Investigating the Use of Prolia (Denosumab) in the Treatment of Acute Charcot Neuroarthropathy

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1 INTRODUCTION

1.1 Background

Charcot neuroarthropathy (CN) is a debilitating disease primarily affecting poorly controlled diabetic patients with peripheral neuropathy. The consequences of CN include ulcerations of the foot and ankle, osteomyelitis, and severe musculoskeletal deformity. These consequences frequently lead to below-knee amputation of the affected limb. The literature estimates that CN affects 0.08% of diabetics. However, the prevalence can be as high as 13% among high-risk diabetic patients (1). Patients with CN are reported to have a significant decreased ability to perform activities of daily living and experience a lower quality of life compared to diabetics without pedal complications (2, 3). The disease also has a significant impact on health-care costs, with CN accounting for an estimated $5.4 million in California in-patient health-care costs annually between 2008 and 2012 (4).

CN is characterized by a sequence of inflammation and osseous degradation. The disease process begins in the acute phase, characterized by severe inflammation. Patients suffering from concomitant neuropathy will generally continue to ambulate on the affected limb. This exacerbates the inflammatory process and creates a devastating inflammatory cascade, leading to severe osseous destruction and joint dislocation. The inflammatory cascade will eventually regress naturally, causing the remodeled bone to consolidate in a malalignment. This leads to a characteristic rocker bottom deformity. This resultant deformity is often nonfunctional, and, depending on the severity, may require reconstructive surgery or amputation below the knee (5-7).

Recent breakthroughs in identifying the etiology of CN have clarified the specific influence of a variety of inflammatory molecules and cytokines. In the osteolytic acute phase of CN, there is also an increased level of tumor necrosis factor alpha (TNF-α), which may be causing an inflammatory cascade of events(8). (TNF-α) has been found to increase expression of the receptor activator of nuclear factor kappa-B ligand (RANKL), leading to activation of the nuclear factor kappa-light-chain-enhancer of activated B cells (NF-kB) (9, 10). This ultimately leads to osteoclastogenesis and bone turnover (11). The increase in osteoclast activity, combined with persistent ambulation, leads to further osseous fracture and bone weakening (12, 13). These fractures further stimulate the inflammatory process and exacerbate the devastating cycle (14).

The current gold standard for treatment of acute CN is immediate non-weightbearing in a total contact cast (TCC) (15). This stage of treatment requires frequent changing of the TCC, and can last for up to 6 months (16). Resolution of skin temperature gradient clinically defines the end of the acute phase. Specifically, the acute phase is considered resolved when the skin temperature of the affected foot reaches $\pm$ 2 degrees Celsius of the contralateral foot (7). Once the acute phase concludes, the patient is transitioned to weightbearing status in a patient-specific molded Charcot Restraint Orthotic Walker (CROW).

1.2 Potential Pharmacotherapy

Studies have investigated the use of bisphosphonates in treating the inflammatory stage of CN, but these studies failed to demonstrate a clear clinical benefit (17, 18). TNF-alpha inhibitors, calcitonin, and human parathyroid hormone have also been proposed (19). However, none of these medications have been formally investigated or FDA approved for the treatment of acute CN.

A recent systematic review reinforced the opinion that anti-RANKL agents may represent a breakthrough in the treatment of acute CN, concluding, given the role of the RANK/RANKL pathway in CN pathophysiology, RANKL inhibition may represent an effective treatment for acute CN (20). Nonetheless, to date, there are no studies on the use of anti-RANKL agents for this disease.

1.3 Prolia Description

Prolia (denosumab), a potent RANKL inhibitor, has been observed to reduce bone turnover and increase bone mineral density in both female and male osteoporotic patients. 60 mg Prolia delivered once subcutaneously every 6 months has been shown to be safe to use and is able to reduce fracture risk in osteoporotic patients (21-25). Prolia, when compared to alendronate (a bisphosphonate) has been shown to be more effective at increasing bone mineral density with less adverse events in osteoporotic patients (26, 27).

Inflammatory cytokines stimulate the production of RANKL, which leads to the maturation of osteoclast progenitors. By inhibiting the binding of RANKL to its receptor, denosumab inhibits osteoclast formation, function and survival, which in turn, may arrest the bony destruction that characterizes CN thus making Prolia a major candidate for the treatment of acute CN.

1.4 Prolia Indications and Adverse Effects

Prolia is a RANK ligand (RANKL) inhibitor indicated for:

- Treatment of postmenopausal women with osteoporosis at high risk for fracture
- Treatment to increase bone mass in men with osteoporosis at high risk for fracture
- Treatment to increase bone mass in men at high risk for fracture receiving androgen deprivation therapy for nonmetastatic prostate cancer
- Treatment to increase bone mass in women at high risk for fracture receiving adjuvant aromatase inhibitor therapy for breast cancer

Contraindications:

- Hypocalcemia
- Pregnancy
- Known hypersensitivity to Prolia

- Warnings and precautions:Same Active Ingredient: Patients receiving Prolia should not receive

XGEVA®

- Increased risk of multiple vertebral fractures following Prolia discontinuation
- Hypersensitivity including anaphylactic reactions may occur.

