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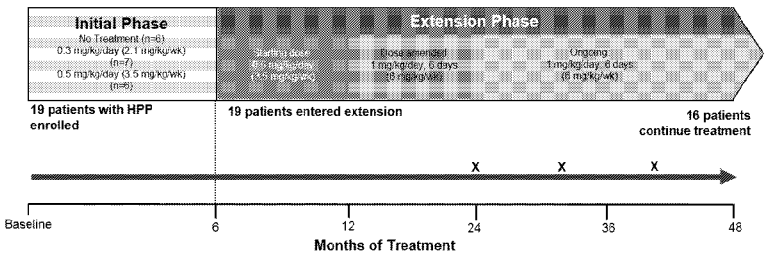

FIG. 1

(57) Abstract: The disclosure features methods for treating hypophosphatasia (HPP) in a patient (e.g., adolescent or adult having HPP) exhibiting physical impairments or decreased walking ability by administering a soluble alkaline phosphatase (sALP) to the patient.

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METHODS FOR TREATING HYPOPHOSPHATASIA IN ADOLESCENTS AND ADULTS

FIELD

The disclosure relates to methods for treating hypophosphatasia (HPP).

BACKGROUND

Hypophosphatasia (HPP) is a rare, heritable skeletal disease with an incidence of 1 per 100,000 births for the most severe forms of the disease. The disorder results from loss-of-function mutations in the gene coding for tissue-nonspecific alkaline phosphatase (TNALP). HPP exhibits a remarkable range of symptoms and severity, from rickets to almost complete absence of bone mineralization *in utero*.

Most patients having HPP display skeletal changes, short stature, painful lower limbs, gait disturbance, and premature shedding of teeth. During adolescence, symptoms of HPP can include rickets (osteomalacia); short stature; skeletal deformities, such as bowed legs and enlarged wrists, knees, and ankles as a result of flared metaphyses; and the premature loss of deciduous teeth due to aplasia, hypoplasia, or dysplasia of dental cementum that connects the tooth root with the periodontal ligament. Adults having HPP usually present symptoms during middle age, although frequently there is a history of rickets and/or premature loss of teeth, such as during adolescence. Recurrent metatarsal stress fractures are common and calcium pyrophosphate dihydrate deposition can cause arthritis and pyrophosphate arthropathy in adults having HPP. Due to physical impairments associated with HPP, adolescent and adult patients afflicted with HPP often exhibit decreased walking ability relative to healthy subjects.

Notably, the efficacy and safety of treatment of HPP, particularly physical impairments and decreased walking ability associated with adolescent and adult forms of HPP, with a therapeutic for an extended period of time is unknown. Thus, there exists a need for methods that can be used to treat and monitor HPP in patients, such as adolescents and adults having HPP, for extended durations so that these patients can live with decreased physical impairments and regain walking ability.

SUMMARY

Disclosed are (1) methods to identify (a) adolescents having hypophosphatasia (HPP; e.g., adolescents having HPP of about 12 to about 18 years of age) and (b) adults having HPP (e.g., adults having HPP older than about 18 years of age) for treatment with a soluble alkaline phosphatase (sALP; e.g., SEQ ID NO: 1), and (2) treatment of such patients identified in (1) and (2) with a sALP.

Exemplary metrics useful for evaluating the need for or the efficacy of treatment using a sALP (e.g., SEQ ID NO: 1) include (1) the Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2), (2) the Six Minute Walk Test (6MWT), and (3) plasma PPi and PLP concentrations. The methods further include the use of one or more of the described metrics (e.g., the BOT-2, 6MWT, or plasma PPi and PLP concentrations) singly or in combination to assess treatment efficacy using a sALP for a patient having HPP in which improvements relative to a certain score or value demonstrate that the sALP is effective for treating HPP. Additionally, methods further include changing the dosage and/or frequency of sALP (e.g., SEQ ID NO: 1) administration in order to determine the effective amount of the sALP to administer to an adolescent having HPP or adult having HPP.

A first aspect features a method of treating HPP in a patient of about 12 years of age or older than about 12 years of age, which includes administering a sALP to the patient at a dosage providing about 6 mg/kg/week of the sALP. In particular, the sALP includes an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1 (e.g., asfotase alfa). Administration of the sALP (e.g., SEQ ID NO: 1) results in one or more of the following: (i) an improvement in walking ability that is sustained during a treatment period of at least one year (e.g., at least two years, at least three years, at least four years, or longer) of the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult older than about 18 years of age treated with a sALP) relative to walking ability of an untreated subject having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age); (ii) an average decrease in PPi concentrations in a plasma sample from the patient that is sustained during a treatment period of at least one year relative to PPi concentrations in a plasma sample from an untreated subject having HPP; and/or (iii) an average decrease in PLP concentrations in a plasma sample from the patient that is sustained during a treatment period of at least one year relative to PLP concentrations in a plasma sample from an untreated subject having HPP.

For instance, administration of the sALP (e.g., SEQ ID NO: 1) results in an average increase in a 6MWT value of the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) relative to the 6MWT value of the patient prior to administration of the sALP. In particular, the average increase in the 6MWT value is to about 80% or greater of the predicted 6MWT value of the patient, in which the 6MWT value of the patient was less than about 80% of the predicted 6MWT value of the patient prior to administration of the sALP. Additionally, the average increase in the 6MWT value of the patient is sustained during a treatment period of at least two years, at least three years, at least four years, or longer (e.g., at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or more than ten years, such as for the lifetime of the patient). The patient also exhibits decreased reliance on an assistive device (e.g., a wheelchair, braces, crutches, or an orthotic) for mobility after administration of the sALP.

As a result of the methods, the average decrease in PPi concentrations in a plasma sample from the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult older than about 18 years of age treated with a sALP) is about 25% or greater (e.g., 30%, 35%, 40%, 45%, 50%, 55%, 60%, or more than 60%) relative to PPi concentrations in a plasma sample from the patient prior to administration of the sALP (e.g., SEQ ID NO: 1). Moreover, the average decrease in PPi concentrations in a plasma sample from the patient having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult older than about 18 years of age treated with a sALP) is determined relative to PPi concentrations in a plasma sample from an untreated subject having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult older than about 18 years of age). The average decrease in PPi concentrations in a plasma sample from the patient is sustained during a treatment period of at least two years, at least three years, at least four years, or longer (e.g., at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or more than ten years, such as for the lifetime of the patient).

As a result of the methods, the average decrease in PLP concentrations in a plasma sample from the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) is about 50% or greater (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more than 95%) relative to PLP concentrations in a plasma sample from the patient prior to administration of the sALP (e.g., SEQ ID NO: 1). In particular, the average decrease in PLP concentrations in a plasma sample from the patient is determined relative to PLP concentrations in a plasma sample from an untreated subject having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age). Additionally, the average decrease in PLP concentrations in a plasma sample from the patient is sustained during a treatment period of at least two years, at least three years, at least four years or longer (e.g., at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or more than ten years, such as for the lifetime of the patient).

A second aspect features a method of treating HPP in a patient of about 12 years of age or older than about 12 years of age having a medial total BOT-2 running speed and agility score of less than about 7. The method includes administering a sALP (e.g., SEQ ID NO: 1) to the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) at a dosage providing about 6 mg/kg/week of the sALP. In particular, the sALP includes an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1. Administration of the sALP results in an average increase in the BOT-2 running speed and agility medial total score to about 9 or greater than about 9 that is sustained during a treatment period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or more than ten years, such as for the lifetime of the patient).

Additionally, the medial total BOT-2 running speed and agility score of the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult older than about 18 years of age treated with a sALP) is determined relative to a BOT-2 running speed and agility medial total score of an untreated subject having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult older than about 18 years of age). Moreover, the average increase in the BOT-2 running speed and agility medial total score is sustained during a treatment period of at least two years, at least three years, at least four years, or longer (e.g., at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or more than ten years, such as for the lifetime of the patient). The medial total BOT-2 running speed and agility score of the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age treated with a sALP) is determined from measurements selected from the group consisting of stepping over a balance beam, shuttle run, two-legged side hop, and one-legged side hop.

A third aspect features a method of treating HPP in a patient of about 12 years of age or greater than about 13 years of age having a medial total BOT-2 strength score of less than about 14, in which the method includes administering a sALP to the patient at a dosage providing about 6 mg/kg/week of the sALP. In particular, the sALP includes an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1. Administration of the sALP (e.g., asfotase alfa) for at least

two years (e.g., at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or more than ten years, such as for the lifetime of the patient) results in an average increase in the BOT-2 strength medial total score to about 18 or greater than about 18.

Additionally, the medial total BOT-2 strength score of the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age treated with a sALP) is determined relative to a BOT-2 strength medial total score of an untreated subject (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age). Moreover, the medial total BOT-2 strength score of the patient is determined relative to a BOT-2 strength score of an untreated subject having HPP. The medial total BOT-2 strength score of the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult older than about 18 years of age) is determined from measurements selected from the group consisting of sit-ups, V-ups, standing long jump, wall sit, and push-ups.

In any of the above aspects, the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) is about 12 years of age, older than about 12 years of age, about 18 years of age, or older than about 18 years of age. Furthermore, the patient may be one that has not been previously administered the sALP (e.g., SEQ ID NO: 1).

In any of the above aspects, the sALP (e.g., SEQ ID NO: 1) is formulated for daily or weekly administration, such as twice a week, three times a week, four times a week, five times a week, six times a week, or seven times a week. For example, the sALP (e.g., SEQ ID NO: 1) is formulated at a dosage of 2 mg/kg for administration three times a week, a dosage of 3 mg/kg for administration three times a week, or a dosage of 1 mg/kg for administration six times a week. Moreover, the sALP (e.g., SEQ ID NO: 1) is formulated for administration once daily and/or is formulated for administration on consecutive or alternating days. Additionally, the sALP (e.g., SEQ ID NO: 1) is administered for a treatment period of at least four, least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer, such as for the lifetime of the patient).

In any of the above aspects, the sALP (e.g., SEQ ID NO: 1) includes or consists of the amino acid sequence of SEQ ID NO: 1. Furthermore, the sALP (e.g., SEQ ID NO: 1) is administered in an amount that is therapeutically effective to treat at least one symptom of HPP (e.g., one or more of rickets, premature loss of deciduous teeth, incomplete bone mineralization, elevated blood and/or urine levels of phosphoethanolamine (PEA), hypomineralization, rachitic ribs, hypercalciuria, short stature, skeletal deformity, waddling gait, bone pain, bone fracture, HPP-related seizure, inadequate weight gain, and calcium pyrophosphate dihydrate crystal deposition).

In any of the above aspects, the sALP (e.g., SEQ ID NO: 1) is administered at an initial dosage of about 2.1 mg/kg/week or about 3.5 mg/kg/week and then is increased to a dosage of about 6 mg/kg/week or more (e.g., about 9 mg/kg/week). For example, the dosage is increased to a dosage of about 6 mg/kg/week or more after administration of the sALP (e.g., SEQ ID NO: 1) for at least six months, at least one year, at least two years, at least three years, at least four years, or longer (e.g., at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or more than ten years, such as for the lifetime of the patient).

In any of the above aspects, symptoms of HPP (e.g., rickets, premature loss of deciduous teeth, incomplete bone mineralization, elevated blood and/or urine levels of phosphoethanolamine (PEA), hypomineralization, rachitic ribs, hypercalciuria, short stature, skeletal deformity, waddling gait, bone pain, bone fracture, HPP-related seizure, inadequate weight gain, craniosynostosis, and/or calcium pyrophosphate dihydrate crystal deposition) presented at birth or did not present at birth. For example, symptoms of HPP presented in the HPP patient at 12 years of age or older (e.g., 13 years of age, 14 years of age, 15 years of age, 16 years of age, 17 years of age, 18 years of age, or older).

In any of the above aspects, the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) exhibits tolerability to administration of the sALP (e.g., SEQ ID NO: 1), such as a lack of or decreased incidence of adverse events selected from the group consisting of injection site erythema, decrease in hemoglobin, pyrexia, pneumonia, upper respiratory tract infection, otitis media, vomiting, constipation, diarrhea, tooth loss, nasopharyngitis, rash, dental carries, and irritability.

In any of the above aspects, the sALP (e.g., SEQ ID NO: 1) is formulated in a pharmaceutical composition, with at least one pharmaceutically acceptable carrier, such as saline (e.g., sodium chloride and sodium phosphate). For example, the at least one pharmaceutically acceptable carrier includes 150 mM sodium chloride and 25 mM sodium phosphate. Moreover, the pharmaceutical composition is formulated for subcutaneous, intramuscular, intravenous, oral, nasal, sublingual, intrathecal, or intradermal administration. In particular, the pharmaceutical composition is formulated for subcutaneous administration.

In any of the above aspects, the sALP (e.g., SEQ ID NO: 1) is physiologically active toward PEA, PPi, and PLP, catalytically competent to improve skeletal mineralization in bone, and/or is the soluble extracellular domain of an alkaline phosphatase.

Definitions

As used herein, “a” or “an” means “at least one” or “one or more” unless otherwise indicated. In addition, the singular forms “a”, “an”, and “the” include plural referents unless the context clearly dictates otherwise.

As used herein, “about” refers to an amount that is ± 10% of the recited value and is preferably ± 5 % of the recited value, or more preferably ± 2 % of the recited value.

As used herein, “at least” refers to an amount that is ≤ 10% of the recited value and is preferably ≤ 5 % of the recited value, or more preferably ≤ 2 % of the recited value.

By “asfotase alfa” is meant a human TNALP (hTNALP) fusion protein formulated for the treatment of HPP. Asfotase alfa (STRENSIQ®, Alexion Pharmaceuticals, Inc.) is a fusion protein including a soluble glycoprotein of two identical polypeptide chains, in which each polypeptide chain includes amino acid residues 1-726 of SEQ ID NO: 1. The structure of each polypeptide chain includes the catalytic domain of hTNALP, the human immunoglobulin G_1 Fc domain, and a deca-aspartate peptide used as a bone targeting domain (the structure hTNALP-Fc-D_{10}). The two polypeptide chains are covalently linked by two disulfide bonds. Asfotase alfa has been approved under the trade name STRENSIQ® in the United States, Europe, Japan, Canada, Israel, Australia, and Korea.

As used herein, "average" refers to a numerical value expressing the mean or median of a data set. The mean of a data set is calculated by dividing the sum of the values in the set by their number. The median of a date set is calculated by determining the middle value in a list of odd numbers or by determining the mean of the two data values in the middle in a list of even numbers.

The term "bone-targeting moiety," as used herein, refers to an amino acid sequence of between 1 and 50 amino acid residues (such as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 15, 16, 18, 20, 22, 24, 25, 26, 28, 30, 32, 34, 35, 36, 38, 40, 42, 44, 45, 46, 48, or 50 amino acid residues) in length having a sufficient affinity to the bone matrix, such that the bone-targeting moiety, singularly, has an *in vivo* binding affinity to the bone matrix that is about 10^{-6} M to about 10^{-15} M (e.g., 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M, 10^{-14} M, or 10^{-15} M). For example, the bone-targeting moiety can include a series of consecutive aspartate (D) and/or glutamate (E) residues, in which n = 1 to 50, e.g., n = 3-30, e.g., 5-15, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 , 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 36, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

The terms "Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition" or "BOT-2," as used herein, refer to the second edition of a standardized test of gross and fine motor performance for patients, e.g., including adolescent and adult patients having HPP. See Bruininks, R. H. (2005). *Bruininks-Oseretsky Test of Motor Proficiency, (BOT-2)*. Minneapolis, MN: Pearson Assessment, hereby incorporated by reference in its entirety. The BOT-2 is administered individually to assess gross and fine motor skills of a range of patients. The BOT-2, for example, can be used to evaluate physical impairments and mobility restrictions in patients having HPP (e.g., adults and adolescents having HPP). The BOT-2 provides composite BOT-2 scores in the following exemplary areas: strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination. For example, a BOT-2 strength total score can be determined by having a patient perform sit-ups, v-ups, standing long jump, wall sit, and push-ups. A running speed and agility total score can be determined by having a patient step over a balance beam or perform a shuttle run, two-legged side hop, or one-legged side hop. Both BOT-2 total strength and BOT-2 running speed and agility total scores range from 0 to 25, in which a score of about 10 to 25 is considered representative of healthy subjects. Normative scores for the strength and running speed and agility are 15 +/- 5. Adult and adolescent scores are not normed (does not use the scaled score), and thus higher point values represent better performance. Either average or median scores may be used, with median scores preferred for smaller sample sizes or smaller data sets.

