, regardless of expectedness or causality, must be reported to the IRB within 10 working days. Copies of each report and documentation of IRB notification and receipt will be kept in the Clinical Investigator's binder.

8.3.3 UPIRTSO Events

Investigators are required to submit a report of UPIRTSO events to the IRB within 10 working days of first learning of the event.

8.3.4 Pregnancy

If a female subject becomes pregnant during the study, she will be withdrawn from the study immediately. Though pregnancy is not by definition an SAE, it will be subject to SAE reporting requirements.

Though the subject will be withdrawn from the study, permission to record survival and adverse event (malignancy, hospitalization for infection) data for the mother to the end of the pregnancy and of survival data for the baby at birth will be requested.

8.4 Safety Monitoring Plan

The plan for ensuring subject safety will include the following elements:

1. Clinical procedures will only be performed by properly trained personnel who are qualified by training and licensure to perform the procedures.

2. Investigator and all study personnel have completed required training regarding Human Subject Protections. All personnel will comply with all related regulations and laws.

3. Patients will be rigorously screened against inclusion/exclusion criteria to ensure that their participation is safe.

4. Adverse events and SAEs will be assessed and followed throughout study. Subjects will have contact information to enable them to contact study personnel easily and quickly.

5. Study data and information will be kept confidential and managed in accordance with requirements of Health Insurance Portability and Accountability Act of 1996 (HIPAA). All data will be stored in locked offices and not released without subject permission.

6. Subject may discontinue participation at any time, for any reason. Any subject who fails to complete the research procedures within acceptable parameters, or is unable to safely tolerate participation in the study will be withdrawn.

7. Institutional Review Board approval will be obtained.

8. Monitoring will be conducted by an external Data and Safety Monitor as described in the Data and Safety Monitoring Plan (appendix A).

8.4.1 Anticipated Risks / Risk Mitigation

8.4.1.1 Venipuncture

The risks of venipuncture are transient pain, bleeding, lightheadedness, bruising, possible vasovagal reaction, and infection. Only trained certified nursing assistants, nurses, nurse practitioners, or physicians will be allowed to perform venipuncture to minimize the risk of complication.

8.4.1.2 pQCT and DXA

The pQCT and DXA scan will expose the subject to ionizing radiation. The level of exposure for this study will be approximately 3% of that received from natural sources of radiation by a northern US resident in 1 year. Trained staff will perform the scans to minimize the risk of additional exposure.

8.4.1.3 Provisions to Monitor the Data to Ensure the Safety of Subjects

Genetic studies have raised concern as to whether the studies would place research subjects at risk for discrimination based on genetics. The federal Genetic Information Nondiscrimination Act (GINA) was passed to address this concern. GINA makes it illegal for medical insurance companies and most employers to discriminate based on genetic information. The protections of GINA do not apply to life, disability, or long-term-care insurance.

8.4.2 Study Stopping Rules

This study may be terminated by the PI at any time. Reasons for terminating the study may include the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to subjects.
- Subject enrollment is unsatisfactory.
- Data recording is inaccurate or incomplete.
- 1 patient develops a CTCAE Grade 4 or higher.

8.4.3 Anticipated / Expected Adverse Events

The following AEs have been reported in individuals treated with ACTIMMUNE: fever (52%), headache (33%), rash (17%), chills (14%), injection-site erythema or tenderness (14%), fatigue (14%), diarrhea (14%), vomiting (13%), and nausea (10%). Less common adverse events observed more frequently in the IFN-γ 1b group compared with placebo (occurring in <10% of patients) included myalgia, arthralgia, back pain, abdominal pain, and depression.

Adverse events related to osteopetrosis are expected. These expected events include but are not limited to, pain, fracture, infection, osteonecrosis, anemia, neutropenia, thrombocytopenia, hepatosplenomegaly, cranial nerve impingement, and hypocalcemia. These events will be considered expected for the purposes of expedited SAE reporting.

8.5 Data Safety Monitoring Plan

Data safety monitoring plan is described in detail in Appendix A.