Discontinue permanently if a clinically significant reaction occurs

- Hypocalcemia: Must be corrected before initiating Prolia. May worsen, especially in patients with renal impairment. Adequately supplement patients with calcium and vitamin D. In patients pre-disposed to hypocalcemia and disturbances of mineral metabolism, clinical monitoring of calcium and mineral levels (phosphorus and magnesium) is highly recommended within 14 days of Prolia injection.
- Osteonecrosis of the jaw: Has been reported with Prolia. Monitor for symptoms
- Atypical femoral fractures: Have been reported. Evaluate patients with thigh or groin pain to rule out a femoral fracture
- Serious infections including skin infections: May occur, including those leading to hospitalization. Advise patients to seek prompt medical attention if they develop signs or symptoms of infection, including cellulitis
- Dermatologic reactions: Dermatitis, rashes, and eczema have been reported. Consider discontinuing Prolia if severe symptoms develop
- Severe Bone, Joint, Muscle Pain may occur. Discontinue use if severe symptoms develop
- Suppression of bone turnover: Significant suppression has been demonstrated. Monitor for consequences of bone oversuppression

Adverse Reactions:

- Postmenopausal osteoporosis: Most common adverse reactions (> 5% and more common than placebo) were: back pain, pain in extremity, hypercholesterolemia, musculoskeletal pain, and cystitis. Pancreatitis has been reported in clinical trials
- Male Osteoporosis: Most common adverse reactions (> 5% and more common than placebo) were: back pain, arthralgia, and nasopharyngitis
- Bone loss due to hormone ablation for cancer: Most common adverse reactions (≥ 10% and more common than placebo) were: arthralgia and back pain. Pain in extremity and musculoskeletal pain have also been reported in clinical trials

2 STUDY DESIGN

2.1 Study Objective

The primary objective of this study is to assess the safety of denosumab 60 mg, in conjunction with a TCC, as a treatment for acute CN. This pilot study will inform feasibility of an appropriately designed randomized, controlled study. This study will be a prospective, single-arm, open-label, Phase 1 trial.

Safety will be assessed by reported and observed adverse events. These will be recorded at each follow-up visit. Objectively, efficacy will be defined clinically as the reduction in foot temperatures compared to a baseline value.

The clinical importance of foot temperatures in the context of acute Charcot neuroarthropathy has been established in the medical literature. Skin temperature was formally described as a measure of acute phase activity in 1972 (28). Subsequent studies provided further validation, identifying the correlation of skin temperature with disease activity (29, 33, 34). Clinical studies, including those evaluating pharmaceutical interventions, have predominantly relied upon skin temperature in defining the resolution of the acute phase (17-18, 28-35).

Lab values will be used as markers of bone turnover and inflammation. These will be measured at the initial and each follow-up visit. Bone specific alkaline phosphatase (BSAP) is a marker of bone turnover. While a marker of bone formation, BSAP has been shown to be elevated in acute Charcot neuroarthropathy reflecting ongoing bone turnover and remodeling. Subjectively efficacy will be determined by recording symptom score on an 100-point Visual Analog Scale (VAS, 100mm scale). The surveys will be taken at the initial visit and at each follow-up visit. A study schema is provided in section 5.6. All enrolled patients will be receiving both pharmaceutical treatment (Prolia) and standard of care.

As this study is a pilot study and therefore explicitly underpowered, a power analysis was not performed. Statistical analysis will consist of descriptive statistics of the subject demographics, mean temperature differential of the subjects stratified by length of follow-up, mean BSAP levels stratified by length of follow-up, mean time until normalization of skin temperature gradient (within 2 degrees Celsius), and mean change in VAS stratified by length of follow-up. Graphical representations of temperature differential and BSAP by length of follow-up among subjects will be presented.

2.1.1 Use of Historical Controls

The future, adequately-powered study, which this pilot serves as a precursor to, will focus on efficacy rather than safety as the primary endpoint. Data from this pilot study, in combination with study data from the existing literature will be used to ensure the future study will be adequately powered. The rationale for using a historical control is to allocate more novel resources to the patients enrolled in this study. This will also help increase the power of the study as well as reduce type 1 error. Data from the following studies which will be included for this purpose is listed below:

Bem R, et al. Intranasal calcitonin in the treatment of acute Charcot neuroosteoarthropathy: a randomized controlled trial. Diabetes Care. 2006 Jun;29(6):1392-4.
A 2006 study by Bem et al investigating the use of intranasal calcitonin included 16 control patients affected by Charcot neuroarthropathy. Initial skin temperature differential between the affected and non-affected limb was 3.6 +/- 0.8° C in the control group. At 3 months, the skin differential between the affected and non-affected limb was 1.5 ± 0.5°C which is a 2.1°C improvement. Skin temperature difference was also calculated at 1, 2 and 6 months after baseline. All patients were treated with off-loading by removable contact cast or cast walker.

Pitocco D, et al. Six-month treatment with alendronate in acute Charcot neuroarthropathy: a randomized controlled trial. Diabetes Care. 2005 May;28(5):1214-5.

A 2005 study by Pitocco et al included 9 control patients. Initial skin temperature differential was 3.4 +/- 1.2° C in the control group and 3.6 +/- 1.1° C in the treatment group. At 6 months, there was a 1.5° C improvement in the control group and a 1.7° C improvement in the treatment group (temperature differential of 1.9° C in each group). There was no difference between the temperature differential between control and treatment groups at 6 months. Patients were treated with a total contact cast for the first 2 months and then a pneumatic walker for 4 months, though the treatment group was also administered alendronate.

Jude EB1, et al. Bisphosphonates in the treatment of Charcot neuroarthropathy: a double-blind randomised controlled trial. Diabetologia. 2001 Nov;44(11):2032-7.
A 2001 study by Jude et al included 18 control patients. Initial skin temperature differential was 3.3° +/- 1.4° C in the control group. After 4 weeks, the skin temperature differential was improved by 1.4 +/- 0.7° C in the control group, with an overall reduction in skin temperature to 1.9° C between the affected and non-affected limb. Skin temperature gradient among the control was also portrayed graphically at 2, 4, 6, 8, 10, 12, 24, 36, and 52 weeks. Control patients were offloaded using either a scotch cast boot, pneumatic walker, or total contact cast.