The term "catalytically competent," as used herein, refers to a sALP that hydrolyzes the bone mineralization inhibitor inorganic pyrophosphate (PPi) to provide inorganic phosphate (Pi), thereby decreasing the extracellular concentrations of PPi. Thus, the catalytically competent sALP improves skeletal mineralization in bone by regulating the concentration of PPi.

By "extracellular domain" is meant any functional extracellular portion of the native protein, e.g., alkaline phosphatase. In particular, the extracellular domain lacks the signal peptide.

By "Fc" is meant a fragment crystallizable region of an immunoglobulin, e.g., IgG-1, IgG-2, IgG-3, IgG-3 or IgG-4, including the CH2 and CH3 domains of the immunoglobulin heavy chain. Fc may also include any portion of the hinge region joining the Fab and Fc regions. The Fc can be of any mammal,

including human, and may be post-translationally modified (e.g., by glycosylation). In a non-limiting example, Fc can be the fragment crystallizable region of human IgG-1 having the amino acid sequence of SEQ ID NO: 20.

By "fragment" is meant a portion of a polypeptide or nucleic acid molecule that contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain, e.g., 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 400, 500, 600, 700, or more amino acid residues, up to the entire length of the polypeptide. Exemplary sALP fragments have amino acid residues 18-498, 18-499, 18-500, 18-501, 18-502, 18-503, 18-504, 18-505, 18-506, 18-507, 18-508, 18-509, 18-510, 18-511, or 18-512 of an ALP (e.g., SEQ ID NOs: 2-6), and may include additional C-terminal and/or N-terminal portions. Biological activity of such fragments is tested in standard assays known in the art, e.g., by a non-compartmental analysis (NCA) to calculate pharmacokinetic parameters of a sALP fragment.

The terms "hypophosphatasia" or "HPP," as used herein, refer to a rare, heritable skeletal disorder caused by, e.g., one or more loss-of-function mutations in the ALPL (alkaline phosphatase, liver/bone/kidney) gene, which encodes tissue-nonspecific alkaline phosphatase (TNALP). HPP may be further characterized as infantile HPP, childhood HPP, perinatal HPP (e.g., benign perinatal HPP or lethal perinatal HPP), odonto-HPP, adolescent HPP, or adult HPP. For instance, "adolescent HPP" describes a patient having HPP that is about 12 years of age to about 18 years of age, whereas "adult HPP" describes a patient having HPP that is older than about 18 years of age.

The term "HPP phenotype," as used herein, refers to any one of rickets (defect in growth plate cartilage), osteomalacia, elevated blood and/or urine levels of inorganic pyrophosphate (PPi), phosphoethanolamine (PEA), or pyridoxal 5'-phosphate (PLP), seizure, bone pains, and calcium pyrophosphate dihydrate crystal deposition (CPPD) in joints leading to chondrocalcinosis, craniosynostosis, and premature death. Without being so limited, a HPP phenotype can be documented by one or more of growth retardation with a decrease of long bone length (including but not limited to femur, tibia, humerus, radius, and/or ulna), a decrease of the mean density of total bone and a decrease of bone mineralization in bones such as femur, tibia, ribs and metatarsi, and phalange, a decrease in teeth mineralization, and a premature loss of deciduous teeth (e.g., aplasia, hypoplasia, or dysplasia of dental cementum). Without being so limited, correction or prevention of bone mineralization defect may be observed by one or more of the following: an increase of long bone length, an increase of mineralization in bone and/or teeth, a correction of bowing of the legs, a reduction of bone pain and a reduction of CPPD crystal deposition in joints.

By "naïve patient" is meant a patient having HPP (e.g., an adolescent or adult) that has never received treatment with a sALP such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa).

The terms "peptide," "polypeptide," and "protein" are used interchangeably and refer to any chain of two or more natural or unnatural amino acid residues, regardless of post-translational modification

(e.g., glycosylation or phosphorylation), constituting all or part of a naturally-occurring or non-naturally occurring polypeptide or peptide, as is described herein.

By "pharmaceutically acceptable carrier" or "pharmaceutically acceptable excipient" is meant at least one carrier or excipient, respectively, which is physiologically acceptable to the treated patient while retaining the therapeutic properties of the compound with which it is administered. One exemplary pharmaceutically acceptable carrier substance is physiological saline. For instance, the pharmaceutically acceptable carrier can include sodium chloride (e.g., 150 mM sodium chloride) and sodium phosphate (e.g., 25 mM sodium phosphate). Other physiologically acceptable carriers and their formulations are known to those skilled in the art and described, e.g., in *Remington's Pharmaceutical Sciences* (20th edition), A. Gennaro, Ed., 2000, Lippincott, Williams & Wilkins, Philadelphia, PA.

By "pharmaceutical composition" is meant a composition containing a polypeptide or nucleic acid molecule as described herein formulated with at least one pharmaceutically acceptable excipient, diluent, or carrier. The pharmaceutical composition may be manufactured or sold with the approval of a governmental regulatory agency as part of a therapeutic regimen for the treatment or prevention of a disease or event in a patient. Pharmaceutical compositions can be formulated, for example, for subcutaneous administration, intravenous administration (e.g., as a sterile solution free of particulate emboli and in a solvent system suitable for intravenous use), for oral administration (e.g., a tablet, capsule, caplet, gelcap, or syrup), or any other formulation described herein, e.g., in unit dosage form.

The term "physical impairments," as used herein, refers to a physiological condition, such as bone weakness and muscle weakness, associated with HPP that can restrict or eliminate, e.g., walking ability, functional endurance, and ability to perform activities of daily living (ADL) of a patient. In particular, physical impairments may restrict or eliminate a patient's ability to perform ADL, which are routine activities that healthy subjects perform on a daily basis without requiring assistance, such as functional mobility or transferring (e.g., walking), bathing and showering, dressing, self-feeding, and personal hygiene and grooming. As described herein, therapeutic compositions (e.g., compositions including a sALP fusion polypeptide, such as asfotase alfa) can be administered to a patient (e.g., an adolescent or adult having HPP) to decrease the severity and/or frequency of physical impairments associated with an HPP phenotype and/or to increase the walking ability of the patient (e.g., the walking ability determined from the distance walked by the patient over six minutes).

The term "physiologically active," as used herein, refers to a sALP that hydrolyzes phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and pyridoxal 5'-phosphate (PLP) to provide Pi, thereby decreasing extracellular concentrations of PEA, PPi, and PLP.

The terms "sALP," "soluble alkaline phosphatase," and "extracellular domain of an alkaline phosphatase" are used interchangeably and refer to a soluble, non-membrane bound alkaline phosphatase (ALP) or a domain or a biologically active fragment of the soluble, non-membrane bound ALP. sALPs include, for example, an alkaline phosphatase lacking a C-terminal glycolipid anchor (GPI signal sequence, e.g., polypeptides including or consisting of the amino acid residues 18-502 of a human TNALP (SEQ ID NOs: 2, 3, 4, 5, or 6)). In particular, a TNALP may include, e.g., a polypeptide including or consisting of amino acid residues 1-485 of SEQ ID NO: 1, such as asfotase alfa, or a polypeptide variant having at least 95% sequence identity to the amino acid residues 1-485 of SEQ ID NO: 1. sALPs

further include, for example, mammalian orthologs of human TNALP, such as a rhesus TNALP (SEQ ID NO: 7), a rat TNALP (SEQ ID NO: 8), a canine TNALP (SEQ ID NO: 9), a porcine TNALP (SEQ ID NO: 10), a murine TNALP (SEQ ID NO: 11), a bovine TNALP (SEQ ID NOs: 12-14), or a feline TNALP (SEQ ID NO: 15). sALPs also include soluble, non-membrane-bound forms of human PALP (e.g., polypeptides including or consisting of amino acid residues 18-502 of SEQ ID NOs: 16 or 17), GCALP (e.g., polypeptides including or consisting of amino acid residues 18-502 of SEQ ID NO: 18), and IALP (e.g., polypeptides including or consisting of amino acid residues 18-502 of SEQ ID NO: 19), and additional variants and analogs thereof that retain alkaline phosphatase activity, e.g., the ability to hydrolyze PP_i. A sALP, in particular, lacks the N-terminal signal peptide (e.g., aa 1-17 of SEQ ID NOs: 2-6, 8, 11-13, or 15 or aa 1-25 of SEQ ID NO: 7).

By "sALP fusion polypeptide" is meant a polypeptide having the structure Z-sALP-Y-spacer-X-W_n-V, Z-W_n-X-spacer-Y-sALP-V, Z-sALP-Y-W_n-X-spacer-V, and Z-W_n-X-sALP-Y-spacer-V. In particular, the sALP fusion polypeptide can be Z-sALP-Y-spacer-X-W_n-V or Z-W_n-X-spacer-Y-sALP-V, such as hTNALP-Fc-D_{10} (e.g., asfotase alfa; the amino acid sequence SEQ ID NO: 1). Any one of X, Y, Z, V, the spacer, and/or W_n can be absent or an amino acid sequence of at least one amino acid. For example, X, Y, Z, and V may be a dipeptide sequence (e.g., leucine-lysine or aspartic acid-isoleucine), such as a two residue linker at the Y position (e.g., leucine-lysine) and a two residue linker at the X position (e.g., aspartic acid-isoleucine). Spacers include, for example, a Fc region of an immunoglobulin, such as the amino acid sequence of SEQ ID NO: 20. W_n can be a bone-targeting moiety as defined herein, e.g., having a series of consecutive aspartate (D) or glutamate (E) residues, in which n = 1 to 50, e.g., n = 3-30, e.g., 5-15, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 , 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 36, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50.

By "signal peptide" is meant a short peptide (5-30 amino acids long) at the N-terminus of a polypeptide that directs a polypeptide towards the secretory pathway (e.g., the extracellular space). The signal peptide is typically cleaved during secretion of the polypeptide. The signal sequence may direct the polypeptide to an intracellular compartment or organelle, e.g., the Golgi apparatus. A signal sequence may be identified by homology, or biological activity, to a peptide with the known function of targeting a polypeptide to a particular region of the cell. One of ordinary skill in the art can identify a signal peptide by using readily available software (e.g., Sequence Analysis Software Package of the Genetics Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, Wis. 53705, BLAST, or PILEUP/PRETTYBOX programs). A signal peptide can be one that is, for example, substantially identical to amino acid residues 1-17 of SEQ ID NOs: 2-6 or amino acid residues 1-25 of SEQ ID NO: 7.

As used herein, when a polypeptide or nucleic acid sequence is referred to as having "at least X% sequence identity" to a reference sequence, wherein "X" is a real number, it is meant that at least X percent of the amino acid residues or nucleotides in the polypeptide or nucleic acid are identical to those of the reference sequence when the sequences are optimally aligned. An optimal alignment of sequences can be determined in various ways that are within the skill in the art, for instance, the Smith Waterman alignment algorithm (Smith et al., *J. Mol. Biol.* 147:195-7, 1981) and BLAST (Basic Local Alignment Search Tool; Altschul et al., *J. Mol. Biol.* 215: 403-10, 1990). These and other alignment

algorithms are accessible using publicly available computer software such as "Best Fit" (Smith and Waterman, Advances in Applied Mathematics, 482-489, 1981) as incorporated into GeneMatcher Plus (Schwarz and Dayhoff, Atlas of Protein Sequence and Structure, Dayhoff, M.O., Ed pp 353-358, 1979), BLAST, BLAST-2, BLAST-P, BLAST-N, BLAST-X, WU-BLAST-2, ALIGN, ALIGN-2, CLUSTAL, Megalign (DNASTAR), or other software/hardware for alignment. In addition, those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve optimal alignment over the length of the sequences being compared.

The terms "patient" or "subject" refer to a mammal, including, but not limited to, a human or a non-human mammal, such as a bovine, equine, canine, ovine, or feline.

"Parenteral administration," "administered parenterally," and other grammatically equivalent phrases, as used herein, refer to modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, subcutaneous, intradermal, intravenous, intranasal, intraocular, pulmonary, intramuscular, intra-arterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intrapulmonary, intraperitoneal, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intracerebral, intracranial, intracarotid, and intrasternal injection and infusion.

By "therapeutically effective amount" is meant an amount of a polypeptide or nucleic acid molecule described herein that is sufficient to substantially improve, treat, prevent, delay, suppress, or arrest at least one symptom of HPP. A therapeutically effective amount of a composition described herein may depend on the severity of the disorder being treated and the condition, weight, and general state of the patient and can be determined by an ordinarily-skilled artisan with consideration of such factors. A therapeutically effective amount of a composition described herein can be administered to a patient in a single dose or in multiple doses administered over a period of time.

By "treating," "treat," or "treatment" is meant the medical management of a patient with the intent to cure, ameliorate, stabilize, reduce the likelihood of, or prevent HPP and/or management of a patient exhibiting or likely to have HPP, e.g., by administering a pharmaceutical composition. This term includes active treatment, that is, treatment directed specifically toward the improvement or associated with the cure of a disease, pathological condition, disorder, or event, and also includes causal treatment, that is, treatment directed toward removal of the cause of the associated disease, pathological condition, disorder, or event. In addition, this term includes palliative treatment, that is, treatment designed for the relief or improvement of at least one symptom rather than the curing of the disease, pathological condition, disorder, or event; symptomatic treatment, that is, treatment directed toward constitutional symptoms of the associated disease, pathological condition, disorder, or event; preventative treatment, that is, treatment directed to minimizing or partially or completely inhibiting the development of the associated disease, pathological condition, disorder, or event, e.g., in a patient who is not yet ill, but who is susceptible to, or otherwise at risk of, a particular disease, pathological condition, disorder, or event; and supportive treatment, that is, treatment employed to supplement another specific therapy directed toward the improvement of the associated disease, pathological condition, disorder, or event.

As used herein, "walking ability" refers to the ability of a patient (e.g., an adolescent or adult having HPP) to lift and set down each foot in turn. Walking ability may be assessed by tests, in particular,

the Six Minute Walk Test (6MWT). See the American Thoracic Society statement: guidelines for the six-minute walk test (*American Journal of Respiratory and Critical Care Medicine*, 166(1):111-7, 2002), hereby incorporated by reference in its entirety. The 6MWT is determined from the distance (e.g., in meters) that a patient walks on a flat, hard surface in a period of six minutes. The 6MWT value is then compared to the predicted 6MWT value of an untreated subject (e.g., an untreated subject of about the same age, height, and/or gender) or a healthy subject (e.g., a healthy subject of about the same age, height, and/or gender), which may be expressed as a percentage of the predicted 6MWT value.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an image showing the study design for administering asfotase alfa to adolescents and adults having hypophosphatasia (HPP) over a time period of four years, including an initial phase of treatment with asfotase alfa (from baseline) to six months and an extension phase of treatment with asfotase alfa (from six months of treatment to four or more years of treatment). An X represents that a patient discontinued treatment with asfotase alfa.

Figures 2A-2D are graphs showing the PPi and PLP concentrations for individual adolescent and adult patients (Fig. 2A), the median change in PPi and PLP concentrations after six months of treatment with asfotase alfa (Fig. 2B), and the median inorganic pyrophosphate (PPi; Fig. 2C) and median pyridoxal 5'-phosphate (PLP; Fig. 2D) concentrations in plasma samples from adolescent and adult HPP patients during treatment with asfotase alfa over a time period of four years. The upper normal limit and lower normal limit of both PPi and PLP concentrations for healthy subjects of about 12 to 18 years of age and patients over about 18 years of age are shown. The number (n) of patients assessed for plasma PPi and PLP concentrations is shown above each time point. A * indicates that the dosage groups (2.1 mg/kg/week and 3.5 mg/kg/week) were combined. The initial 6 control patients do not have data at year 4 due to the 6 month offset. The time period during which treatment with asfotase alfa was increased to a dosage of 6 mg/kg/week via protocol amendment is shown. $P < 0.0001$ for the change in median plasma PPi and PLP concentrations from baseline at Month 6.