9 Data Handling and Record Keeping

9.1 Confidentiality

Study data and information will be kept confidential and managed in accordance with requirements of Health Insurance Portability and Accountability Act of 1996 (HIPAA). We will follow institutional policies regarding HIPAA, we will answer any questions/concerns the subjects and their parents may have in regards to their privacy, address items, and document. If at any time subject/parent/family/ or child appear uneasy during exams, questions, etc. the team member will stop the visit to refer to PI or doctor performing visit to determine if the study visit can continue. Research team is permitted to access sources of information about the subjects for research purposes only, document all requests if not in protocol.

HIPAA regulations require a signed subject authorization informing the subject of the following:

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

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

The samples sent to laboratories will include the subject's ID number and not their name. Those who have access to data and specimens are the research team who all have HIPAA training; these same people are response for receipt and/or transmission of data and specimens.

All interactions with the subject and his/her parents will take place in a private setting, e.g., a private exam room.

The genetic information obtained in this study is kept in strict confidence. The laboratories in which genotyping and assays are performed use only study ID codes and no other identifiers. The information is identified in the database by a unique code. The genotyping results are merged with the phenotype data for statistical genetic analysis using only study ID codes to identify individuals. We will secure hard copy information with keys and computer databases with password protection so that only research personnel related to this project will have access to information.

9.2 Source Documents

Source Data are the clinical findings and observations, laboratory and test data, and other information contained in *Source Documents*. *Source Documents* are the original records (and certified copies of original records); including, but not limited to, hospital medical records, physician or office charts, physician or nursing notes, subject diaries or evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, x-rays, etc. When applicable, information recorded on the CRF shall match the *Source Data* recorded on the *Source Documents*.

9.3 Case Report Forms

The study CRF is the primary data collection instrument for the study. All data requested on the CRF must be recorded. All missing data must be explained. If a space on the CRF is left blank because the

procedure was not done or the question was not asked, "N/D" will be entered. If the item is not applicable to the individual case, "N/A" will be entered. All entries should be printed legibly in black ink. If any entry error has been made, to correct such an error, a single straight line will be drawn through the incorrect entry and the correct data entered above it. All such changes must be initialed and dated. DO NOT ERASE OR WHITE OUT ERRORS. For clarification of illegible or uncertain entries, the clarification will be printed above the item, then initialed and dated.

A Case Report Form will be completed for each subject enrolled into the clinical study. The Investigator-Sponsor will review, approve and sign/date each completed CRF; the Investigator-Sponsor's signature serving as attestation of the Investigator-Sponsor's responsibility for ensuring that all clinical and laboratory data entered on the CRF are complete, accurate and authentic.

All data including source documents and CRFs will be maintained in a locked filing cabinet in a locked office with limited access to only those involved in the research. Individual subject files will be labeled with the subjects unique study identification number and not their name.

9.4 Clinical Reports

An annual progress report will be submitted to the funding source and the IRB. Investigators will submit a final report of the clinical study to the reviewing IRB within 3 months of termination or completion of the clinical study or the Investigator's part of the clinical study.

9.5 Records Retention

The Investigator-Sponsor will maintain a file of all documents concerning the use of human subjects in research for 3 years from completion of IRB related work and 6 years for HIPAA.

9.6 Data and Specimen Banking

Stored samples will be identified by an indirect identifier (study ID) indefinitely. The study PI and study coordinator will have access to the link between subject information and study ID.

If stored samples are used in the future to look at new markers the subject or parent/guardian will not be notified nor will they receive the results from the analysis. If the samples are used by other research groups for analysis, the subject and guardian will not be notified. However, any samples that are sent to another researcher will be identified only with a number and will not be able to be traced back to the subject. The subject will not be identified in any publication or report from this study. The subject or guardian can request that blood, urine, RNA, and DNA samples be destroyed at any time by contacting the study PI.

10 Study Auditing, and Inspecting

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

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

11 Ethical Considerations

This study is to be conducted according to US and international standards of GCP (Code of Federal Regulations [CFR] 21 CFR 312 and International Conference on Harmonization [ICH] guidelines), applicable government regulations and Institutional research policies and procedures.

This protocol and any amendments will be submitted to a properly constituted independent IRB, in agreement with local legal prescriptions, for formal approval of the study conduct. The decision of the IRB concerning the conduct of the study will be made in writing to the Investigator and a copy of this decision will be provided to the Sponsor before commencement of this study.

12 Study Finances

12.1 Funding Source

Research related costs will be paid for through an investigator initiated grant from Horizon Pharma. ACTIMMUNE will be provided by Horizon Pharma.