Armstrong DG, Lavery LA. Monitoring healing of acute Charcot's arthropathy with infrared dermal thermometry. J Rehabil Res Dev. 1997 Jul;34(3):317-21.
A 1997 study by Armstrong and Lavery followed 39 acute Charcot patients, without an intervention group. Patients were offloaded using either total contact casts or removable cast walkers. Mean temperature differential was reported on a monthly basis for 12 months. While a graphical representation of the monthly mean temperature difference is provided in the study (using point estimates), explicit numerical values are not provided.

Armstrong DG1, Lavery LA, Liswood PJ, Todd WF, Tredwell JA. Infrared dermal thermometry for the high-risk diabetic foot. Phys Ther. 1997 Feb;77(2):169-75; discussion 176-7.
This 1997 study, also led by Armstrong, followed 21 acute Charcot patients. The study compared the patients with Charcot against two separate groups: an asymptomatic neuropathy group, and a neuropathic ulcer group. The study reported an initial temperature difference of 8.3°F (90.4°F

versus 82.1°F). The only further temperature gradient was reported as 0.04°F at 12 months, though the exact background temperature values were not reported.

Armstrong DG, Todd WF, Lavery LA, Harkless LB, Bushman TR. The natural history of acute Charcot's arthropathy in a diabetic foot specialty clinic. J Am Podiatr Med Assoc. 1997 Jun;87(6):272-8.
Game FL, et al. Audit of acute Charcot's disease in the UK: the CDUK study. Diabetologia. 2012 Jan;55(1):32-5.
Two additional studies reported time-to-resolution of the acute phase, though did not report temperature gradient after baseline. Time-to-resolution data is clinically useful, providing an estimate of the length of the acute phase period. As a marker of disease activity, however, skin temperature gradient is of potentially greater use. Time-to-resolution data only reflects the end of the acute phase period; this data does not indicate if the clinical response, for example, occurred mostly during certain time intervals or was more gradual in nature. Skin temperature gradient, reported at defined intervals, can provide a more complete representation of disease activity. This information can be used to correlate the clinical response to external events, such as the administration of medication or a change in off-loading modalities.

A study by Armstrong et al in 1997 followed 55 patients without an intervention group, with an average initial temperature gradient of 5.1 +/- 1.4° C. Patients were non-weight-bearing and immobilized in serial casts for an average acute phase period of 18.5 +/- 10.6 weeks. An observational study by Game et al in 2012 included a large volume of patients, though the study was uncontrolled and affected by a range of design concerns. Off-loading consisted initially of a non-removable device in 35.4% of cases and with a removable device in 50% of cases. The median time-to-resolution of the 121 patients who were treated without bisphosphonates was 10 months (range: 2-29 months).

While skin temperature gradients serve as the secondary efficacy endpoints at 6 and 12 months, the skin temperature gradients will be calculated at every follow-up appointment. This data in comparison with historical controls will be utilized in future power analyses to determine sample size.

2.2 Treatment Group

Subjects will be assigned to a single treatment group and will receive offloading with a TCC and a 60 mg SC dose of Prolia. The TCC offloading device will be identical for all subjects. As the goal will be for 6 subjects to complete the trial, 7 subjects will be enrolled to allow dropout of 14.3% (1 subject). The historical controls may have a potential source of variation given the differences in offloading devices, but the variability is minimal and should not be a major source of confounding.

2.3 Study Duration

Each subject will participate in this Phase 1 trial for a total of 1 year. There will be a screening visit with podiatry, 2 additional screenings with physical therapy and dentistry respectively, and biweekly

follow-ups thereafter with podiatry and either endocrinology or rheumatology until the 3rd month. The subject will also return for 6th, 9th and 12th month follow-ups where they will be monitored for adverse events; pain will be assessed using the Visual Analog Scale, scored out of 100. Office visits will be jointly conducted by a podiatrist and, alternatingly, either a rheumatologist (Dr. Emmanuel Katsaros) or an endocrinologist (Dr. Airani Sathananthan). The evaluation by the rheumatologist and endocrinologist will focus on vigilant monitoring for adverse events from treatment.

2.4 Study Endpoints

2.4.1 Primary Endpoints - Safety

1. Subject incidence of adverse events and changes in lab values.

2.4.2 Secondary Endpoints - Efficacy

1. Change in skin temperature difference in degrees Celsius between the affected and non-affected limb at 6 months and 12 months. For each individual, the following difference will be calculated:
Temperature (affected limb, °C) minus temperature (unaffected limb, °C) at month 6.
Temperature (affected limb, °C) minus temperature (unaffected limb, °C) at month 12.
There are therefore effectively two secondary efficacy endpoints.

2.4.3 Exploratory Endpoints

1. Change from baseline Visual Analog Scale (VAS, 100mm scale) survey scores at 6 months and 12 months.

2.5 Measurement Methods

At each study visit, the treating podiatrist will visually and physically assess patients. Skin temperature will be recorded using an infrared thermometer. The same infrared thermometer will be used at each patient encounter to maintain consistency. Laboratory values will be obtained at every visit. See Section 4.1.2.C for list of tests. The Visual Analog Scale pain survey will be administered at every visit to subjectively measure patient pain.

3 POTENTIAL RISKS AND BENEFITS

3.1 Adverse effects of Prolia

See Section 1.4. Subjects will be advised to contact study coordinators if they notice signs of any of the adverse effects mentioned in Section 1.4. Comprehensive dental will be provided prior to enrollment in the study. Subjects will be advised to maintain proper oral hygiene during the study. If subjects require dental care following the dental screening, they will be advised to inform their dentist that they are receiving Prolia and should therefore avoid invasive dental procedures during treatment (due to the risk of osteonecrosis of the jaw).