Figures 3A-3B are graphs showing the median change from baseline in the 6MWT value of adolescent and adult HPP patients at six months of treatment with asfotase alfa (Fig. 3A) and the median Six Minute Walk Test (6MWT) value of adolescent and adult HPP patients during treatment with asfotase alfa over a time period of four years (Fig. 3B). The median 6MWT value is shown as the percentage of the predicted 6MWT relative to the normal range for healthy subjects of about the same age and height. The normal range for healthy subjects of the same age and height is 80 to 100% of the predicted median distance walked (shown in green). A * indicates that the dosage groups (2.1 mg/kg/week and 3.5 mg/kg/week) were combined. The initial 6 control patients do not have data at year 4 due to the 6 month offset. The time period during which treatment with asfotase alfa was increased to a dosage of 6 mg/kg/week via protocol amendment is shown (Fig. 3B).

Figures 4A-4C are images and a graph of the Bruininks-Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2) strength tests (Fig. 4A), BOT-2 running speed and agility tests (Fig. 4B), and the average BOT-2 strength scores and BOT-2 running speed and agility scores of adolescent and adult HPP patients after administration of asfotase alfa over a time period of four years (Fig. 4C). The median of the BOT-2

scores for each time interval (baseline, 6 months, 1 year, 2 years, 3 years, 4 years) are shown. A * indicates that the dosage groups (2.1 mg/kg/week and 3.5 mg/kg/week) were combined. The initial 6 control patients did not have data for the year 4 time point due to the 6 month offset.

DETAILED DESCRIPTION

We have discovered that asfotase alfa (SEQ ID NO: 1, STRENSIQ®, Alexion Pharmaceuticals, Inc.) can be used effectively to treat hypophosphatasia (HPP), its symptoms, and physical impairments associated therewith, in both adolescents having HPP (e.g., adolescents having HPP of about 12 to about 18 years of age) and in adults having HPP (e.g., adults having HPP older than about 18 years of age) for an extended period of time (e.g., at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). In particular, asfotase alfa (SEQ ID NO: 1) can be administered to treat adolescents and adults with HPP exhibiting physical impairments (e.g., bone or muscle weakness) and decreased walking ability relative to an untreated subject having HPP (e.g., an untreated subject having HPP of about the same age, same gender, and/or height). Furthermore, the adolescent having HPP or adult having HPP can be a naïve patient that has not previously received treatment with asfotase alfa (SEQ ID NO: 1).

Methods for administering asfotase alfa (SEQ ID NO: 1) to an adolescent having HPP or an adult having HPP that result in an improvement in walking ability of the adolescent or adult having HPP are described. For example, asfotase alfa (SEQ ID NO: 1) can be administered to an adolescent having HPP or an adult having HPP with decreased walking ability, such that the Six Minute Walk Test (6MWT) value of the adolescent or adult is less than about 80% of the predicted 6MWT value, such as the predicted 6MWT value for an untreated subject having HPP (e.g., an untreated subject having HPP of about the same age, same gender, and/or height). For instance, the adult or adolescent having HPP exhibits decreased reliance on an assistive device for mobility generally or for walking specifically, such as a wheelchair, a wheeled walker, a cane, or an orthotic, after administration of the sALP.

Methods for administering asfotase alfa (SEQ ID NO: 1) to an adolescent having HPP or an adult having HPP having an average Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2) score indicative of physical impairments (e.g, an average BOT-2 score of less than about 7 in one or more BOT-2 score areas of strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination) are also described. For example, asfotase alfa (SEQ ID NO: 1) can be administered to an adolescent having HPP or an adult having HPP having an average BOT-2 running speed and agility score of less than about 7. Furthermore, asfotase alfa (SEQ ID NO: 1) can be administered to an adolescent having HPP or an adult having HPP having an average BOT-2 strength score of less than about 14.

Additionally, methods for administering asfotase alfa (SEQ ID NO: 1) to an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age) that exhibits elevated inorganic pyrophosphate (PPi) concentrations in a plasma sample from the adolescent or adult having HPP relative to PPi concentrations in a plasma sample from an untreated subject having HPP (e.g., an untreated subject

having HPP of about the same age, same gender, and/or height) are described. For instance, a plasma sample from the adolescent or adult may have a PPi concentration of about 5 μM to about 6 μM. Additionally, a plasma sample from the patient may have a PLP concentration of about 50 ng/ml to about 300 ng/ml.

In any of these methods, asfotase alfa (SEQ ID NO: 1) may be administered to an adolescent having HPP or an adult having HPP for an extended period of time, e.g., at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). Furthermore, given the results described herein using asfotase alfa, other sALPs (such as a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1) may be used to treat an adolescent having HPP or an adult having HPP for an extended period of time, e.g., at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

Methods of Treatment

Provided herein are methods for treating an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age). Adolescents having HPP can be treated by administering a sALP (such as TNALP, for example, a sALP fusion polypeptide, such as the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) across a range of ages, e.g., about 12 to about 13 years of age, about 13 to about 15 years of age, about 14 to about 16 years of age, about 12 to about 16 years of age, about 15 to about 16 years of age, about 14 to about 17 years of age, about 15 to about 17 years of age, about 16 to about 18 years of age, or about 14 to about 18 years of age. Likewise, adults having HPP can be treated by administering a sALP (such as TNALP, for example, a sALP fusion polypeptide, such as the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) across a range of ages, e.g., about 18 to about 20 years of age, about 20 to about 25 years of age, about 25 to about 30 years of age, about 30 to about 35 years of age, about 35 to about 40 years of age, about 40 to about 45 years of age, about 45 to about 50 years of age, about 50 to about 55 years of age, about 60 to about 65 years of age, about 20 to about 30 years of age, about 30 to about 40 years of age, about 40 to about 50 years of age, about 50 to about 60 years of age, about 60 to about 70 years of age, about 20 to about 65 years of age, about 30 to about 65, years of age, or older than 65 years of age.

Adolescents (e.g., adolescents having HPP of about 12 to about 18 years of age) and adults (e.g., adults having HPP older than about 18 years of age) can be diagnosed with HPP prior to administration of a sALP (such as TNALP, for example, a sALP fusion polypeptide, such as the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). An adolescent having HPP or an adult having HPP can exhibit, e.g., physical impairments and impaired walking ability relative to an adolescent without HPP or

an adult without HPP, respectively. Additionally, the adolescent having HPP or adult having HPP can be a naïve patient that has not previously received treatment with a sALP (such as TNALP, for example, a sALP fusion polypeptide, such as the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). The method involves administering a sALP (such as TNALP, for example, a sALP fusion polypeptide, such as the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) to an adolescent having HPP or an adult having HPP, such as administering a sALP for a period of least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

In particular, a sALP, such as asfotase alfa, can be administered for a period of time to an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age) previously determined to have a 6MWT value of less than about 80% of the predicted 6MWT value of the patient (e.g., relative to an untreated subject of about the same age, the same gender, and/or the same height), a plasma PPi concentration of about 5 μM or greater, a plasma PLP concentration of about 50 ng/ml or greater, an average BOT-2 running speed and agility score of less than about 7, and/or an average BOT-2 strength score of less than about 14. Moreover, the 6MWT value of the patient (e.g., the adolescent or adult having HPP) can be compared to the 6MWT value at baseline of the patient. Additionally, the 6MWT value of the adolescent or adult having HPP can be compared to the 6MWT value of a healthy patient. In particular, the sALP (e.g., asfotase alfa) can be administered for a period of least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). Alternatively, the methods can include determining the 6MWT, BOT-2 running speed and agility score, BOT-2 strength score, plasma PPi concentration, and/or plasma PLP concentration prior to administering a sALP, such as asfotase alfa, as described herein.

Additionally, each of the described metrics (e.g., the 6MWT value, the BOT-2 strength score, BOT-2 running speed and agility score, plasma PPi concentrations, and plasma PLP concentrations) for a patient having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) can be used singly or in combination to assess treatment efficacy using a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), in which improvements relative to a certain value or score demonstrate that the sALP is effective for treating HPP.

For example, when administration of a sALP to an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age) results in an average increase in the BOT-2 running speed and agility score to about 9 or greater, in which the adolescent or adult previously had an average BOT-2 strength score of less than about 7, then the sALP treatment is effective at treating, e.g., physical impairments

associated with HPP. Alternatively, when administration of a sALP does not result in an average increase in the BOT-2 running speed and agility score to about 9 or greater, the dosage and/or frequency of sALP administration can be changed in order to determine the effective amount of the sALP for the adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age). For instance, the dosage of the sALP such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 2.1 mg/kg/week or about 3.5 mg/kg/week to about 6 mg/kg/week or about 9 mg/kg/week.

Hypophosphatasia in adolescents and adults

Asfotase alfa is administered, as described herein, to treat adolescent HPP or adult HPP. In particular, patients having adolescent HPP (e.g., adolescents having HPP of about 12 to about 18 years of age) or adult HPP (e.g., adults having HPP older than about 18 years of age) can be treated with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years (e.g., the lifetime of the patient). In preferred embodiments, an HPP phenotype of, e.g., adolescent HPP or adult HPP, is treated with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa).

Accordingly, the methods are useful for alleviating any of the symptoms of HPP described herein, particularly when the sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) is administered for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

For instance, the methods are useful for treating symptoms of adolescent and adult HPP, including, but not limited to, premature loss of teeth due to aplasia, hypoplasia, or dysplasia of dental cementum that connects the tooth root with the periodontal ligament; rickets (osteomalacia); skeletal deformities, such as bowed legs and enlarged wrists, knees, and ankles as a result of flared metaphyses; short stature; muscle weakness, skeletal pain; stiffness; bone fractures; pseudofractures; arthritis; pyrophosphate arthropathy; calcium pyrophosphate dihydrate crystal deposition; altered physical function including walking ability; elevated blood and/or urine levels of inorganic pyrophosphate (PP_i), phosphoethanolamine (PEA), and/or urine levels of pyridoxal 5'-phosphate (PLP); hypomineralization; rachitic ribs; hypercalciuria; HPP-related seizure; inadequate weight gain; and/or calcium pyrophosphate dihydrate crystal deposition. Any of the above mentioned symptoms can be treated with a sALP for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five

years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

Exemplary metrics useful for evaluating the need for or the efficacy of treatment using a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) include (1) the Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2), (2) the Six Minute Walk Test (6MWT), and (3) plasma PPi and PLP concentrations, which are described in further detail below.

Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2)

Adolescents having HPP (e.g., adolescents having HPP of about 12 to about 18 years of age) or adults having HPP (e.g., adults having HPP older than about 18 years of age) can be identified for treatment with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) using the BOT-2). In particular, the BOT-2 can be used to evaluate physical impairments and mobility restrictions in an adolescents having HPP or an adult having HPP to generate a BOT-2 score for the adolescent or adult.

The BOT-2 includes a range of tests to evaluate physical impairments of a patient having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age), which can be performed with, e.g., a kit including the tests. The BOT-2 provides composite BOT-2 scores in the following areas: strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination. For example, the adolescent or adult having HPP can perform sit-ups, v-ups, standing long jump, wall sit, and/or push-ups to determine the BOT-2 strength score; the adolescent or adult having HPP can step over a balance beam and/or perform a shuttle run, two-legged side hop, and/or one-legged side hop to determine the BOT-2 running speed and agility score; the adolescent or adult having HPP can cut out a circle and/or connect dots to determine the BOT-2 fine motor precision score; the adolescent or adult having HPP can copy a star and/or copy a square to determine the BOT-2 fine motor integration score; the adolescent or adult having HPP can transfer pennies, sort cards, and/or string blocks to determine the manual dexterity score; the adolescent or adult having HPP can tap his or her foot and finger and/or perform jumping jacks to determine the BOT-2 bilateral coordination score; the adolescent or adult having HPP can walk forward on a line and/or stand on one leg on a balance beam to determine the BOT-2 balance score; and the adolescent or adult having HPP can throw a ball at a target and/or catch a tossed ball to determine the BOT-2 upper-limb coordination score. The BOT-2 score is an additive total of each area assessed. Moreover, the BOT-2 score used to assess the physical proficiency of the patient can be the raw additive score or a normative score.

An adolescent having HPP (e.g., adolescents having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., adults having HPP older than about 18 years of age) could perform tests in one or more of described areas (strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination) to generate a BOT-2 score indicative of physical impairments in the adolescent or adult. Within each BOT-2 area

(strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination), an adolescent or adult having HPP could perform one or more tests to determine the BOT-2 score of the adolescent or adult, e.g., the adolescent or adult could perform one or more of sit-ups, v-ups, standing long jump, wall sit, and push-ups to determine the BOT-2 strength score. If desired, only a single test (e.g., one test selected from the group of sit-ups, v-ups, standing long jump, wall sit, and push-ups) can be performed to determine the BOT-2 score (e.g., a BOT-2 strength score) of an adolescent or adult having HPP.

Each of the BOT-2 scores (strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination) of the patient having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) can be compared to the BOT-2 score of patients without HPP (e.g., an adolescent without HPP of about 12 to about 18 years of age or an adult without HPP older than about 18 years of age) to, e.g., determine the standard deviation of the BOT-2 score. Each of the BOT-2 scores (e.g., strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination) of the patient having HPP can be compared to the BOT-2 score of other HPP patients to, e.g., determine the average BOT-2 score for the HPP patient.

BOT-2 scores (e.g., strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination scores) range from about 0 to equal to or less than about 25, in which a score of greater than about 10 is considered representative of healthy subjects (e.g., patients without HPP). Patients with an average BOT-2 score (e.g., strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination scores) of less than about 10 can be treated with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as by administering a sALP for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

For example, an HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) with a BOT-2 running speed and agility score of less than 10 (e.g, about 0, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, or about 10) can be treated with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). Similarly, an HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) with a BOT-2 strength score of less than 14 (e.g, about 0, about 1, about 2, about 3, about 4, about 5, about 6, about 7, about 8, about 9, about 10, about 11, about 12, about 13, or about 14) can then be treated with a sALP (such as TNALP, for example

the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

The methods result in an improvement in the BOT-2 score (e.g., strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and/or upper-limb coordination score) of a HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age). For example, treatment with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with a sALP for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient), result in an average increase in the BOT-2 strength score to about 10 to about 20 or greater (e.g. about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, or about 25). Additionally, treatment with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with a sALP for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient), result in an average increase in the BOT-2 running speed and agility score to about 9 to about 20 or greater (e.g. about 9, about 10, about 11, about 12, about 13, about 14, about 15, about 16, about 17, about 18, about 19, about 20, about 21, about 22, about 23, about 24, or about 25).

The increase in the BOT-2 score (e.g., strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and/or upper-limb coordination score) can be sustained throughout administration of the sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), e.g., for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). Likewise, the decrease in physical impairments can be sustained throughout administration of the sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), e.g., for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

The BOT-2 scores (strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination scores) of a

HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) can be used singly or in combination to assess treatment efficacy using a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), in which improvements relative to a certain test score demonstrate that the sALP is effective for treating physical impairments associated with HPP. For example, when administration of a sALP to a HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) results in an average increase in the BOT-2 running speed and agility score to about 9 or greater, in which the patient previously had an average BOT-2 running speed and agility score of less than about 7, then the sALP is considered to be effective at, e.g., treating physical impairments associated with HPP. Alternatively, an increase of at least two points or more (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 points) over the BOT-2 running speed and agility score prior to treatment indicates efficacy (e.g., when coupled with a sustained high score for greater than 1 year of treatment).