12.2 Conflicts of Interest

Any Investigator who has a conflict of interest with this study (patent ownership, royalties, or financial gain greater than the minimum allowable by their institution, etc.) must refer to the Regents Policies on Individual Conflict of Interest Policy or Institutional Conflict of Interest Policy. These policies require University Faculty and staff to report external professional activities and business and significant financial interests related to his or her University activities by submitting a REPA (Report of External Professional Activities) at least once per year. Faculty and staff should also file a REPA when substantial changes in business or financial interests occur, when an activity that presents a potential conflict of interest is anticipated, or when submitting an application for research support or technology transfer, submitting research protocols to the IRB, or receiving financial contributions. All investigators will follow their University's conflict of interest policy.

13 Publications Plan

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

References

1. Tolar J, Teitelbaum SL, Orchard PJ. Osteopetrosis. N Engl J Med. 2004 Dec 30;351(27):2839–49.

2. Gonen KA, Yazici Z, Gokalp G, Ucar AK. Infantile osteopetrosis with superimposed rickets. Pediatr Radiol. 2013 Jan;43(2):189–95.

3. Tolar J, Bonfim C, Grewal S, Orchard P. Engraftment and survival following hematopoietic stem cell transplantation for osteopetrosis using a reduced intensity conditioning regimen. Bone Marrow Transplant. 2006 Dec;38(12):783–7.

4. Schroder K, Hertzog PJ, Ravasi T, Hume DA. Interferon-gamma: an overview of signals, mechanisms and functions. J Leukoc Biol. 2004 Feb;75(2):163–89.

5. Garrett IR, Boyce BF, Oreffo RO, Bonewald L, Poser J, Mundy GR. Oxygen-derived free radicals stimulate osteoclastic bone resorption in rodent bone in vitro and in vivo. J Clin Invest. 1990 Mar;85(3):632–9.

6. Madyastha PR, Yang S, Ries WL, Key LLJ. IFN-gamma enhances osteoclast generation in cultures of peripheral blood from osteopetrotic patients and normalizes superoxide production. J Interferon Cytokine Res. 2000 Jul;20(7):645–52.

7. Rodriguiz RM, Key LL, Ries WL. Combination macrophage-colony stimulating factor and interferon-gamma administration ameliorates the osteopetrotic condition in microphthalmic (mi/mi) mice. Pediatr Res. 1993 Apr;33(4 Pt 1):384–9.

8. Key LL, Ries WL, Rodriguiz RM, Hatcher HC. Recombinant human interferon gamma therapy for osteopetrosis. J Pediatr. 1992 Jul;121(1):119–24.

9. Key LLJ, Rodriguiz RM, Willi SM, Wright NM, Hatcher HC, Eyre DR, et al. Long-term treatment of osteopetrosis with recombinant human interferon gamma. N Engl J Med. 1995 Jun 15;332(24):1594–9.

10. Driessen GJ, Gerritsen EJ, Fischer A, Fasth A, Hop WC, Veys P, et al. Long-term outcome of haematopoietic stem cell transplantation in autosomal recessive osteopetrosis: an EBMT report. Bone Marrow Transplant. 2003 Oct;32(7):657–63.

11. Maluish AE, Urba WJ, Longo DL, Overton WR, Coggin D, Crisp ER, et al. The determination of an immunologically active dose of interferon-gamma in patients with melanoma. J Clin Oncol. 1988 Mar;6(3):434–45.

12. Meyskens FL, Kopecky KJ, Taylor CW, Noyes RD, Tuthill RJ, Hersh EM, et al. Randomized trial of adjuvant human interferon gamma versus observation in high-risk cutaneous melanoma: a Southwest Oncology Group study. J Natl Cancer Inst. 1995 Nov 15;87(22):1710–3.

13. Dawson R, Condos R, Tse D, Huie ML, Ress S, Tseng C-H, et al. Immunomodulation with recombinant interferon-gamma1b in pulmonary tuberculosis. PLoS ONE. 2009;4(9):e6984.

14. Muir AJ, Sylvestre PB, Rockey DC. Interferon gamma-1b for the treatment of fibrosis in chronic hepatitis C infection. J Viral Hepat. 2006 May;13(5):322–8.