In consideration of the potential musculoskeletal risks of Prolia, including muscle weakness, comprehensive physical therapy screenings will also be coordinated. Physical therapy evaluations will occur prior to enrollment, will subsequent appointments determined based on the needs of the individual subject.

3.2 Contraindications to Prolia administration

- *See section 1.4*

3.3 Benefits of Study

Because recent research supports the role of inflammatory cytokines in the pathogenesis of the disease, it is important to explore treatment options for the disease which can decrease inflammation and halt the disease process. By inhibiting RANK/RANKL pathway in CN pathophysiology, it is hypothesized that the acute phase of the disease will be arrested. This can prevent the development of debilitating deformities and the possible consequences therein, including medial arch collapse, a rocker-bottom foot, bony deformity, ulceration, infection, and eventual amputation.

It is believed that the potential benefits of this study outweigh the potential risks.

4 SUBJECT SELECTION

Prior to screening for eligibility for the study, an informed consent must be obtained. Eligibility will be determined by a set of inclusion and exclusion criteria outlined in Sections 4.2 and 4.3.

4.1 Informed Consent/Screening Visit

Subjects will complete a written informed consent prior to screening for eligibility. This will be administered by the investigators at the study site in the patient's preferred language (English or Spanish). The informed consent must be signed and dated. Each subject will be assigned a number in ascending order beginning from 01. A screening log of all the subjects will be obtained for each written informed consent. The screening log includes: screening number, first and last name, age, gender, eligibility status or reason for ineligibility. This information will be stored in a locked security box, and kept in a locked cabinet at the Patient Care Center. This will be accessible by Dr. David Shofler and specifically designated study personnel.

4.1.1 Medical History Screening

Subject eligibility will be determined by the inclusion/exclusion criteria. Record if the subject does or does not meet the criteria.

1. Record date of visit, subject enrollment number, and subject initials
2. Verify subject eligibility based on inclusion and exclusion criteria. Adherence to criteria will be noted.
3. Record subject demographics, including date of birth, gender, race, and weight, and if there were any treatments attempted prior to enrollment.

4.1.2 Physical Assessment Screening

Subject eligibility will be determined by the inclusion and exclusion criteria. Subject adherence to inclusion or exclusion criteria will be recorded.

4.1.2.A Criteria for Diagnosis of Acute Charcot Neuroarthropathy

Subjects must be diagnosed with acute CN. The subject must have been diagnosed with acute CN within 1 month. Any prior treatments will be noted on the screening log. At least two of the clinical criteria must be met, one of which must include elevated limb temperature. Radiographic staging will be recorded as well.

Clinical criteria:

- Erythema and/or
- Edema and/or
- Heat: Temperature gradient difference of $\geq$ 2 degrees Celsius of affected limb to non-affected limb, with the affected limb having the higher temperature

Radiographic criteria:

- Modified Eichenholtz Stage 0: Radiographs are negative or
- Modified Eichenholtz Stage 1: Subchondral bone fragmentation, periarticular fracture with or without joint subluxation/dislocation

1. The physician will visually assess for the presence of erythema and edema.

2. The temperature gradient between the affected and non-affected limb will be assessed with an infrared thermometer.
3. X-rays will be taken of the foot in AP, lateral, and oblique views.
4. Ankle x-rays will be taken in AP, lateral, and mortise views.

4.1.2.B Criteria for Diagnosis of Peripheral Neuropathy

- Patients must be either previously diagnosed with neuropathy or meet the criteria of the following screening exams for neuropathy
    - Semmes Weinstein Monofilament Test (SWMF) with loss of protective sensation in $\geq$ 4 of 10 locations of the affected limb OR
    - Vibration perception threshold biothesiometer mean reading of > 25 mV to the great toe

  - If a previous diagnosis of neuropathy is used to meet this criteria, a physician's record will be required and will be incorporated into the patient's file.

4.1.2.C Preliminary Lab Tests (Initial Visit)

- Pregnancy test (if appropriate)
- CBC with differential
- CRP
- ESR
- Chem-7 panel
    - (Sodium, Potassium, Chloride, Bicarbonate, BUN, Creatinine, Glucose)
- Liver function tests
- Calcium
- HbA1c
- Fasting blood glucose
- Bone specific alkaline phosphatase (BSAP)

4.1.2.D Dental Health Screening

Subject's dental health will be evaluated

- Consultation with Dr. Diana Folmsbee, DMD
- Radiographic studies
    - Panoramic Radiograph
    - 4 Bitewing Radiographs
    - 2 Periapical Radiographs – on a case-by-case basis

4.1.3.E Physical Therapy Screening

Subject's physical strength will be evaluated, and mobility protocol completed

- Consultation with Dr. Lindsey Liggan, PT, DPT

4.2 Inclusion Criteria

Subjects must meet the following inclusion criteria in order to qualify for enrollment:

1. Men or women > 30 years old
2. Subject is able and willing to comply with study procedures, and is able to give signed and dated consent

3. Subject meets criteria for diagnosis of Diabetes Mellitus Type 1 or 2, active Charcot neuroarthropathy, and peripheral neuropathy
4. Subjects with serum calcium or albumin-adjusted serum calcium ≥2.0 mmol/L (8.0mg/dL)

To recruit potential subjects, podiatric offices local to a 15 miles radius will be informed of the study. The primary investigator will reach out to the podiatric offices directly, and a flyer advertisement will be distributed. Subjects will be enrolled at the Foot and Ankle Clinic of the WesternU Patient Care Center. The period of enrollment for the study will be up to 2 years. For subjects that meet the inclusion criteria, a written and oral consent will be collected. All office visits will occur at the WesternU Patient Care Center.