Additionally, within each BOT-2 area (strength, running speed and agility, fine motor precision, fine motor integration, manual dexterity, bilateral coordination, balance, and upper-limb coordination), a HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) could perform one or more tests to determine the BOT-2 score of the patient. For instance, the adolescent or adult having HPP could perform one or more of sit-ups, v-ups, standing long jump, wall sit, and push-ups to determine the BOT-2 strength score and assess the treatment efficacy of sALP administration. The adolescent or adult having HPP could perform one or more of balance beam, a shuttle run, two-legged side hop, and/or one-legged side hop to determine the BOT-2 running speed and agility score and assess the treatment efficacy of sALP administration. The adolescent or adult having HPP can cut out a circle and/or connect dots to determine the BOT-2 fine motor precision score and assess the treatment efficacy of sALP administration. The adolescent or adult having HPP can copy a star and/or copy a square to determine the BOT-2 fine motor integration score and assess the treatment efficacy of sALP administration. The adolescent or adult having HPP could perform one or more of transferring pennies, sorting cards, and stringing blocks to determine the BOT-2 manual dexterity score and assess the treatment efficacy of sALP administration. The adolescent or adult having HPP can tap his or her foot and finger and/or perform jumping jacks to determine the BOT-2 bilateral coordination score and assess the treatment efficacy of sALP administration. The adolescent or adult having HPP can walk forward on a line and/or stand on one leg on a balance beam to determine the BOT-2 balance score and assess the treatment efficacy of sALP administration. The adolescent or adult having HPP can throw a ball at a target and/or catch a tossed ball to determine the BOT-2 upper-limb coordination score and assess the treatment efficacy of sALP administration.

Alternatively, when administration of a sALP does not result in an average increase in the BOT-2 running speed and agility score to greater than about 9 (e.g., an increase of at least 2 to 10 points (e.g., 2, 3, 4, 5, 6, 7, 8, 9, or 10 points) over the BOT-2 running speed and agility score prior to treatment with the sALP), the dosage and/or frequency of sALP administration can be changed in order to determine the effective amount of the sALP for the HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age). For instance, the dosage of the

sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 2.1 mg/kg/week or about 3.5 mg/kg/week to about 6 mg/kg/week or about 9 mg/kg/week.

Six Minute Walk Test (6MWT)

Adolescents having HPP (e.g., adolescents having HPP of about 12 to about 18 years of age) or adults having HPP (e.g., adults having HPP older than about 18 years of age) can be identified for treatment with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) using the 6MWT. In particular, the 6MWT can be used to evaluate walking ability in an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age) to generate a 6MWT value for the adolescent or adult.

The 6MWT can be performed indoors or outdoors using a flat, straight, enclosed corridor (e.g., of about 30 meters in length) with a hard surface. A stopwatch or other timer can be used to track the time and a mechanical counter or other device can be used to determine the distance (e.g., in meters) that the HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) walks. For instance, the length of the corridor can be marked every three meters to determine the number of meters walked by the HPP patient, with the turnaround point at 30 meters and the starting line also marked. The distance walked by the patient in six minutes can then be compared to the predicted number of meters walked, e.g., by an untreated subject of about the same age, the same gender, and/or the same height, and expressed as a percentage value to generate the 6MWT value of the patient. The 6MWT value of the patient (e.g., the adolescent or adult having HPP) can be compared to the 6MWT value at baseline of the patient. Additionally, the 6MWT value of the adolescent or adult having HPP can be compared to the 6MWT value of a healthy patient.

HPP patients (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) with an average 6MWT of less than about 80% of the predicted 6MWT value can be treated with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as by administering a sALP for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). For example, an HPP patient with an average 6MWT of less than about 80% of the predicted 6MWT value (e.g., about 50%, about 55%, about 60%, about 65%, about 70%, or about 75% of the predicted 6MWT value) can be treated with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at

least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

The methods can result in an improvement in the 6MWT value of a HPP patient (e.g., an adolescent having HPP of about 118 years of age or an adult having HPP older than about 18 years of age). For example, treatment with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with a sALP for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient), result in an average increase in the 6MWT value to about 80% or greater of the predicted 6MWT value of the patient (e.g. about 82%, about 84%, about 86%, about 88%, about 90%, about 92%, about 94%, about 96%, about 98%, or more of the predictive 6MWT value).

The increase in the 6MWT value of the HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) can be sustained throughout administration of the sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), e.g., for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). For instance, the 6MWT value increases to greater than about 80% of the predicted 6 MWT value of the patient and remains at ± 10% of the increased 6MWT value during treatment with the sALP (e.g., asfotase alfa).

Likewise, the improvement in walking ability of the HPP patient can be sustained throughout administration of the sALP, e.g., for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). For instance, the HPP patient exhibits decreased reliance on an assistive device for walking, such as a wheelchair, a wheeled walker, a cane, or an orthotic during treatment with the sALP.

Alternatively, when administration of a sALP does not result in an average increase in the 6MWT value to greater than 80% of the predicted 6MWT value (e.g., of an untreated subject having HPP of about the same age, same gender, and/or height), the dosage and/or frequency of sALP administration can be changed in order to determine the effective amount of the sALP for the HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age). For instance, the dosage of the sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 2.1 mg/kg/week or about 3.5 mg/kg/week to about 6 mg/kg/week or about 9 mg/kg/week.

Plasma Inorganic Pyrophosphate (PPi) and Pyridoxal 5'-phosphate (PLP) Concentrations

Patients having HPP (e.g., adolescents having HPP of about 12 to about 18 years of age or adults having HPP older than about 18 years of age) can be identified for treatment with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) by determining the PPi and/or PLP concentrations in a plasma sample from the patient. Any method known to those of skill in the art can be used to quantify the PPi and PLP concentrations in a plasma sample or alternatively in a urine sample, as described in detail in Whyte et al., 1995 (*J. Clin. Invest.* 95(4): 1440–1445), hereby incorporated by reference in its entirety. In particular, PPi and PLP concentrations in a plasma sample can be used to evaluate ALP activity for the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age).

In comparison to healthy subjects (e.g., healthy subjects of about the same age, same gender, and/or same height), HPP patients typically exhibit elevated plasma concentrations of PPi and PLP, such as a PPi concentration of about 5 μM or greater and/or a PLP concentration of about 50 ng/ml or greater. The lower normal limit for plasma PPi concentrations of healthy adults is about 1 μM, while the upper normal limit is about 5.9 μM. The lower normal limit for plasma PPi concentrations of adolescent adults is about 0.8 μM, while the upper normal limit is about 4.9 μM. The lower normal limit for plasma PLP concentrations of healthy adults is less than about 10 ng/ml, while the upper normal limit is about 60 ng/ml. The lower normal limit for plasma PLP concentrations of adolescent adults is less than about 10 ng/ml, while the upper normal limit is less than about 25 ng/ml.

HPP patients (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) with elevated plasma concentrations of PPi and/or PLP can be treated with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as by administering a sALP for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

For example, an HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) with a PPi concentration of about 5 μM or greater can be treated with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). Likewise, an HPP patient with a PLP concentration of about 50 ng/ml or greater can be treated with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years,

at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

The methods result in an average decrease in PPi and/or PLP concentrations in a plasma sample from the patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age). For example, treatment with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with a sALP for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient), results in an average decrease in PPi concentrations in a plasma sample from the patient of about 25% or greater (e.g., 30%, 35%, 40%, 45%, 50%, 55%, 60%, or more than 60%) relative to PPi concentrations in a plasma sample from the patient prior to administration of the sALP. Likewise, treatment with a sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), such as treatment with a sALP for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient), results in an average decrease in PLP concentrations in a plasma sample from the patient of about 50% or greater (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or more than 95%) relative to PLP concentrations in a plasma sample from the patient prior to administration of the sALP.

The decrease in the plasma PPi and/or PLP concentrations of the HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) can be sustained throughout administration of the sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), e.g., for a period of at least one year (e.g., at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient). For instance, the plasma PPi concentration decreases by about 25% and remains at ± 10% of the decreased plasma PPi concentration during treatment with the sALP and/or the plasma PLP concentration decreases by about 50% and remains at ± 10% of the decreased plasma PLP concentration during treatment with the sALP

Alternatively, when administration of a sALP does not result in an average decrease in PPi concentrations in a plasma sample from the patient by about 25% or greater, the dosage and/or frequency of sALP administration can be changed in order to determine the effective amount of the sALP for the HPP patient (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age). Likewise, when administration of a sALP does not result in an average decrease in PLP concentrations in a plasma sample from the patient by about 50% or greater, the dosage and/or frequency of sALP administration can be changed in order to determine the effective amount of the sALP for the HPP patient (e.g., an adolescent having HPP of about 12 to about 18

years of age or an adult having HPP older than about 18 years of age). For instance, the dosage of the sALP (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be increased from, e.g., about 2.1 mg/kg/week or about 3.5 mg/kg/week to about 6 mg/kg/week or about 9 mg/kg/week.

Alkaline Phosphatase

Asfotase alfa is a human TNALP (hTNALP; SEQ ID NO: 1) fusion polypeptide formulated for the treatment of HPP. In particular, asfotase alfa (SEQ ID NO: 1) can be used effectively to treat hypophosphatasia (HPP), its symptoms, and physical impairments associated therewith in an adolescent or adult having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age) for an extended period of time (e.g., at least one year, at least two years, at least three years, at least four years, at least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer than ten years, such as for the lifetime of the patient).

Given the results described herein, the treatment methods are not limited to administration of a particular alkaline phosphatase (ALP) or nucleic acid sequence encoding an ALP. Alkaline phosphatases encompass a group of enzymes that catalyze the cleavage of a phosphate moiety (e.g., hydrolysis of pyrophosphate, PP_i). There are four known mammalian alkaline phosphatase (ALP) isozymes: tissue nonspecific alkaline phosphatase (TNALP; described further below), placental alkaline phosphatase (PLALP) (e.g., Accession Nos. P05187, NP_112603, and NP_001623), germ cell alkaline phosphatase (GALP) (e.g., Accession No. P10696), and intestinal alkaline phosphatase (IALP) (e.g., Accession Nos. P09923 and NP_001622). In addition to the exemplary ALPs discussed above, any polypeptide having the identical or similar catalytic site structure and/or enzymatic activity of ALP can be used (e.g., as a sALP or a sALP fusion polypeptide as defined herein) for treating HPP patients, such as adolescents or adults having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age or an adult having HPP older than about 18 years of age). Bone delivery conjugates including sALP are further described in PCT publication Nos: WO 2005/103263 and WO 2008/138131.

TNALPs that can be used according to the methods described herein include, e.g., human TNALP (Accession Nos. NP_000469, AAI10910, AAH90861, AAH66116, AAH21289, and AAI26166); rhesus TNALP (Accession No. XP_01109717); rat TNALP (Accession No. NP_037191); dog TNALP (Accession No. AAF64516); pig TNALP (Accession No. AAN64273), mouse (Accession No. NP_031457), cow TNALP (Accession Nos. NP_789828, NP_776412, AAM 8209, and AAC33858), and cat TNALP (Accession No. NP_001036028). In particular, TNALP can be a recombinant human TNALP (e.g., SEQ ID NO: 1, asfotase alfa; see U.S. Patent Nos. 7,763,712 and 7,960,529, incorporated herein by reference in their entirety) used for the treatment of HPP patients, such as adolescents with HPP (e.g., adolescents having HPP of about 12 to about 18 years of age) or adults with HPP (e.g., adults having HPP older than about 18 years of age). The TNALP can also be one that exhibits at least about 95% sequence identity to the polypeptide or nucleic acid sequence of the above-noted TNALPs.

Soluble Alkaline Phosphatases

The ALPs that can be used in the methods described herein include soluble (e.g., extracellular or non-membrane-bound) forms of any of the alkaline phosphatases described herein. The sALP can be, for example, a soluble form of human tissue non-specific alkaline phosphatase (human TNALP (hTNALP)). The methods are not limited to a particular sALP and can include any sALP that is physiologically active toward, e.g., phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and pyridoxal 5'-phosphate (PLP). In particular, a sALP is one that is catalytically competent to improve skeletal mineralization in bone. The methods further include nucleic acids encoding the sALPs described herein that can be used to treat the conditions described herein, e.g., HPP, such as adolescents with HPP (e.g., adolescents having HPP of about 12 to about 18 years of age) or adults with HPP (e.g., adults having HPP older than about 18 years of age).

TNALP is a membrane-bound protein anchored by a glycolipid moiety at the C-terminal (Swiss-Prot, P05186). This glycolipid anchor (GPI) is added post-translationally after the removal of a hydrophobic C-terminal end, which serves both as a temporary membrane anchor and as a signal for the addition of the GPI. While the GPI anchor is located in the cell membrane, the remaining portions of TNALP are extracellular. In particular, TNALP (e.g., human TNALP (hTNALP)) can be engineered to replace the first amino acid of the hydrophobic C-terminal sequence (an alanine) with a stop codon, thereby producing an engineered hTNALP that contains all amino acid residues of the native anchored form of TNALP and lacks the GPI membrane anchor. One skilled in the art will appreciate that the position of the GPI membrane anchor will vary in different ALPs and can include, e.g., the last 10, 12, 14, 16, 18, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 32, 34, 36, 38, 40, 45, 50, or more amino acid residues on the C-terminus of the polypeptide. Recombinant sTNALP can include, e.g., amino acids 1 to 502 (18 to 502 when secreted), amino acids 1 to 501 (18 to 501 when secreted), amino acids 1 to 504 (18 to 504 when secreted), amino acids 1 to 505 (18-505 when secreted), or amino acids 1 to 502. Thus, the C-terminal end of the native ALP can be truncated by certain amino acids without affecting ALP activity.

In addition to the C-terminal GPI anchor, TNALP also has an N-terminal signal peptide sequence. The N-terminal signal peptide is present on the synthesized protein when it is synthesized, but cleaved from TNALP after translocation into the ER. The sALPs include both secreted (i.e., lacking the N-terminal signal) and non-secreted (i.e., having the N-terminal signal) forms thereof. One skilled in the art will appreciate that the position of the N-terminal signal peptide will vary in different alkaline phosphatases and can include, for example, the first 5, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 27, 30, or more amino acid residues on the N-terminus of the polypeptide. One of skill in the art can predict the position of a signal sequence cleavage site, e.g., by an appropriate computer algorithm such as that described in Bendtsen et al. (*J. Mol. Biol.* 340(4):783-795, 2004) and available on the Web at www.cbs.dtu.dk/services/SignalP/.

The methods can also be performed using sALP consensus sequences derived from the extracellular domain of ALP isozymes (e.g., TNALP, PALP, GCALP, IALP, etc.). Thus, similar to sTNALP discussed above, the present disclosure also provides other soluble human ALP isozymes, i.e., without the peptide signal, preferably comprising the extracellular domain of the ALPs. The sALPs also include polypeptide sequences satisfying a consensus sequence derived from the ALP extracellular domain of

human ALP isozymes and of mammalian TNALP orthologs (human, mouse, rat, cow, cat, and dog) or a consensus derived from the ALP extracellular domain of just mammalian TNALP orthologs (human, mouse, rat, cow, cat, and dog). The sALPs also include those which satisfy similar consensus sequences derived from various combinations of these TNALP orthologs or human ALP isozymes. Such consensus sequences are given, for example, in WO 2008/138131.

sALPs of the present methods can include not only the wild-type sequence of the sALPs described above, but any polypeptide having at least 50% (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to these alkaline phosphatases (e.g., SEQ ID NOs: 1-24; for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). Examples of mutations that can be introduced into an ALP sequence are described in US Publication No. 2013/0323244, hereby incorporated by reference in its entirety. A sALP can optionally be glycosylated at any appropriate one or more amino acid residues. In addition, an sALP can have at least 50% (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to any of the sALPs described herein (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa). A sALP can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, or more additions, deletions, or substitutions relative to any of the sALPs described herein (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa).

sALP Fusion Polypeptides

Any of the sALPs (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa), linkers, spacers (e.g., Fc regions), and bone-targeting moieties described herein can be combined in a fusion polypeptide, which includes the structures Z-sALP-Y-spacer-X-W_n-V, Z-W_n-X-spacer-Y-sALP-V, Z-sALP-Y-W_n-X-spacer-V, and Z-W_n-X-sALP-Y-spacer-V. In particular, the structure of the sALP fusion polypeptide can be Z-sALP-Y-spacer-X-W_n-V or Z-W_n-X-spacer-Y-sALP-V. The sALP of the sALP fusion polypeptide can be the full-length ALP or functional fragments of ALPs, such as the soluble, extracellular domain of the ALP, as is described herein (e.g., TNALP, PALP, GCALP and IALP).