15. Li JT, Yunginger JW, Reed CE, Jaffe HS, Nelson DR, Gleich GJ. Lack of suppression of IgE production by recombinant interferon gamma: a controlled trial in patients with allergic rhinitis. J Allergy Clin Immunol. 1990 May;85(5):934–40.

16. Bitran JD, Green M, Perry M, Hollis DR, Herndon JE. A phase II study of recombinant interferon-gamma following combination chemotherapy for patients with extensive small cell lung cancer. CALGB. Am J Clin Oncol. 1995 Feb;18(1):67–70.

17. Ghanei M, Panahi Y, Mojtahedzadeh M, Khalili ARH, Aslani J. Effect of gamma interferon on lung function of mustard gas exposed patients, after 15 years. Pulm Pharmacol Ther. 2006;19(2):148–53.

18. Maciejewski J, Selleri C, Anderson S, Young NS. Fas antigen expression on CD34+ human marrow cells is induced by interferon gamma and tumor necrosis factor alpha and potentiates cytokine-mediated hematopoietic suppression in vitro. Blood. 1995 Jun 1;85(11):3183–90.

19. Selleri C, Maciejewski JP, Sato T, Young NS. Interferon-gamma constitutively expressed in the stromal microenvironment of human marrow cultures mediates potent hematopoietic inhibition. Blood. 1996 May 15;87(10):4149–57.

20. Wanachiwanawin W, Wiener E, Siripanyaphinyo U, Chinprasertsuk S, Mawas F, Fucharoen S, et al. Serum Levels of Tumor Necrosis Factor-alpha, Interleukin-1, and Interferon-gamma in beta-Thalassemia/HbE and Their Clinical Significance. Journal of Interferon & Cytokine Research. 1999 Feb 1;19(2):105–11.

21. Butthep P, Wisedpanichkij R, Jindadamrongwech S, Fucharoen S. Elevated erythropoietin and cytokines levels are related to impaired reticulocyte maturation in thalassemic patients. Blood Cells, Molecules, and Diseases. 2015 Feb;54(2):170–6.

22. Varni JW, Thompson KL, Hanson V. The Varni/Thompson Pediatric Pain Questionnaire. I. Chronic musculoskeletal pain in juvenile rheumatoid arthritis. Pain. 1987 Jan;28(1):27–38.

23. Cohen LL, Lemanek K, Blount RL, Dahlquist LM, Lim CS, Palermo TM, et al. Evidence-based assessment of pediatric pain. J Pediatr Psychol. 2008 Oct;33(9):939-955-957.

24. Thompson KL, Varni JW, Hanson V. Comprehensive assessment of pain in juvenile rheumatoid arthritis: an empirical model. J Pediatr Psychol. 1987 Jun;12(2):241–55.

25. Burckhardt CS, Jones KD. Adult measures of pain: The McGill Pain Questionnaire (MPQ), Rheumatoid Arthritis Pain Scale (RAPS), Short-Form McGill Pain Questionnaire (SF-MPQ), Verbal Descriptive Scale (VDS), Visual Analog Scale (VAS), and West Haven-Yale Multidisciplinary Pain Inventory (WHYMPI). Arthritis Care & Research. 2003;49.S5:S96–104.

26. Hawker GA, Mian S, Kendzerska T, French M. Measures of adult pain: Visual Analog Scale for Pain (VAS Pain), Numeric Rating Scale for Pain (NRS Pain), McGill Pain Questionnaire (MPQ), Short-Form McGill Pain Questionnaire (SF-MPQ), Chronic Pain Grade Scale (CPGS), Short Form-36 Bodily Pain Scale (SF-36 BPS), and Measure of Intermittent and Constant Osteoarthritis Pain (ICOAP). Arthritis Care Res (Hoboken). 2011 Nov;63 Suppl 11:S240-252.

27. Devane JG, Martin ML, Matson MA. A short 2 week dose titration regimen reduces the severity of flu-like symptoms with initial interferon gamma-1b treatment. Curr Med Res Opin. 2014 Jun;30(6):1179–87.