For Women of Child bearing potential:

Must agree to practice abstinence (not have sex) or must agree to use a highly effective method of birth control during treatment with denosumab and for an additional 5 months after the last dose of denosumab. Postmenopausal women are those who fit into one of the following categories:

- Age ≥ 55 years, with cessation of menses for 12 or more months.
- Age < 55 years, but no spontaneous menses for at least 2 years.
- Age < 55 years and spontaneous menses within the past 1 year, but currently amenorrheic (for example, spontaneous or secondary to hysterectomy), AND with documented postmenopausal gonadotropin levels (luteinizing hormone and follicle-stimulating hormone levels > 40 IU/L) or postmenopausal estradiol levels (< 5 ng/dL) or according to the definition of "postmenopausal range" for the laboratory involved.
- Underwent a bilateral oophorectomy.

Highly effective methods of birth control include:

- Combined (estrogen and progestogen) hormonal methods (pills, vaginal ring, or skin patch)
- Single hormonal methods (progesterone) to release the egg from the ovary (pills, shots/injections, or implants placed under the skin by a healthcare provider)
- Intrauterine device (IUD)
- Intrauterine hormonal-releasing system (IUS)
- Surgery to tie both fallopian tubes (bilateral tubal ligation/occlusion)
- Your male partner has had a vasectomy and testing shows there is no sperm in the semen

4.3 Exclusion Criteria

Subjects will not be enrolled in the study if any of the following criteria are met

1. Unable to provide signed and dated consent.
2. Charcot neuroarthropathy of the ipsilateral lower extremity, diagnosed over 1 month prior.
3. Prior foot or ankle surgery of the ipsilateral lower extremity.
4. Prior amputation at any level of either lower extremity.
5. Prior foot or ankle fracture of the ipsilateral lower extremity unrelated to the current acute CN episode.
6. Currently has any of the following:

a. Infection
b. Foot ulceration
c. Hypocalcemia
d. Creatinine clearance less than 30 mL/min or on dialysis
e. Pre-existing disturbance of mineral metabolism (e.g., hypoparathyroidism unstable on therapy, thyroid or parathyroid surgery, vitamin D deficiency, malabsorption syndromes, excision of small intestine, history of diseases affecting bone metabolism) that has not been effectively corrected or treated.

7. Have undergone revascularization procedures of the lower extremities.
8. Female subjects who are pregnant or planning to breastfeed should not participate in this study.
9. Determined to have poor oral hygiene after dental screening or are at increased risk for developing osteonecrosis of the jaw.
10. History of osteonecrosis of the jaw.
11. History of tooth extraction or other dental surgery within the prior 6 months.
12. Invasive dental work planned in the next 2 years.
13. Have a known hypersensitivity to Prolia.
14. Known use of a bone active medication within the 6 months prior to enrollment.
15. Liver disease, defined as AST > 2.0x ULN, ALT > 2.0x ULN, TBL > 1.5x ULN.
16. Malignancy within the last 5 years (except cervical carcinoma in situ or basal cell carcinoma)

4.4 Method of Subject Assignment to Treatment Group

Subjects will be enrolled into the study after verification of eligibility according to the inclusion and exclusion criteria. Written informed consent will also be obtained from subjects. Each subject will receive a unique enrollment number. This will be an open label Phase 1 trial. Both the investigators and the subjects will be aware of the treatment the subjects will be receiving. There will be only one treatment group. The length of time considered acceptable between screening tests and study enrollment will be 30 days.

5 STUDY PROCEDURES

5.1 Surveys

Surveys will be performed during screening visits.

5.2 Initial Visit

5.2.1 Enrollment

Refer to section 4

5.2.2 Medical History

1. Record date of visit, subject enrollment number, and subject initials
2. Verify subject eligibility based on inclusion and exclusion criteria. Adherence to criteria will be noted.
3. Record subject demographics, including date of birth, gender, race
4. Past medical history will be recorded in detail, including a careful screening relevant to future potential adverse events.
5. Subject will complete a VAS survey. The 100-point Visual Analog Scale (VAS, 100mm scale) pain survey will be administered by the podiatrist overseeing care of the subjects at the initial and every follow-up visit.

5.2.3 Physical Examination

Refer to section 4.1.2

- Evaluate change in inflammation
    - Describe location and extent of erythema
    - Grade edema (mild, moderate, severe)
    - Measure temperature gradient difference between affected and non-affected limb using infrared thermometer
- Test for peripheral neuropathy

5.2.4 Laboratory Procedures

The following lab results will be obtained

- Pregnancy test (if appropriate)
- CBC with differential
- CRP
- ESR
- Chem-7 panel
    - (Sodium, Potassium, Chloride, Bicarbonate, BUN, Creatinine, Glucose)
- Liver function tests
- Serum Calcium
- Serum Phosphorus
- HbA1c
- Fasting blood glucose
- Bone specific alkaline phosphatase (BSAP)

5.2.5 Radiographic Procedures

- Foot x-rays 3 views
- Ankle x-rays 3 views

5.2.6 Adverse Events Pre-screen

The medical history recorded during the initial visit will be carefully performed in order to evaluate the significance of adverse events in the future. Both a rheumatologist and endocrinologist will be present at every visit, alongside a podiatrist, to monitor for adverse events.

5.2.7 Treatment

5.2.7.A Administration of Prolia Solution

All subjects will receive Prolia at the second visit. Refer to section 5.3.6.A and section 5.5. This will occur no later than 30 days after screening.

5.2.7.B Administration of Total Contact Cast

All subjects will receive a total contact cast (TCC) at the initial visit. Refer to section 5.4.

5.2.7.C Scheduling of Screening visits

Physical therapy and dental follow-up visits will be scheduled during the initial visit.