Any one of X, Y, Z, and V and/or the spacer can be absent or a linker region including an amino acid sequence of at least one amino acid. For example, X, Y, Z, and V may be a dipeptide sequence (e.g., leucine-lysine or aspartic acid-isoleucine), such as a two residue linker at the Y position (e.g., leucine-lysine) or a two residue linker at the X position (e.g., aspartic acid-isoleucine). For example, sALP fusion polypeptides can have the structure hTNALP-Fc-D_{10} (e.g., a sALP fusion polypeptide including the amino acid sequence of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa).

The linker region can be of any sequence and length that allows the sALP to remain biologically active, e.g., not sterically hindered. Exemplary linker lengths are between 1 and 200 amino acid

residues, e.g., 1-5, 6-10, 11-15, 16-20, 21-25, 26-30, 31-35, 36-40, 41-45, 46-50, 51-55, 56-60, 61-65, 66-70, 71-75, 76-80, 81-85, 86-90, 91-95, 96-100, 101-110, 111-120, 121-130, 131-140, 141-150, 151-160, 161-170, 171-180, 181-190, or 191-200 amino acid residues. For instance, linkers include or consist of flexible portions, e.g., regions without significant fixed secondary or tertiary structure. Exemplary flexible linkers are glycine-rich linkers, e.g., containing at least 50%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, or even 100% glycine residues. Linkers can also contain, e.g., serine residues. In some cases, the amino acid sequence of linkers consists only of glycine and serine residues. A linker can optionally be glycosylated at any appropriate one or more amino acid residues. Additionally, a linker as described herein can include any other sequence or moiety, attached covalently or non-covalently. The linker can also be absent, in which the spacer (e.g., the Fc region) and the sALP are fused together directly, with no intervening residues.

Useful spacers include, but are not limited to, polypeptides comprising a Fc region. For example, a sALP can be a fusion polypeptide including an Fc region of an immunoglobulin at the N-terminal or C-terminal domain. An immunoglobulin molecule has a structure that is well known in the art. It includes two light chains (~23 kD each) and two heavy chains (~50-70 kD each) joined by inter-chain disulfide bonds. Immunoglobulins are readily cleaved proteolytically (e.g., by papain cleavage) into Fab (containing the light chain and the VH and CH1 domains of the heavy chain) and Fc (containing the CH2 and CH3 domains of the heavy chain, along with adjoining sequences). Useful Fc fragments as described herein include the Fc fragment of any immunoglobulin molecule, including IgG, IgM, IgA, IgD, or IgE, and their various subclasses (e.g., IgG-1, IgG-2, IgG-3, IgG-4, IgA-1, IgA-2), from any mammal (e.g., human). For instance, the Fc fragment is human IgG-1. The Fc fragments can include, for example, the CH2 and CH3 domains of the heavy chain and any portion of the hinge region. The Fc region can optionally be glycosylated at any appropriate one or more amino acid residues known to those skilled in the art. In particular, the Fc fragment of the fusion polypeptide has the amino acid sequence of SEQ ID NO: 20, or has at least 50% (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to SEQ ID NO: 20. Engineered, e.g., non-naturally occurring, Fc regions can be incorporated into the sALP fusion polypeptides described herein, e.g., those described in International Application Pub. No. WO2005/007809, which is hereby incorporated by reference. An Fc fragment as described herein can have 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 30, 35, 40, 50, or more additions, deletions, or substitutions relative to any of the Fc fragments described herein.

W_n can be a bone-targeting moiety, e.g., having a series of consecutive aspartate (D) or glutamate (E) residues, in which n = 1 to 50, e.g., n = 3-30, e.g., 5-15, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 , 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 36, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50. The bone-targeting moiety, if present, can be positioned anywhere in the fusion polypeptide, e.g., at or near the N-terminal or C-terminal end, and/or in the linker region. For instance, the bone-targeting moiety can be present at the C-terminal end of a sALP fusion polypeptide. sALPs and fusion polypeptides can also lack a bone-targeting moiety.

Additional amino acid residues can be introduced into the polypeptide according to the cloning strategy used to produce the fusion polypeptides. For instance, the additional amino acid residues do not

provide an additional GPI anchoring signal so as to maintain the polypeptide in a soluble form. Furthermore, any such additional amino acid residues, when incorporated into the polypeptide of the methods, do not provide a cleavage site for endoproteases of the host cell. The likelihood that a designed sequence would be cleaved by the endoproteases of the host cell can be predicted as described, e.g., by Ikezawa (*Biol. Pharm. Bull.* 25:409-417, 2002).

The sALP fusion polypeptides (such as a TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be associated into dimers or tetramers. For example, two sALP-Fc monomers can covalently be linked through two disulfide bonds located in the hinge regions of the Fc fragments. Additionally, the sALP fusion polypeptide (e.g., a sALP or a sALP fusion polypeptide) can be glycosylated or PEGylated.

Production of Nucleic Acids and Polypeptides

The nucleic acids encoding sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be produced by any method known in the art. Typically, a nucleic acid encoding the desired fusion polypeptide is generated using molecular cloning methods, and is generally placed within a vector, such as a plasmid or virus. The vector is used to transform the nucleic acid into a host cell appropriate for the expression of the fusion polypeptide. Representative methods are disclosed, for example, in Maniatis et al. (Cold Springs Harbor Laboratory, 1989). Many cell types can be used as appropriate host cells, although mammalian cells are preferable because they are able to confer appropriate post-translational modifications. Host cells can include, e.g., Chinese Hamster Ovary (CHO) cell, L cell, C127 cell, 3T3 cell, BHK cell, COS-7 cell or any other suitable host cell known in the art. For example, the host cell is a Chinese Hamster Ovary (CHO) cell (e.g., a CHO-DG44 cell).

The sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be produced under any conditions suitable to effect expression of the sALP polypeptide in the host cell. Such conditions include appropriate selection of a media prepared with components such as a buffer, bicarbonate and/or HEPES, ions like chloride, phosphate, calcium, sodium, potassium, magnesium, iron, carbon sources like simple sugars, amino acids, potentially lipids, nucleotides, vitamins and growth factors like insulin; regular commercially available media like alpha-MEM, DMEM, Ham's-F12, and IMDM supplemented with 2-4 mM L-glutamine and 5% Fetal bovine serum; regular commercially available animal protein free media like Hyclone™ SFM4CHO, Sigma CHO DHFR$^-$, Cambrex POWER™ CHO CD supplemented with 2-4 mM L-glutamine. These media are desirably prepared without thymidine, hypoxanthine and L-glycine to maintain selective pressure, allowing stable protein-product expression.

Pharmaceutical compositions and formulations

A composition that can be used in the methods described herein (e.g., including a sALP or sALP fusion polypeptide, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide

variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. The route of administration can depend on a variety of factors, such as the environment and therapeutic goals. In particular, the polypeptides and fusion polypeptides described herein can be administration by any route known in the art, e.g., subcutaneous (e.g., by subcutaneous injection), intravenously, orally, nasally, intramuscularly, sublingually, intrathecally, or intradermally. By way of example, pharmaceutical compositions that can be used in the methods described herein can be in the form of a liquid, solution, suspension, pill, capsule, tablet, gelcap, powder, gel, ointment, cream, nebulae, mist, atomized vapor, aerosol, or phytosome.

Dosage

Any amount of a pharmaceutical composition (e.g., including a sALP or sALP fusion polypeptide, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be administered to an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age). The dosages will depend on many factors including the mode of administration and the age of the patient. Typically, the amount of the composition (e.g., a sALP or sALP fusion polypeptide, such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) contained within a single dose will be an amount that is effective to treat a condition (e.g., HPP) as described herein without inducing significant toxicity.

For example, the sALP polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) described herein can be administered to an HPP patient, such as an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age), in individual doses ranging, e.g., from 0.01 mg/kg to 500 mg/kg (e.g., from 0.05 mg/kg to 500 mg/kg, from 0.1 mg/kg to 20 mg/kg, from 5 mg/kg to 500 mg/kg, from 0.1 mg/kg to 100 mg/kg, from 10 mg/kg to 100 mg/kg, from 0.1 mg/kg to 50 mg/kg, 0.5 mg/kg to 25 mg/kg, 1.0 mg/kg to 10 mg/kg, 1.5 mg/kg to 5 mg/kg, or 2.0 mg/kg to 3.0 mg/kg) or from 1 μg/kg to 1,000 μg/kg (e.g., from 5 μg/kg to 1,000 μg/kg, from 1 μg/kg to 750 μg/kg, from 5 μg/kg to 750 μg/kg, from 10 μg/kg to 750 μg/kg, from 1 μg/kg to 500 μg/kg, from 5 μg/kg to 500 μg/kg, from 10 μg/kg to 500 μg/kg, from 1 μg/kg to 100 μg/kg, from 5 μg/kg to 100 μg/kg, from 10 μg/kg to 100 μg/kg, from 1 μg/kg to 50 μg/kg, from 5 μg/kg to 50 μg/kg, or from 10 μg/kg to 50 μg/kg).

Exemplary doses of a sALP include, e.g., 0.01, 0.05, 0.1, 0.5, 1, 2, 2.5, 5, 10, 20, 25, 50, 100, 125, 150, 200, 250, or 500 mg/kg; or 1, 2, 2.5, 5, 10, 20, 25, 50, 100, 125, 150, 200, 250, 500, 750, 900, or 1,000 μg/kg. For all dosages or ranges recited herein, the term "about" can be used to modify these dosages by ±10% of the recited values or range endpoints. In particular, compositions (e.g., including sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa)) in

accordance with the present disclosure can be administered to patients in doses ranging from about 0.001 mg/kg/day to about 500 mg/kg/day, about 0.01 mg/kg/day to about 100 mg/kg/day, or about 0.01 mg/kg/day to about 20 mg/kg/day. For example, the sALP compositions (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be administered to patients in a weekly dosage ranging, e.g., from about 0.5 mg/kg/week to about 140 mg/kg/week, e.g., about 0.8 mg/kg/week to about 50 mg/kg/week, or about 1 mg/kg/week to about 10 mg/kg/week (e.g., about 6 or about 9 mg/kg/week). In particular, the sALP (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be administered at a dosage of 2 mg/kg three times a week (total dose 6 mg/kg/week), 1 mg/kg six times a week (total dose 6 mg/kg/week), 3 mg/kg three times a week (total dose 9 mg/kg/week), 0.5 mg/kg three times a week (total dose of 1.5 mg/kg/week), or 9.3 mg/kg three times a week (total dose 28 mg/kg/week). The dosage will be adapted by the clinician in accordance with conventional factors such as the extent of the disease and different parameters from the HPP patient, such as an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age).

Dosages of compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be provided in either a single or multiple dosage regimens. Doses can be administered, e.g., hourly, bihourly, daily, bidaily, twice a week, three times a week, four times a week, five times a week, six times a week, weekly, biweekly, monthly, bimonthly, or yearly. Alternatively, doses can be administered, e.g., twice, three times, four times, five times, six times, seven times, eight times, nine times, 10 times, 11 times, or 12 times per day. In particular, the dosing regimen is once weekly. The duration of the dosing regimen can be, e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 day(s), week(s), or month(s), or even for the remaining lifespan of the HPP patient, such as an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age). The amount, frequency, and duration of dosage will be adapted by the clinician in accordance with conventional factors such as the extent of the disease and different parameters from the HPP patient, such as an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age).

For example, a sALP or sALP fusion polypeptide (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated as a solution for injection, which is a clear, colorless to slightly yellow, aqueous solution, pH 7.4. The sALP or sALP polypeptide (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) may be formulated at a concentration of 12mg/0.3mL,18mg/0.45mL, 28mg/0.7mL, 40mg/1ml, or 80mg/0.8mL. In particular, the composition can be formulated as a 40 mg/ml solution for injection, in which each ml of solution contains

40 mg of sALP or sALP polypeptide (e.g., each vial contains 0.3 ml solution and 12 mg of sALP (40 mg/ml), each vial contains 0.45 ml solution and 18 mg of sALP (40 mg/ml), each vial contains 0.7 ml solution and 28 mg of sALP(40 mg/ml), or each vial contains 1.0 ml solution and 40 mg of asfotase alfa (40 mg/ml)). A sALP or sALP polypeptide (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated as a solution for injection at a concentration of 100 mg/ml, in which each 1 ml of solution contains 100 mg of sALP or sALP polypeptide (e.g., each vial contains 0.8 ml solution and 80 mg of asfotase alfa (100 mg/ml)).

For example, the recommended dosage of a sALP or sALP fusion polypeptide ((such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) is 2 mg/kg of body weight administered subcutaneously three times per week, or a dosage regimen of 1 mg/kg of body weight administered subcutaneously six times per week. Additional dosage information is provided below (Table 1).

Table 1. DOSING OF ASFOTASE ALFA

Body Weight (kg)	If injecting 3x per week			If injecting 6 x per week		
	Dose to be injected	Volume to be injected	Vial type used for injection	Dose to be injected	Volume to be injected	Vial type used for injection
3	6 mg	0.15 ml	0.3 ml			
4	8 mg	0.20 ml	0.3 ml			
5	10 mg	0.25 ml	0.3 ml			
6	12 mg	0.30 ml	0.3 ml	6 mg	0.15 ml	0.3 ml
7	14 mg	0.35 ml	0.45 ml	7 mg	0.18 ml	0.3 ml
8	16 mg	0.40 ml	0.45 ml	8 mg	0.20 ml	0.3 ml
9	18 mg	0.45 ml	0.45 ml	9 mg	0.23 ml	0.3 ml
10	20 mg	0.50 ml	0.7 ml	10 mg	0.25 ml	0.3 ml
11	22 mg	0.55 ml	0.7 ml	11 mg	0.28 ml	0.3 ml
12	24 mg	0.60 ml	0.7 ml	12 mg	0.30 ml	0.3 ml
13	26 mg	0.65 ml	0.7 ml	13 mg	0.33 ml	0.45 ml
14	28 mg	0.70 ml	0.7 ml	14 mg	0.35 ml	0.45 ml
15	30 mg	0.75 ml	1 ml	15 mg	0.38 ml	0.45 ml
16	32 mg	0.80 ml	1 ml	16 mg	0.40 ml	0.45 ml
17	34 mg	0.85 ml	1 ml	17 mg	0.43 ml	0.45 ml
18	36 mg	0.90 ml	1 ml	18 mg	0.45 ml	0.45 ml
19	38 mg	0.95 ml	1 ml	19 mg	0.48 ml	0.7 ml
20	40 mg	1.00 ml	1 ml	20 mg	0.50 ml	0.7 ml
25	50 mg	0.50 ml	0.8 ml	25 mg	0.63 ml	0.7 ml
30	60 mg	0.60 ml	0.8 ml	30 mg	0.75 ml	1 ml
35	70 mg	0.70 ml	0.8 ml	35 mg	0.88 ml	1 ml
40	80 mg	0.80 ml	0.8 ml	40 mg	1.00 ml	1 ml
50				50 mg	0.50 ml	0.8 ml
60				60 mg	0.60 ml	0.8 ml
70				70 mg	0.70 ml	0.8 ml
80				80 mg	0.80 ml	0.8 ml
90				90 mg	0.90 ml	0.8 ml (x2)
100				100 mg	1.00 ml	0.8 ml (x2)

Formulations

The compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated according to standard methods. Pharmaceutical formulation is a well-established art, and is further described in, e.g., Gennaro (2000) *Remington: The Science and Practice of Pharmacy*, 20th Edition, Lippincott, Williams & Wilkins (ISBN: 0683306472); Ansel et al. (1999) *Pharmaceutical Dosage Forms and Drug Delivery Systems*, 7th Edition, Lippincott Williams & Wilkins Publishers (ISBN: 0683305727); and Kibbe (2000) *Handbook of Pharmaceutical Excipients American Pharmaceutical Association*, 3rd Edition (ISBN: 091733096X). For instance, a sALP composition (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a

polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated, for example, as a buffered solution at a suitable concentration and suitable for storage at 2-8℃ (e.g., 4℃). A composition can also be formulated for storage at a temperature below 0℃ (e.g., -20℃ or -80℃). A composition can further be formulated for storage for up to 2 years (e.g., one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, 10 months, 11 months, 1 year, 1½ years, or 2 years) at 2-8℃ (e.g., 4℃). Thus, the compositions described herein can be stable in storage for at least 1 year at 2-8℃ (e.g., 4℃).

The compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be in a variety of forms. These forms include, e.g., liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends, in part, on the intended mode of administration and therapeutic application.

For example, compositions intended for systemic or local delivery can be in the form of injectable or infusible solutions. Accordingly, the compositions (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated for administration by a parenteral mode (e.g., subcutaneous, intravenous, intraperitoneal, or intramuscular injection).

The compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP fusion polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable for stable storage at high concentration. Sterile injectable solutions can be prepared by incorporating a composition described herein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating a composition described herein into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods for preparation include vacuum drying and freeze-drying that yield a powder of a composition described herein plus any additional desired ingredient (see below) from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition a reagent that delays absorption, for example, monostearate salts, and gelatin.

The compositions described herein can also be formulated in immunoliposome compositions. Such formulations can be prepared by methods known in the art such as, e.g., the methods described in Epstein et al. (1985) *Proc Natl Acad Sci USA* 82:3688; Hwang et al. (1980) *Proc Natl Acad Sci USA* 77:4030; and U.S. Patent Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in, e.g., U.S. Patent No. 5,013,556.

Compositions including sALPs and sALP fusion polypeptides (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can also be formulated with a carrier that will protect the composition (e.g., a sALP polypeptide or sALP fusion polypeptide) against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are known in the art. See, e.g., J.R. Robinson (1978) *Sustained and Controlled Release Drug Delivery Systems*, Marcel Dekker, Inc., New York.

When compositions are to be used in combination with a second active agent, the compositions can be co-formulated with the second agent, or the compositions can be formulated separately from the second agent formulation. For example, the respective pharmaceutical compositions can be mixed, e.g., just prior to administration, and administered together or can be administered separately, e.g., at the same or different times.

Carriers/vehicles

Preparations containing a sALP or sALP fusion polypeptide (such as TNALP, for example the sALP polypeptide of SEQ ID NO: 1 or a polypeptide variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, e.g., asfotase alfa) can be provided to HPP patients, such as an adolescent having HPP (e.g., an adolescent having HPP of about 12 to about 18 years of age) or an adult having HPP (e.g., an adult having HPP older than about 18 years of age), in combination with pharmaceutically acceptable sterile aqueous or non-aqueous solvents, suspensions or emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil, fish oil, and injectable organic esters. Aqueous carriers include water, water-alcohol solutions, emulsions or suspensions, including saline and buffered medical parenteral vehicles including sodium chloride solution, Ringer's dextrose solution, dextrose plus sodium chloride solution, Ringer's solution containing lactose, or fixed oils. For example, the pharmaceutically acceptable carrier can include sodium chloride and/or sodium phosphate, in which the composition includes, e.g., about 150 mM sodium chloride and/or about 25 mM sodium phosphate, pH 7.4.

Intravenous vehicles can include fluid and nutrient replenishers, electrolyte replenishers, such as those based upon Ringer's dextrose, and the like. Pharmaceutically acceptable salts can be included therein, for example, mineral acid salts such as hydrochlorides, hydrobromides, phosphates, sulfates, and the like; and the salts of organic acids such as acetates, propionates, malonates, benzoates, and the like. Additionally, auxiliary substances, such as wetting or emulsifying agents, pH buffering substances, and the like, can be present in such vehicles. A thorough discussion of pharmaceutically acceptable carriers is available in *Remington's Pharmaceutical Sciences* (Mack Pub. Co., N.J. 1991).

The following examples are intended to illustrate, rather than limit, the disclosure. These studies feature the administration of asfotase alfa (SEQ ID NO: 1) to adolescents of about 12 to about 18 years of age and adults older than about 18 years of age to treat HPP, its symptoms, and physical impairments associated therewith for an extended period of time.

Example 1. Study Design

Adolescents and adults with hypophosphatasia (HPP) of about 12 years to about 66 years of age participated in an initial phase study to determine the efficacy, safety, and tolerability of treatment with a soluble alkaline phosphatase (sALP) (asfotase alfa; SEQ ID NO: 1) for 6 months (Fig. 1). During the initial phase study, 13 patients with HPP (3 adolescent and 10 adults) received treatment with asfotase alfa (the treatment group) and 6 patients with HPP (3 adolescents and 3 adults) did not receive treatment with asfotase alfa (the control group). The age of onset of HPP symptoms in the treatment group was less than 18 years of age for 10 patients, and was greater than or equal to about 18 years of age for 2 patients (Table 2). The age of onset of HPP symptoms was unknown for 1 patient. The age of onset of HPP symptoms in the control group was less than 18 years of age for 6 patients.

Table 2. Baseline characteristics for HPP patients prior to treatment with asfotase alfa. Dosage treatment groups (2.1 mg/kg/week and 3.5 mg/kg/week) were combined for all results.

Characteristic	Initial group assignment		Overall (N=19)
	Control (n=6)	Combined treatment (n=13)	
Age at enrollment, years	21 (13, 58)	55 (14, 66)	**53 (13, 66)**
Age group, n (%) Adolescent (age 12–18 years) Adult (age ≥18 years)	 3 (50) 3 (50)	 3 (23) 10 (77)	 **6 (32)** **13 (68)**
Age at symptom onset, years	1.5 (0.2, 4)	2.0 (0, 36)	**2.0 (0, 36)**
Age at symptom onset, n <18 years of age ≥18 years of age Unknown	 6 (100) 0 (0) 0 (0)	 10 2 1	 **16 (84)** **2 (11)** **1 (1)**
Female, n (%)	2 (33)	10 (77)	**12 (63)**
White, n (%)	5 (83)	13 (100)	**18 (95)**

HPP patients selected for treatment with asfotase alfa had serum ALP below the age-adjusted normal range, plasma pyridoxal 5'-phosphate (PLP) concentrations of at least twice the upper normal limit, and evidence of osteopenia or osteomalacia in skeletal radiographs and/or bone biopsies. Patients were excluded from the study for serum calcium or phosphate levels below the normal range, serum vitamin D levels less than 20 ng/mL, serum creatinine or parathyroid hormone levels above the upper limit of normal, or a medical condition or other extenuating circumstances that could significantly interfere with patient compliance with the study protocol. HPP patient characteristics prior to treatment with asfotase alfa included fractures, bone pain, muscle complaints, joint complaints, unusual gait, and requiring assistive devices for mobility or ambulation (Table 3).

Table 3: Characteristics of HPP patients prior to treatment with asfotase alfa. Dosage treatment groups (2.1 mg/kg/week and 3.5 mg/kg/week) were combined for all results.

Characteristic	Initial group assignment		Overall (N=19)
	Control (n=6)	Combined treatment (n=13)	
Fractures	6 (100)	12 (92)	**18 (95)**
Fracture fixation			
Steel, titanium rods	4 (67)	7 (54)	**11 (58)**
Plates, screws	3 (50)	3 (23)	**6 (32)**
Bone pain severe enough to limit activity	5 (83)	13 (100)	**18 (95)**
Muscle complaints			
Weakness	5 (83)	12 (92)	**17 (90)**
Pain	4 (67)	10 (77)	**14 (74)**
Joint complaints			
Pain	5 (83)	12 (92)	**17 (90)**
Swelling	2 (33)	5 (39)	**7 (37)**
Unusual gait	4 (67)	11 (85)	**15 (79)**
Assistive devices for ambulation	2 (33)	3 (23)	**5 (26)**

Prior to treatment with asfotase alfa, fractures occurred in 12 patients in the treatment group and in 6 patients in the control group. Fracture fixation was accomplished with steel, titanium rods, and/or plates with screws. Muscle complaints included weakness (5 control patients and 12 patients treated with asfotase alfa) in addition to pain (4 control patients and 10 patients treated with asfotase alfa). Unusual gait was exhibited by 4 patients in the control group and 11 patients in the treatment group. The use of assistive devices for mobility or ambulation was required for 2 patients in the control group and 3 patients in the treatment group, ranging from wheelchairs, wheeled walkers, canes, or orthotics. Additionally, 3 patients in the control group were unable to walk the full 6 minutes at baseline.

During the initial phase of treatment with asfotase alfa for 6 months, 7 patients received asfotase alfa at a dosage of 0.3. mg/kg/day (2.1 mg/kg/week) and 6 patients received asfotase alfa at a dosage of 0.5 mg/kg/day (3.5 mg/kg/week) via subcutaneous administration. The 6 patients in the initial control group did not receive asfotase alfa treatment. Following the initial phase study, both treatment and control group patients (19 total patients) received treatment with asfotase alfa. Asfotase alfa was administered via subcutaneous administration at an initial dose of 0.5 mg/kg/day (3.5 mg/kg/week), which was increased after 1 year to 1 mg/kg/day administered via subcutaneous administration once daily over 6 days (6 mg/kg/week). The dosage of asfotase alfa was maintained at 6 mg/kg/week for the remainder of the extension phase. During the extension phase, 3 patients discontinued treatment with asfotase alfa due to injection site reactions (2 patients) and non-compliance with the treatment protocol (1 patient).

Metrics used during the extension phase to assess treatment of HPP with asfotase alfa included: 1) changes in inorganic pyrophosphate (PPi) and pyridoxal 5'-phosphate (PLP) concentrations in patient

plasma samples to assess ALP activity; 2) Six Minute Walk Test (6MWT) values to assess walking ability; and 3) Bruininks-Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2) scores to assess physical function.

Example 2. Plasma inorganic pyrophosphate (PPi) and pyridoxal 5'-phosphate (PLP) concentrations

ALP activity in plasma samples of the HPP patients was assessed by quantifying the concentrations of the ALP substrates PPi and PLP, as described in Whyte et al., 1995 (*J. Clin. Invest.* 95(4): 1440–1445), hereby incorporated by reference in its entirety. PPi and PLP concentrations in plasma samples from the control group and group treated with asfotase alfa were elevated at baseline from the normal range (Table 4). Similarly, ALP activity values were relatively low (23.5 U/L for the control group and 18.0 U/L for the treatment group) compared to age- and gender-adjusted ALP activity values (40 U/L to 160 U/L).

Table 4: Measurements of alkaline phosphatase (ALP) activity and plasma inorganic pyrophosphate (PPi) and pyridoxal 5'-phosphate (PLP) concentrations prior to treatment with asfotase alfa.

Characteristic	Initial group assignment		Overall (N=19)
	Control (n=6)	Combined treatment (n=13)	
ALP, U/L LLN (age- and gender-adjusted) 12–13 y: 160 (M), 110 (F) 14–15 y: 130 (M), 55 (F) 16–19 y: 60 (M), 40 (F) ≥ 20 y: 40 (M, F)	23.5 (18, 45)	18.0 (18, 35)	18.0 (18, 45)
PPi, µM Normal range 13–18y: <0.75–4.78 >18y: 1.00–5.82	6.2 (4.2, 12.1)	5.1 (2.2, 8.2)	5.2 (2.2, 12.1)
PLP, ng/mL Normal range: 5–18y: 5.7–61.2 >18y: 2.8–26.7	237.0 (106.0, 906.0)	267.0 (28.8, 1590.0)	267.0 (28.8, 1590.0)

For all subsequent measurements, plasma PPi and PLP concentrations were combined for the treatment group and control group at each respective time point (6 month, 1 year, 2 years, and 3 years of asfotase alfa treatment), since the control group received treatment with asfotase alfa after the initial phase of 6 months. Due to the 6 month offset in treatment with asfotase alfa of the control group, plasma PPi and PLP are not available for the control group patients at 4 years. Measurements of plasma PPi and PLP concentrations were combined for of all patients (treatment and control groups) at baseline (Fig. 2A). The median plasma PPi concentration was 5.26 µM (minimum plasma PPi concentration of 2.15 µM; maximum plasma PPi concentration of 8.2 µM), while the median plasma PLP concentration was 267.0 ng/ml (minimum plasma PLP concentration of 28.8 ng/ml; a maximum plasma PLP concentration of 1590.0 ng/ml).

Plasma PPi concentrations decreased after 6 months of treatment with asfotase alfa, as indicated by a median change from baseline of -2.19 for patients treated with asfotase alfa compared to a change of -0.18 for the control group (Fig. 2B). Likewise, plasma PLP concentrations decreased after 6 months of treatment with asfotase alfa, as indicated by a median change from baseline of -254.5 for patients treated with asfotase alfa compared to a change of -11 for the control group (Fig. 2B).

The decrease in plasma PPi concentrations was sustained throughout the extension phase of treatment with asfotase alfa (Fig. 2C). The median plasma PPi concentration decreased to 3.39 µM after 6 months of treatment with asfotase alfa (minimum plasma PPi concentration of 0.75 µM; maximum plasma PPi concentration of 6.00 µM). The median PPi concentration was 3.94 µM at 1 year (minimum plasma PPi concentration of 1.62 µM; maximum plasma PPi concentration of 6.82 µM); the median PPi concentration was 2.63 µM at 2 years (minimum plasma PPi concentration of 0.85 µM; maximum plasma PPi concentration of 10.90 µM); the median PPi concentration was 2.03 µM at 3 years (minimum plasma PPi concentration of 1.20 µM; maximum plasma PPi concentration of 4.58 µM); and the median PPi concentration was 2.56 µM at 4 years (minimum plasma PPi concentration of 1.09 µM; maximum plasma PPi concentration of 3.47 µM).

Likewise, the decrease in plasma PLP concentrations was sustained throughout the extension phase of treatment with asfotase alfa (Fig. 2D). The median plasma PLP concentration was 39.9 ng/ml after 6 months of treatment with asfotase alfa (minimum plasma PLP concentration of 2.5 ng/ml; maximum plasma PLP concentration of 419.0 ng/ml). The median PLP concentration was 96.1 ng/ml at 1 year (minimum plasma PLP concentration of 6.3 ng/ml; maximum plasma concentration of 367.0 ng/ml); the median PLP concentration was 21.9 ng/ml at 2 years (minimum plasma PLP concentration of 2.7 ng/ml; maximum plasma PLP concentration of 192.0 ng/ml); the median PLP concentration was 14.1 ng/ml at 3 years (minimum plasma PLP concentration of 2.5 ng/ml; maximum plasma PLP concentration of 96.7 ng/ml); and the median PLP concentration was 12.5 ng/ml at 4 years (minimum plasma PLP concentration of 3.9 ng/ml; maximum plasma PLP concentration of 38.4 ng/ml).

Example 3. Six Minute Walk Test (6MWT) Values

Physical function and walking ability of the HPP patients was assessed with the Six Minute Walk Test (6MWT). Patients improved from a walking distance of 355 meters in 6 minutes at baseline (minimum distance of 10, maximum distance of 620; n=19) to a walking distance of 450 meters in 6 minutes after 6 months of treatment with asfotase alfa (minimum distance of 193, maximum distance of 640; n=19). The median change from baseline after 6 months of treatment with asfotase alfa was a distance of 35.0 meters walked in 6 minutes, while the median change from baseline in the control group was -6.5 meters walked in 6 minutes (Fig. 3A). This change includes the 3 patients in the control group that were unable to walk the full 6 minutes at baseline. Thus, HPP patients after 6 months of treatment with asfotase exhibited an improvement in walking ability, while the walking ability of the control group patients worsened.