28. Mosteller RD. Simplified calculation of body-surface area. N Engl J Med. 1987 Oct 22;317(17):1098.

APPENDIX A:

Data and Safety Monitoring Plan (Multi-Centered, No DSMB)

Project No. : 21549-01
Protocol Title: Open-label Phase 1/2 Study with a Single Arm of Interferon Gamma-1b Treatment of Osteopetrosis
Principal Investigator: Lynda Polgreen, MD, MS
Monitor: Peter Liu, MD

UCLA CTSI: ☐ UCLA-CHS ☒ LA Biomed/ Harbor-UCLA ☐ Cedar-Sinai Medical Center ☐ Charles Drew University

Version: 1.0 Version Date: June 23, 2015

1. Categorization of risk level (low, medium, or high) and a discussion of the relevant specific considerations.

Risk Level: ☐ Low ☒ Moderate ☐ High

Justification of Risk Level:
Potential for direct subject benefit.

2. Specific information to be provided at the time of each periodic review and the bases for selecting this data set.

Enrollment Data:
The investigator will prepare a periodic report to include **Local** target, interval and cumulative data in a tabular format as follows:

- ☒ Number of subjects screened
- ☒ Number of subjects enrolled
- ☒ Number of screen failures with reason for screen failure
- ☒ Dropout number with reason for dropout and stage of study at dropout
- ☒ Completion number
- ☒ Other study specific enrollment data such as subset enrolled in each group, site, etc. Specify below: enrollment at each site

For Entire Study:

- ☒ Number of subjects enrolled
- ☒ Completion number

Local Adverse Event Reporting:

☒ **All study-related adverse events** (including loss of confidentiality and moderate-severe discomfort with study sample collection) with severity and attribution (possibly, probably or definitely related).

☒ The rates of routine illnesses (URI, headaches, muscle aches, etc.) and traumatic injuries are not expected to be different than usual and will not be reported or tracked as unexpected AEs but will be tracked and reported as AEs for the DSM Reports.

Serious Adverse Event and Unexpected Adverse Events:

☒ For all study-related serious adverse events (SAEs) and study-related unexpected adverse events (UAEs), the clinical summary will also be provided in a cumulative fashion.

☒ Due to the large number of disease-related SAEs such as

fracture, bone pain, osteonecrosis, anemia, neutropenia, thrombocytopenia, cranial nerve impingement, and hypocalcemia,

these serious adverse events will be tracked and evaluated by treatment group at each DSM periodic reporting but will not be reported by expedited reporting to the IRB.

Entire Study Serious Adverse Event Reporting:

☒ All **study-related** serious adverse events (SAEs) and **study-related** unexpected adverse events (UAEs)

☒ For all study-related serious adverse events (SAEs) and study-related unexpected adverse events (UAEs), the clinical summary will also be provided in a cumulative fashion.

The investigator will comply with the Policies for Reporting of Adverse Events to the local IRB that requires reporting of adverse events that in the opinion of the investigator may represent unanticipated problems involving risks to the other subjects in the research.

☒ The investigator will provide a tabulation of the unanticipated problems that meet the IRB reporting requirement with their evaluation of each events in the DSM Report

Monitor's Review:

☒ The investigator will provide the report to the Monitor at least 30 day before the CTRC due date for DSM Report submission.

☒ The Monitor will review the report and may request additional information from the investigator as needed to evaluate the safety and progress of the study. The monitor will submit the periodic report to the PI with the following by the due date.

- ☒ Narrative summary of information presented.
- ☒ Any concerns regarding adverse event rates, recruitment rates, drop-out rates, study integrity, etc
- ☒ Any concerns regarding adverse event rates, recruitment rates, drop-out rates, study integrity, etc. with statement of how these concerns were resolved with the PI.
- ☒ Recommendations for future study conduct (continuation, modification or termination).

☒ The PI will submit the DSM Report with the Monitor's review to the CTRC by the due date via iMedris.

3. Frequency of review, and rationale for recommended frequency.

Frequency of Review: Every 6 months .
Rationale for Review Frequency: Limited number of subjects in the study (10 will be enrolled over 18 months) and the level of moderate risk.

4. Designation of individual(s) as independent monitor(s) reviewing the information. An explicit review of any conflict of interest issues should be presented.

Dr. Jennifer Yee will act as the Monitor for this clinical study. Dr. Yee is a faculty member at Harbor-UCLA with an appointment in the Department of Pediatrics, division of Endocrinology. She has expertise in the area of clinical trials and metabolic bone disease. She has no conflict of interest with the local PI or the study sponsor.