5.3 Follow-up Visits

5.3.1 Patient Information

1. Record date of visit, subject enrollment number, and subject initials.
2. Subject will complete a VAS survey.

5.3.2 Physical Examination

Refer to section 4.1.2 for more detail.

- Evaluate change in inflammation
    - Describe location and extent of erythema
    - Grade edema (mild, moderate, severe)
    - Measure skin temperature of the affected limb and the non-affected limb using an infrared thermometer
- Test for peripheral neuropathy

5.3.3 Laboratory Procedures

- CBC with differential
- Chem-7 panel
- Serum Calcium
- Serum Phosphorus
- HbA1c (if previous test was in excess of 3 months prior)
- Fasting blood glucose
- Bone specific alkaline phosphatase (BSAP)

5.3.4 Radiographic Procedures

- Radiographs of the foot and ankle will be ordered on an as-needed basis
- Foot x-rays 3 views
- Ankle x-rays 3 views

5.3.5 Adverse Events

- Both the rheumatologist and endocrinologist will be present at every visit, alongside the podiatrist, to monitor for adverse events

5.3.6 Treatment

5.3.6.A Administration of Prolia Solution

All subjects will receive one injection of Prolia, occurring at the second visit (the first follow-up visit). Refer to section 5.5. This will occur no later than 30 days after screening.

5.3.6.B Administration of Total Contact Cast

All subjects will receive a total contact cast at each follow-up visit. Refer to section 5.4.

5.4 Total Contact Casting Methods

A total contact cast will be applied to all participants at all visits until the active phase of CN has subsided. All subjects will be non-weightbearing while wearing the total contact cast, with the assistance of a wheelchair, knee-walker, or crutches (weight-bearing to non-affected limb) while wearing the total contact cast. The ability to maintain non-weightbearing status using crutches will be verified, and if there is any doubt a wheelchair or knee-scooter will be utilized instead.

5.4.1 Application of Total Contact Cast

The podiatrist will be directly involved in application of the total contact cast. Application should be quick and smooth to avoid weakening of the cast. An assistant will be involved with cast application to ensure proper positioning of the affected lower limb.

Casting will be applied with the patient in the supine position. The affected limb will be covered with a light dressing if applicable, and skin preparation will be performed as needed. A stocking will be used as a first layer, extending from the toes to the tibial tuberosity. Cast-padding will be applied to the lower extremity, including bony prominences of the limb. Fiberglass material will then be applied from the toes to the tibial tuberosity.

5.4.2 Removal of Total Contact Cast

The podiatrist will be directly involved in removal of the total contact cast. A cast saw will be used to remove the cast. Proper technique will be used to ensure safe removal.

5.5 Administration of Prolia Methods

All subjects will receive a subcutaneous injection of Prolia at the second visit (the first follow-up visit). Subjects will be given Calcium and Vitamin D supplementations (≥1000 mg elemental calcium and ≥800 IU vitamin D) during denosumab treatment.

5.5.1 Storage and Handling of Prolia

Prolia is supplied in a single-use prefilled syringe with a safety guard (60 mg/1 mL in a single-use prefilled syringe 1 per carton NDC 55513-710-01). Prolia is stored in a refrigerator at 2°C to 8°C (36°F to 46°F) in the original carton. Do not freeze. Prior to administration, Prolia may be allowed to reach room temperature (up to 25°C/77°F) in the original container. Once removed from the refrigerator, Prolia must not be exposed to temperatures above 25°C/77°F and must be used within 14 days. If not used within the 14 days, Prolia should be discarded. Prolia is not to be used after the expiry date printed on the label. Prolia is to be protected from direct light and heat, and vigorous shaking avoided. The pharmacist will maintain a secured log of refrigerated medications, and the refrigerator will be secured. The pharmacist alone will have access to the medication log and the refrigerator.