For 6MWT values after baseline, 6MWT values were combined for the treatment group and control group at each respective time point (6 month, 1 year, 2 years, and 3 years of asfotase alfa treatment), since the control group received treatment with asfotase alfa after the initial phase of 6

months. Due to the 6 month offset in treatment with asfotase alfa of the control group, 6MWT values are not available for the control group patients at 4 years.

The change in the 6MWT represented an increase from 76% of the predicted 6MWT value (minimum predicted 6MWT value of 42%, predicted 6MWT maximum value 101; n=15) at baseline to 85% of the predicted 6MWT value (minimum predicted 6MWT value of 29%, maximum predicted 6MWT value of 109; n=16) after 6 months of treatment with asfotase alfa (Fig. 3B). The increase in the 6MWT value was sustained throughout treatment with asfotase alfa at 81.9% of the predicted 6MWT value (minimum predicted 6MWT value of 23.9%; maximum predicted 6MWT value of 121.3%) after 1 year; 90.3% of the predicted 6MWT value (minimum predicted 6MWT value of 63.3%; maximum predicted 6MWT value of 120.8%) after 2 years; 91.8% of the predicted 6MWT value (minimum predicted 6MWT value of 64.7%; maximum predicted 6MWT value of 121.6%) after 3 years; and 84.4% of the predicted 6MWT value (minimum predicted 6MWT value of 59.0%; maximum predicted 6MWT value of 96.7%) after 4 years. In summary, the 6MWT value of the patients having HPP treated with asfotase alfa improved from 76% of the predicted 6MWT value at baseline to 85% of the predicted 6MWT value, which is within the normal range of healthy subjects of the same age and height, by 6 months. The improvement in the 6MWT value was sustained throughout 4 years of treatment with asfotase alfa.

Additionally, all 5 patients requiring the use of assistive devices to walk prior to treatment with asfotase alfa exhibited an improvement after 96 weeks of treatment with asfotase alfa. In particular, 1 patient progressed from wheelchair to crutches, 1 patient progressed from walker to cane, 1 patient progressed from wheeled walker to independent ambulation, and 2 patients progressed from cane to independent ambulation.

Example 4. Bruininks-Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2) Scores

Physical function and impairments of the HPP patients were assessed with the strength test of the Bruininks-Oseretsky Test of Motor Proficiency, 2nd Edition (BOT-2). BOT-2 tests to assess running speed and agility of the HPP patients included the 50 foot shuttle run, sideways steps over balance beam, and one and two legged side hops (Fig. 4A). BOT-2 tests to assess strength of the HPP patients included sit-ups, v-ups, standing long jump, wall sit, and push-ups (Fig. 4B). BOT-2 speed and agility total scores and BOT-2 strength total scores were then determined from the sum of points awarded per BOT-2 tests for each time interval (baseline, 6 months, 1 year, 2 year, 3 years, and 4 years).

For BOT-2 scores after baseline, BOT-2 scores were combined for the treatment group and control group at each respective time point (6 month, 1 year, 2 years, and 3 years of asfotase alfa treatment), since the control group received treatment with asfotase alfa after the initial phase of 6 months. Due to the 6 month offset in treatment with asfotase alfa of the control group, BOT-2 scores are not available for the control group patients at 4 years.

BOT-2 running speed and agility total scores improved after 6 months of treatment with asfotase alfa from a median BOT-2 running speed and agility total score of 7 (minimum BOT-2 score of 0; maximum BOT-2 score of 39; 16 patients) at baseline to 12 (minimum BOT-2 score of 5; maximum BOT-2 score of 40; 13 patients) at 6 months. The improvement in the median BOT-2 running speed and agility total score was sustained throughout the extension phase of treatment with asfotase alfa, with a median

BOT-2 running speed and agility total score of 9 (minimum BOT-2 score of 0; maximum score of 40; 15 patients) at 1 year; a median BOT-2 running speed and agility total score of 11 (minimum BOT-2 score of 3; maximum score of 41; 17 patients) at 2 years; a median BOT-2 running speed and agility total score of 10 (minimum BOT-2 score of 4; maximum score of 42; 13 patients) at 3 years; and a median BOT-2 running speed and agility total score of 9 (minimum BOT-2 score of 4; maximum score of 43; 8 patients) at 4 years. BOT-2 running speed and agility total scores exhibited an improvement from baseline after 2 years of treatment with asfotase alfa, with a median running speed and agility total score of 13.5 (minimum BOT-2 score of 0; maximum score of 33; 18 patients) at baseline to 18 (minimum BOT-2 score of 5; maximum score of 40; 17 patients) at 2 years.

Example 5. Tolerability to long-term treatment with asfotase alfa

Generally, treatment with asfotase alfa was well-tolerated in adolescents and adults having HPP, with most adverse events (AEs) consisting of injection-site reactions (ISRs). A total of 359 injection site reactions occurred in 18 of the 19 patients (92% of total patients; Table 5).

Table 5: Occurrence of injection site reactions in patients treated with asfotase alfa for 4 years.

Injection site reaction	Events, n	Patients, n (%) N=19
Erythema	87	11 (58%)
Discoloration	59	7 (37%)
Hematoma	20	7 (37%)
Pain	23	6 (32%)
Atrophy	8	5 (26%)
Pruritus	20	5 (26%)
Reaction	34	4 (21%)
Swelling	12	4 (21%)
Induration	4	2 (11%)

In particular, ISRs included 87 instances of erythema (58% of total patients); 59 instances of discoloration (37% of total patients); 20 instances of hematoma (37% of total patients); 23 instances of pain (32% of total patients); 8 instances of atrophy (26% of total patients); 20 instances of pruritus (26% of total patients); 34 instances of reactions (21% of total patients); 12 instances of swelling (21% of total patients); and 4 instances of induration (11% of total patients).

OTHER EMBODIMENTS

All publications, patents, and patent applications mentioned in the above specification are hereby incorporated by reference to the same extent as if each individual publication, patent or patent application was specifically and individually indicated to be incorporated by reference in its entirety. Various modifications and variations of the described methods, pharmaceutical compositions, and kits of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the claimed invention. Although the disclosure has been described in connection with specific embodiments, it will be understood that it is capable of further modifications and that the invention as claimed should not be unduly limited to such specific embodiments.

CLAIMS

1. A method of treating hypophosphatasia (HPP) in a patient of about 12 years of age or older than about 12 years of age, wherein the method comprises administering a soluble alkaline phosphatase (sALP) to the patient at a dosage providing about 6 mg/kg/week of the sALP, wherein the sALP comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, and wherein administration of the sALP results in one or more of the following:

(i) an improvement in walking ability that is sustained during a treatment period of at least one year of the patient relative to walking ability of a subject selected from the group consisting of a healthy subject and an untreated subject having HPP;

(ii) an average decrease in inorganic pyrophosphate (PPi) concentrations in a plasma sample that is sustained during a treatment period of at least one year from the patient relative to PPi concentrations in a plasma sample from an untreated subject having HPP; and/or

(iii) an average decrease in pyridoxal 5'-phosphate (PLP) concentrations in a plasma sample that is sustained during a treatment period of at least one year from the patient relative to PLP concentrations in a plasma sample from an untreated subject having HPP.

2. The method of claim 1, wherein administration of the sALP results in an average increase in a Six Minute Walk Test (6MWT) value of the patient relative to the 6MWT value of the patient prior to administration of the sALP.

3. The method of claim 2, wherein the average increase in the 6MWT value is to about 80% or greater than about 80% of the predicted 6MWT value of the patient, wherein the 6MWT value of the patient was less than about 80% of the predicted 6MWT value of the patient prior to administration of the sALP.

4. The method of any one of claims 1 to 3, wherein the average increase in the 6MWT value of the patient is sustained during a treatment period of at least two years, at least three years, at least four years, or longer.

5. The method of any one of claims 1 to 4, wherein the patient exhibits decreased reliance on an assistive device for mobility after administration of the sALP.

6. The method of claim 5, wherein the assistive device for mobility is selected from the group consisting of a wheelchair, braces, crutches, and orthotics.

7. The method of any one of claims 1 to 6, wherein the average decrease in PPi concentrations in a plasma sample from the patient is about 25% or greater than about 25% relative to PPi concentrations in a plasma sample from the patient prior to administration of the sALP.

8. The method of any one of claims 1 to 7, wherein the average decrease in PPi concentrations in a plasma sample from the patient is determined relative to PPi concentrations in a plasma sample from an untreated subject having HPP.

9. The method of any one of claims 1 to 8, wherein the average decrease in PPi concentrations in a plasma sample from the patient is sustained during a treatment period of at least two years, at least three years, at least four years, or longer.

10. The method of any one of claims 1 to 9, wherein the average decrease in PLP concentrations in a plasma sample from the patient is about 50% or greater than about 50% relative to PLP concentrations in a plasma sample from the patient prior to administration of the sALP.

11. The method of any one of claims 1 to 10, wherein the average decrease in PLP concentrations in a plasma sample from the patient is determined relative to PLP concentrations in a plasma sample from an untreated subject having HPP.

12. The method of any one of claims 1 to 11, wherein the average decrease in PLP concentrations in a plasma sample from the patient is sustained during a treatment period of at least two years, at least three years, at least four years, or longer.

13. A method of treating HPP in a patient of about 12 years of age or older than about 12 years of age having a median total Bruininks-Oseretsky Test of Motor Proficiency 2nd Edition (BOT-2) running speed and agility score of less than about 7, wherein the method comprises administering a sALP to the patient at a dosage providing about 6 mg/kg/week of the sALP, wherein the sALP comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, and wherein administration of the sALP results in an average increase in the BOT-2 running speed and agility medial total score to about 9 or greater than about 9 that is sustained during a treatment period of at least one year.

14. The method of claim 13, wherein the median total BOT-2 running speed and agility score of the patient is determined relative to a BOT-2 running speed and agility medial total score of an untreated subject having HPP.

15. The method of claim 13 or 14, wherein the average increase in the BOT-2 running speed and agility medial total score is sustained during a treatment period of at least two years, at least three years, at least four years, or longer.

16. The method of any one of claims 13 to 15, wherein the medial total BOT-2 running speed and agility score of the patient is determined from measurements selected from the group consisting of stepping over a balance beam, shuttle run, two-legged side hop, and one-legged side hop.

17. A method of treating HPP in a patient of about 12 years of age or greater than about 12 years of age having a medial total BOT-2 strength score of less than about 14, wherein the method comprises administering a sALP to the patient at a dosage providing about 6 mg/kg/week of the sALP, wherein the sALP comprises an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, and wherein administration of the sALP for at least two years results in an average increase in the BOT-2 strength score to about 18 or greater than about 18.

18. The method of claim 17, wherein the medial total BOT-2 strength score of the patient is determined relative to a BOT-2 strength medial total score of an untreated subject having HPP.

19. The method of claim 17 or 18, wherein the medial total BOT-2 strength score of the patient is determined relative to a BOT-2 strength medial total score of a healthy subject.

20. The method of any one of claims 17 to 19, wherein the medial total BOT-2 strength score of the patient is determined from measurements selected from the group consisting of sit-ups, V-ups, standing long jump, wall sit, and push-ups.

21. The method of any one of claims 1 to 20, wherein the patient is about 12 years of age.

22. The method of any one of claims 1 to 20, wherein the patient is older than about 12 years of age.

23. The method of any one of claims 1 to 20, wherein the patient is about 18 years of age.

24. The method of any one of claims 1 to 20, wherein the patient is older than about 18 years of age.

25. The method of any one of claims 1 to 24, wherein the patient has not been previously administered the sALP.

26. The method of any one of claims 1 to 25, wherein the sALP is formulated for daily or weekly administration.

27. The method of any one of claims 1 to 26, wherein the sALP is formulated for administration twice a week, three times a week, four times a week, five times a week, six times a week, or seven times a week.

28. The method of any one of claims 1 to 27, wherein the sALP is formulated at a dosage of 2 mg/kg for administration three times a week.

29. The method of any one of claims 1 to 27, wherein the sALP is formulated at a dosage of 3 mg/kg for administration three times a week.

30. The method of any one of claims 1 to 27, wherein the sALP is formulated at a dosage of 1 mg/kg for administration six times a week.

31. The method of any one of claims 27 to 30, wherein the sALP is formulated for administration once daily.

32. The method of any one of claims 27 to 30, wherein the sALP is formulated for administration on consecutive or alternating days.

33. The method of any one of claims 1 to 32, wherein the sALP is administered for a treatment period of at least four, least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer.

34. The method of any one of claims 1 to 33, wherein the sALP comprises the amino acid sequence of SEQ ID NO: 1.

35. The method of any one of claims 1 to 34, wherein the sALP consists of the amino acid sequence of SEQ ID NO: 1.

36. The method of any one of claims 1 to 35, wherein the sALP is administered in an amount that is therapeutically effective to treat at least one symptom of HPP.

37. The method of claim 36, wherein the at least one symptom of HPP comprises one or more of rickets, premature loss of deciduous teeth, incomplete bone mineralization, elevated blood and/or urine levels of phosphoethanolamine (PEA), hypomineralization, rachitic ribs, hypercalciuria, short stature, skeletal deformity, waddling gait, bone pain, bone fracture, HPP-related seizure, inadequate weight gain, and calcium pyrophosphate dihydrate crystal deposition.

38. The method of any one of claims 1 to 37, wherein the sALP is administered at an initial dosage of about 2.1 mg/kg/week or about 3.5 mg/kg/week and then is increased to a dosage of about 6 mg/kg/week or more.

39. The method of claim 38, wherein the dosage is increased to a dosage of about 6 mg/kg/week or more after administration of the sALP for at least six months, at least one year, at least two years, at least three years, at least four years, or longer.

40. The method of any one of claims 1 to 39, wherein symptoms of HPP presented at birth or did not present at birth.

41. The method of any one of claims 1 to 40, wherein symptoms of HPP presented at 12 years of age or older.

42. The method of any one of claims 1 to 41, wherein the patient exhibits tolerability to administration of the sALP.

43. The method of claim 42, wherein the tolerability comprises a lack of or decreased incidence of adverse events selected from the group consisting of injection site erythema, decrease in hemoglobin, pyrexia, pneumonia, upper respiratory tract infection, otitis media, vomiting, constipation, diarrhea, tooth loss, nasopharyngitis, rash, dental carries, and irritability.

44. The method of any one of claims 1 to 43, wherein the sALP is formulated in a pharmaceutical composition, with at least one pharmaceutically acceptable carrier.

45. The method of claim 44, wherein the at least one pharmaceutically acceptable carrier is saline.

46. The method of claim 44, wherein the at least one pharmaceutically acceptable carrier comprises sodium chloride and sodium phosphate.

47. The method of claim 46, wherein the at least one pharmaceutically acceptable carrier comprises 150 mM sodium chloride and 25 mM sodium phosphate.

48. The method of any one of claims 44 to 47, wherein the pharmaceutical composition is formulated for at least one of subcutaneous, intramuscular, intravenous, oral, nasal, sublingual, intrathecal, and intradermal administration.

49. The method of claim 48, wherein the pharmaceutical composition is formulated for subcutaneous administration.

50. The method of any one of claims 1 to 49, wherein the sALP is physiologically active toward PEA, PPi, and PLP.

51. The method of any one of claims 1 to 50, wherein the sALP is catalytically competent to improve skeletal mineralization in bone.

52. The method of any one of claims 1 to 51, wherein the sALP is the soluble extracellular domain of an alkaline phosphatase.

53. The method of claim 1, wherein the patient exhibits decreased reliance on an assistive device for mobility after administration of the sALP.

54. The method of claim 53, wherein the assistive device for mobility is selected from the group consisting of a wheelchair, braces, crutches, and orthotics.

55. The method of claim 1, wherein the average decrease in PPi concentrations in a plasma sample from the patient is about 25% or greater than about 25% relative to PPi concentrations in a plasma sample from the patient prior to administration of the sALP.

56. The method of claim 1, wherein the average decrease in PPi concentrations in a plasma sample from the patient is determined relative to PPi concentrations in a plasma sample from an untreated subject having HPP.

57. The method of claim 1, wherein the average decrease in PPi concentrations in a plasma sample from the patient is sustained during a treatment period of at least two years, at least three years, at least four years, or longer.