APPENDIX B

Schedule of Events (next page)

Schedule of Events											If ADA Positive
			time from 1st IFN- γ 1b dose							time from last IFN- γ 1b dose	
	Screening[a]	Baseline	3 weeks (± 2 days)	4 weeks (±2 days)	6 weeks (±2 days)	3 months (± 1 week)	6 months (± 1 week)	9 months (± 1 week)	12 months (± 1 week)	2-3 weeks	every 4-6 weeks
General											
Medical history	X	X				X	X		X		
Physical exam		X				X	X		X		
Genotype[b]		X									
RNA and DNA samples		X[f]				X	X		X		
Anthropometric measurements	X	X[f]				X	X		X		
Safety Measures[c]											
Basic metabolic panel (BMP)	X	X		X	X	X	X	X	X		
Liver function tests (LFT)	X	X		X	X	X	X	X	X		
Urine pregnancy test (UPT) [e]	X	X				X	X	X	X		
Urinalysis (UA)	X	X		X	X	X	X	X	X		
ECHO with interpretation	X										
EKG with interpretation	X										
Anti-drug antibodies (ADA)		X	X		X	X	X		X	X	X
Bone Biomarker Testing[d,g]											
Formation Testing (bone specific alkaline phosphatase [BAP], type 1 procollagen [P1NP])	-	X				X	X		X		
Resorption testing (C-telopeptide [CTX], Tartrate resistant acid phosphatase type5b [TRAP5b])	-	X				X	X		X		
RANKL, OPG, CK-BB	-	X				X	X		X		
Endocrine Assessment											
Total 25-OH Vitamin D (25OHD)	-	X			X	X	X		X		
Serum ionized calcium	-	X				X	X		X		
Urine calcium: creatinine	-	X				X	X		X		
Intact parathyroid hormone (iPTH)	-	X				X	X		X		
Inflammatory/Immunology											
CBC with differential and platelets	-	X[f]				X	X		X		
Erythrocyte sedimentation rate (ESR)	-	X[f]				X	X		X		
Immunologic phenotyping (CD3, 4, 8, 14, 15, 16, 19, 34, 56)[g]	-	X				X	X		X		
Neuropsychological testing											
Pain assessment	-	X				X	X		X		
Quality of life survey	-	X				X	X		X		
Orthopedic assessment											
Dual energy X-ray absorptiometry (DXA) lumbar spine, total body, total hip and femoral neck (adults ≥18yrs only)	-	X				-	-		X		
Peripheral Quantitative CT (pQCT) left tibia	-	X				-	X		X		
In vitro osteoclast function assays – Mansky Lab[h]											
Gene expression – osteoclast marker genes	-	X				X	X		X		
Osteoclast activity – ability to release calcium phos from coated plate	-	X				X	X		X		
Infectious outcomes											
Number of hospitalizations in prior 3 months	-	X[f]				X	X		X		
Number of days treated with antibiotics in last 3 months	-	X[f]				X	X		X		
Type of infectious organism	-	X[f]				X	X		X		
Summary of samples collected per visit											
Serum (red top)	1	4	1		1	4	4		4	1	1
Plasma (purple top)	-	5	-		-	5	5		5	-	-
Urine (non-sterile)	1	1	-		-	1	1		1	-	-
Whole blood		1				1	1		1		

RANKL=Receptor activator of nuclear factor kappa-B ligand; OPG=osteoprotegerin; CK-BB=creatinine kinase isoenzyme BB.

[a] Screening visit evaluations will count for baseline evaluations where applicable.

[b] Genetic testing for CLCN7, CAII, TCIRG1, OSTM1, and RANKL mutations will be done at the University of Minnesota if not already performed clinically

[c] Subjects will be contacted on a weekly basis either by email or telephone to monitor safety during the initial 4 week dose escalation period.

[d] Sample collection will be postponed if needed to ensure collection is more than 2 weeks since last fever (>100.4°F [38°C] axillary) or fracture.

[e] Post-menarchal females.

[f] Height (for subjects <18 years), CBC, and ESR will be obtain from the patient's medical record when available and number of hospitalizations and infectious data from the patient's medical record or if not available then the patient's recollection at the baseline visit.

[g] Samples batch shipped every 3 months to Troy Lund/Kelly Miettunen at UMN

[h] Whole blood shipped overnight to Kim Mansky at UMN