5.5.2 Subject Education

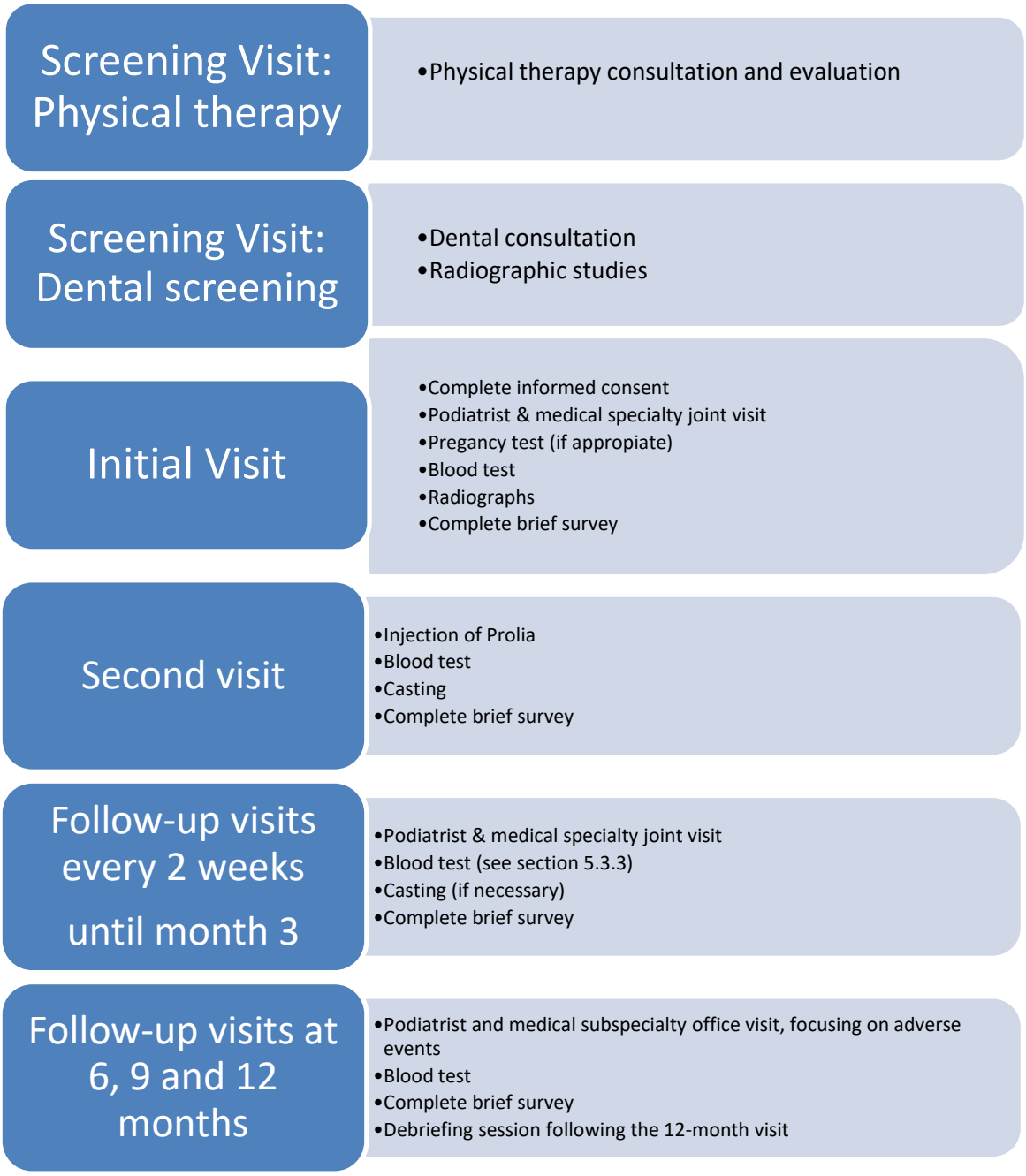
Subjects will be informed of the adverse effects of Prolia and the theoretical benefits of Prolia for the indication under investigation (refer to sections 3.1 and 4).

5.5.2 Administration of Prolia

60 mg of Prolia from a PFS (pre-filled syringe) will be administered to participants during their second visit.

Prior to administration, Prolia will be removed from the refrigerator and brought to room temperature (25 degrees Celsius) in the original container. Prolia will not be heated or warmed. Syringes will be visually inspected to ensure it is a clear to pale yellow solution containing trace amounts of white proteinaceous particles. Syringes will not be used if the solution is discolored or cloudy.

5.6 Flow sheet, Summarizing Office Visits



The physical therapy screening, the dental screening, and the initial visit, are to occur within a two week period. The second visit, which includes injection of Prolia, is intended to occur 1-2 weeks afterwards; as a limit this interval is not to exceed 30 days.

5.7 Subject compensation

Enrolled subjects will be provided a $25 stipend for every visit following the initial visit. This stipend is to compensate patients for associated gas and travel costs.

6 SUBJECT COMPLETION OR DISCONTINUATION

6.1 Subject Completion

The subject will be considered to have completed the study after finishing the safety and efficacy evaluation at 12th months.

6.2 Subject Discontinuation

The subject will be discontinued from the study if the subject withdraws, is withdrawn due to an adverse event, or is no longer able to be located. Any reason for discontinuation will be documented along with information about the health of the subject at the time of withdrawal. All attempts to contact subject must be documented. After 3 documented failed attempts to contact the subject, the subject will be considered lost to follow-up and will be discontinued. As the design of this pilot study includes an allowance of dropout of 1 subject, replacement will be sought if more than 1 subject is discontinued from the study (to yield a total number of subjects no less than 6).

7 SAFETY ASSESSMENTS

A safety evaluation will be conducted at every visit. Both a rheumatologist and an endocrinologist will be present, alongside the podiatrist, at every visit to assess and grade the presence of suspected adverse events.

7.1 Safety Evaluation

Safety will be assessed by recording the nature, intensity, and duration of treatment emergent adverse events.

7.2 Adverse Events

An adverse event (AE) is any unintended and unfavorable sign (e.g., an abnormal laboratory finding), symptom or disease temporally associated with the use of pharmaceutical product, whether or not considered causally related to the product.
Adverse events will be recorded at every visit, including both screening and follow-up visits. Recorded adverse events are not limited to adverse drug reactions (ADRs).

Information collected during subject screening serves to determine if an adverse event started before or during the course of study and to monitor its progression throughout the course of the study. Follow-up evaluation and treatment will continue until the adverse event has resolved.

7.2.2 Adverse Events Causality

Adverse events will be assessed for causality. The event is considered related to the treatment intervention if there is a reasonable possibility that the treatment could have contributed to the event. Evidence supporting the relation between treatment and adverse event can be derived from scientific and medical facts, observation, and professional opinion.

7.3 Serious Adverse Events

An adverse event is considered serious (SAE) if it occurs after signing the informed consent form AND meets any of the following criteria:

- it results in death
- it is life threatening
- it requires hospitalization
- it results in disability or incapacity
- it jeopardizes the health of the subject, such that medical or surgical intervention is needed to prevent one of the aforementioned results. Generally, reconstructive surgery for Charcot neuroarthropathy is avoided during the acute stage. Even in the situation of disease progression, such as advanced foot dislocation or fracture, reconstructive surgical intervention is avoided due to risk of accelerating the disease process during this stage. Surgery of the lower extremity during acute CN may, however, may consist of procedures precipitated by infection, including: incision and drainage procedures, surgical debridement, and bone biopsy. Surgical intervention of this nature will be considered a serious adverse event.

A life-threatening SAE is any SAE that places the subject, in view of the investigator, at immediate risk of death from the reaction as it occurred. It does not include a reaction that, had it occurred in a more serious form, might have caused death.

7.4 Documentation and Reporting of Adverse Events

All involved physicians will invoke the collaborative process in identify any safety concern. Team meetings will occur monthly, and also on as an-needed basis. All adverse events will be discussed as a team, and any ramifications for the remaining patients in the study will be discussed collaboratively as well.

All adverse events will be documented. Serious adverse events will be completely reported to the study grant sponsor, the institutional review board (IRB), and to the University Compliance Office, within 24 hours of becoming aware of the serious adverse event.

7.5 Procedure for Adverse Event Reporting (Individual Case Safety Reports)

Completion of safety assessment forms:

SAEs: Investigators will complete safety assessment forms within 24 hours of the investigator's first knowledge of the SAE. The form must be reported to the institutional review board (IRB) within 5 calendar days.

Non-serious AEs: The safety assessment form must be completed within 5 calendar days of the investigator's first knowledge of the AE.

Reporting of trial product-related SUSARs:

All suspected unexpected serious adverse reactions (SUSARs) will be reported to the Food and Drug Administration (FDA), Amgen as well as the institutional review board (IRB) within 5 calendar days. Cases involving pregnancy or lactation will be reported to Amgen within 10 days of awareness.

An Annual Safety Report will be generated and reported annually. A Final Safety Report will be generated and reported at the conclusion of the study. Additional aggregate safety reports will be considered as the study progresses.

Follow-up of adverse events:

SUSARs: All SUSARs must be followed until the outcome of the event is, "recovered/resolved", "recovered/resolved with sequelae", or "fatal", and until all queries have been resolved. Cases of chronic conditions on-going at the time of death, where death is due to another AE, may be closed with either the outcome "recovering/resolving" or "not recovered/not resolved". If the subject has completed the follow-up period and is expected by the investigator to recover, the case can be closed with the outcome of "recovering/resolving". Follow-up information should only include new information and must be reported within 5 calendar days of the investigator's first knowledge of the information.

Non-serious AEs: All SUSARs must be followed until the outcome of the event is, "recovered/resolved", "recovered/resolved with sequelae", or "fatal", and until all queries have been resolved. Cases of chronic conditions on-going at the time of death, where death is due to another AE, may be closed with either the outcome "recovering/resolving" or "not recovered/not resolved". If the subject has completed the follow-up period and is expected by the investigator to recover, the case can be closed with the outcome of "recovering/resolving".

Queries or follow-up requests from Amgen must be responded to within 14 calendar days from the date of receipt of the request unless otherwise specified in the follow-up request.

8 SUBJECT PROTECTION REQUIREMENTS

The Principal Investigator is required to provide an Institutional Review Board (IRB)/Ethics Committee with necessary materials. The study cannot commence until the IRB/Ethics Committee provides written approval of the proposed Phase 1 trial. Appropriate reports of progress of this study will be provided to the IRB/Ethics Committee in agreement with the policy established by the grant sponsor.

Changes to an approved protocol may only be made by the grant sponsor with IRB authorization, except in instances deemed necessary to eliminate immediate harm to the subjects or when the change involves only administration or logistics.

Significant deviation from the protocol without appropriate approval will be regarded as a protocol violation.

9 REGULATORY REQUIREMENTS

This study will be conducted in accordance with all applicable regulatory requirements, including those promulgated by the U.S. Food and Drug Administration, and with the principles consistent with Good Clinical Practice.

10 RESPONSIBILITIES OF THE INVESTIGATORS

10.1 Obtaining Subject Informed Consent

Information about the study in a language fully understandable by the eligible subject (English or Spanish) will be given in written and oral form by the investigator. It will be explained to subjects that they are free to refuse entry into the study and free to withdraw at any time. Written consent forms will be approved by the grant sponsor. The original consent form will be placed in the subject's study records and a copy will be provided to the subject.

10.2 Subject Confidentiality

The investigators will ensure that privacy of all subjects is maintained. Investigators will assure subjects that their personal identity and all personal medical information will be safeguarded. In all documents submitted to the sponsor, subjects will be identified by a unique identification code. All personal medical information will always be treated as confidential and in compliance with HIPAA regulations.

10.3 Access to Data/Documents

The investigators will have access to data and documents obtained from subjects during the course of the study. Personal medical information may be studied for the purpose of verifying data recorded and subject eligibility and is only accessible to the investigators

10.4 Product Delivery, Storage, and Returns

The investigator is responsible for ensuring that deliveries of all material involved with the study are correctly received and handled. Materials will be properly labeled and safely stored. All unopened materials will be returned to the study grant sponsor.

10.5 Data Handling

All data collected will be included in a report to be submitted to the study grant sponsor. The data will be scanned and sent through secure electronic mail (e-mail). The report will be legible. Any errors will be corrected with a single line strike-through that does not obscure the original entry and annotated with the investigator's initials and current date. No data will be withheld from the report and any missing data will be noted with the reason for why it is missing. Frequency of case report submissions to the grant sponsor will be decided between the investigator and grant sponsor.

10.6 Data Analysis

Given the small sample size, data analysis will by definition be limited to descriptive statistics. The mean, standard deviation, and range will be reported for the change of foot skin temperature compared to the baseline value. Similarly, the mean, standard deviation, and range, will be reported for each of the following dependent variables: skin gradient between the affected and nonaffected limb, bone specific alkaline phosphatase (BSAP), erythrocyte sedimentation rate (ESR), and 100 point Visual Analog Scale survey result. This resultant data will aid in performing a power analysis for the future randomized controlled trial intended. Specifically, selecting an appropriate effect size for power analysis calculations will be more reasonable once the data for this proposed Phase 1 trial has been fully reported.

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