58. The method of claim 1, wherein the average decrease in PLP concentrations in a plasma sample from the patient is about 50% or greater than about 50% relative to PLP concentrations in a plasma sample from the patient prior to administration of the sALP.

59. The method of claim 1, wherein the average decrease in PLP concentrations in a plasma sample from the patient is determined relative to PLP concentrations in a plasma sample from an untreated subject having HPP.

60. The method of claim 1, wherein the average decrease in PLP concentrations in a plasma sample from the patient is sustained during a treatment period of at least two years, at least three years, at least four years, or longer.

61. The method of claim 13, wherein the medial total BOT-2 running speed and agility score of the patient is determined from measurements selected from the group consisting of stepping over a balance beam, shuttle run, two-legged side hop, and one-legged side hop.

62. The method of claim 17, wherein the medial total BOT-2 strength score of the patient is determined from measurements selected from the group consisting of sit-ups, V-ups, standing long jump, wall sit, and push-ups.

63. The method of claim 1, 13, or 17, wherein the patient is about 12 years of age.

64. The method of claim 1, 13, or 17, wherein the patient is older than about 12 years of age.

65. The method of claim 1, 13, or 17, wherein the patient is about 18 years of age.

66. The method of claim 1, 13, or 17, wherein the patient is older than about 18 years of age.

67. The method of claim 1, 13, or 17, wherein the patient has not been previously administered the sALP.

68. The method of claim 1, 13, or 17, wherein the sALP is formulated for daily or weekly administration.

69. The method of claim 1, 13, or 17, wherein the sALP is formulated for administration twice a week, three times a week, four times a week, five times a week, six times a week, or seven times a week.

70. The method of claim 1, 13, or 17, wherein the sALP is formulated at a dosage of 2 mg/kg for administration three times a week.

71. The method of claim 1, 13, or 17, wherein the sALP is formulated at a dosage of 3 mg/kg for administration three times a week.

72. The method of claim 1, 13, or 17, wherein the sALP is formulated at a dosage of 1 mg/kg for administration six times a week.

73. The method of claim 1, 13, or 17, wherein the sALP is formulated for administration once daily.

74. The method of claim 1, 13, or 17, wherein the sALP is formulated for administration on consecutive or alternating days.

75. The method of claim 1, 13, or 17, wherein the sALP is administered for a treatment period of at least four, least five years, at least six years, at least seven years, at least eight years, at least nine years, at least ten years, or longer.

76. The method of claim 1, 13, or 17, wherein the sALP comprises the amino acid sequence of SEQ ID NO: 1.

77. The method of claim 1, 13, or 17, wherein the sALP consists of the amino acid sequence of SEQ ID NO: 1.

78. The method of claim 1, 13, or 17, wherein the sALP is administered in an amount that is therapeutically effective to treat at least one symptom of HPP.

79. The method of claim 1, 13, or 17, wherein the at least one symptom of HPP comprises one or more of rickets, premature loss of deciduous teeth, incomplete bone mineralization, elevated blood and/or urine levels of phosphoethanolamine (PEA), hypomineralization, rachitic ribs, hypercalciuria, short stature, skeletal deformity, waddling gait, bone pain, bone fracture, HPP-related seizure, inadequate weight gain, and calcium pyrophosphate dihydrate crystal deposition.

80. The method of claim 1, 13, or 17, wherein the sALP is administered at an initial dosage of about 2.1 mg/kg/week or about 3.5 mg/kg/week and then is increased to a dosage of about 6 mg/kg/week or more.

81. The method of claim 80, wherein the dosage is increased to a dosage of about 6 mg/kg/week or more after administration of the sALP for at least six months, at least one year, at least two years, at least three years, at least four years, or longer.

82. The method of claim 1, 13, or 17, wherein symptoms of HPP presented at birth or did not present at birth.

83. The method of claim 1, 13, or 17, wherein symptoms of HPP presented at 12 years of age or older.

84. The method of claim 1, 13, or 17, wherein the patient exhibits tolerability to administration of the sALP.

85. The method of claim 84, wherein the tolerability comprises a lack of or decreased incidence of adverse events selected from the group consisting of injection site erythema, decrease in hemoglobin, pyrexia, pneumonia, upper respiratory tract infection, otitis media, vomiting, constipation, diarrhea, tooth loss, nasopharyngitis, rash, dental carries, and irritability.

86. The method of claim 1, 13, or 17, wherein the sALP is formulated in a pharmaceutical composition, with at least one pharmaceutically acceptable carrier.

87. The method of claim 86, wherein the at least one pharmaceutically acceptable carrier is saline.

88. The method of claim 86, wherein the at least one pharmaceutically acceptable carrier comprises sodium chloride and sodium phosphate.

89. The method of claim 88, wherein the at least one pharmaceutically acceptable carrier comprises 150 mM sodium chloride and 25 mM sodium phosphate.

90. The method of claim 1, 13, or 17, wherein the pharmaceutical composition is formulated for at least one of subcutaneous, intramuscular, intravenous, oral, nasal, sublingual, intrathecal, and intradermal administration.

91. The method of claim 90, wherein the pharmaceutical composition is formulated for subcutaneous administration.

92. The method of claim 1, 13, or 17, wherein the sALP is physiologically active toward PEA, PPi, and PLP.

93. The method of claim 1, 13, or 17, wherein the sALP is catalytically competent to improve skeletal mineralization in bone.

94. The method of claim 1, 13, or 17, wherein the sALP is the soluble extracellular domain of an alkaline phosphatase.

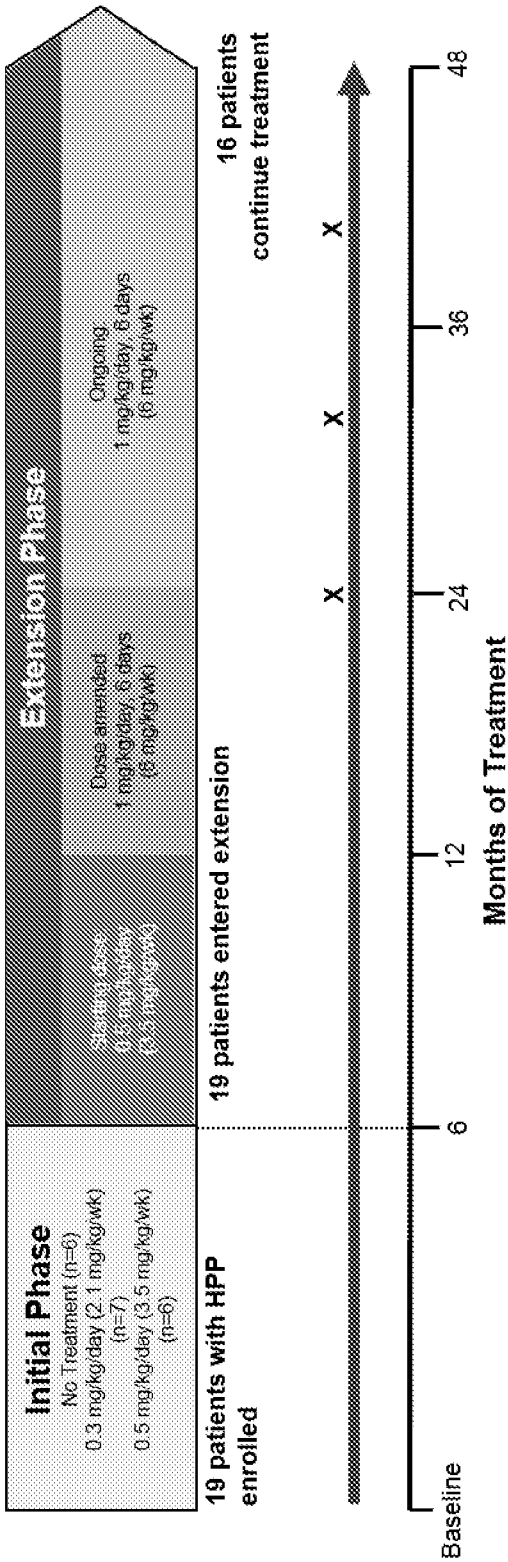
Initial Phase
No Treatment (n=6)
0.3 mg/kg/day (2.1 mg/kg/wk) (n=7)
0.5 mg/kg/day (3.5 mg/kg/wk) (n=6)
Extension Phase
Dose amended 1 mg/kg/day, 6 days (6 mg/kg/wk)
Ongoing 1 mg/kg/day, 6 days (6 mg/kg/wk)
19 patients with HPP enrolled
19 patients entered extension
16 patients continue treatment
X
X
X
Baseline
6
12
24
36
48
Months of Treatment

FIG. 1

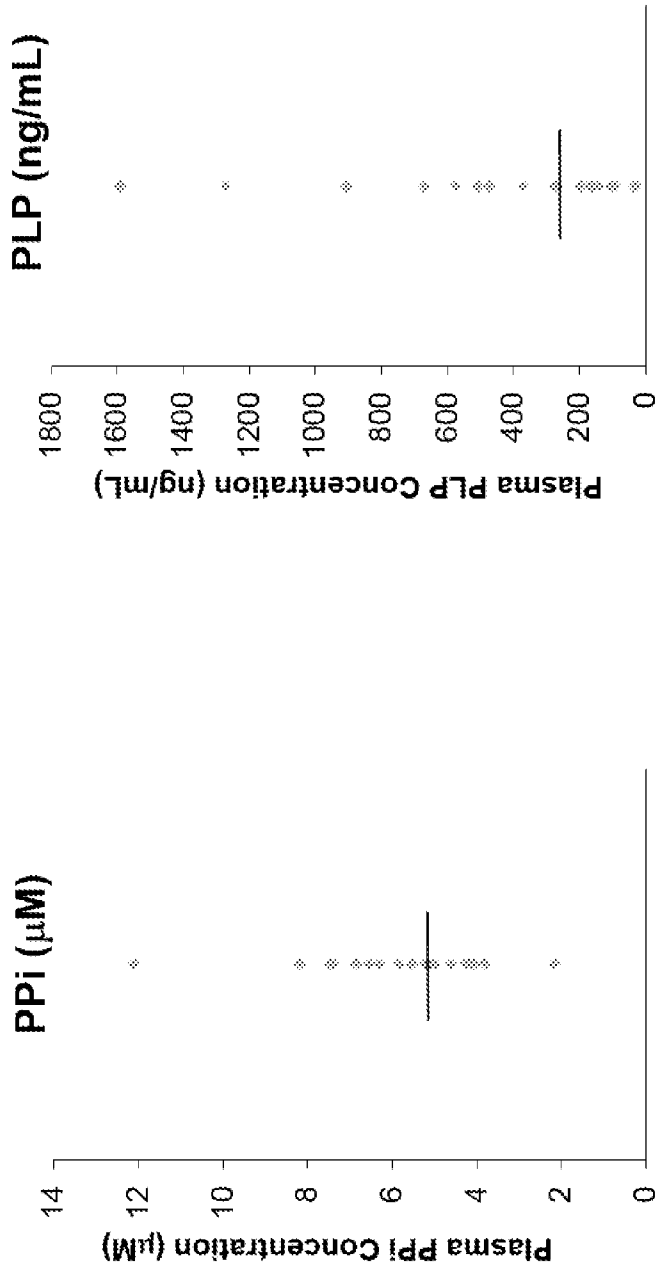
PLP (ng/mL)
Plasma PLP Concentration (ng/mL)
1800
1600
1400
1200
1000
800
600
400
200
0
PPi (μM)
Plasma PPi Concentration (μM)
14
12
10
8
6
4
2
0

FIG. 2A

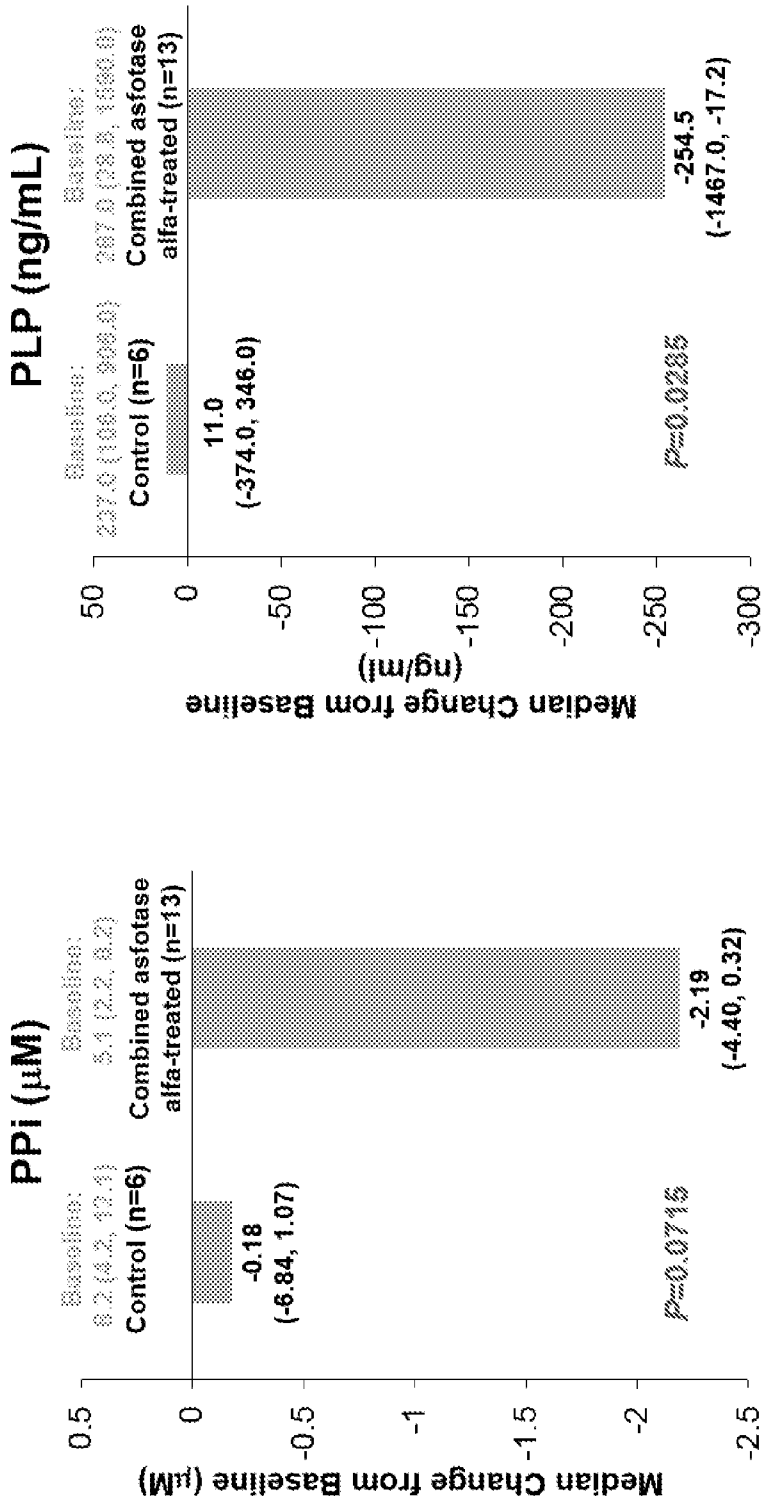
PLP (ng/mL)
Baseline:
237.0 (100.0, 906.0)
Control (n=6)
11.0
(-374.0, 346.0)
Baseline:
287.0 (29.0, 1560.0)
Combined asfotase alfa-treated (n=13)
-254.5
(-1467.0, -17.2)
P=0.0285
Median Change from Baseline (ng/ml)
50
0
-50
-100
-150
-200
-250
-300
PPi (µM)
Baseline:
8.2 (4.2, 12.1)
Control (n=6)
-0.18
(-6.84, 1.07)
Baseline:
5.1 (2.2, 8.2)
Combined asfotase alfa-treated (n=13)
-2.19
(-4.40, 0.32)
P=0.0715
Median Change from Baseline (µM)
0.5
0
-0.5
-1
-1.5
-2
-2.5

FIG. 2B

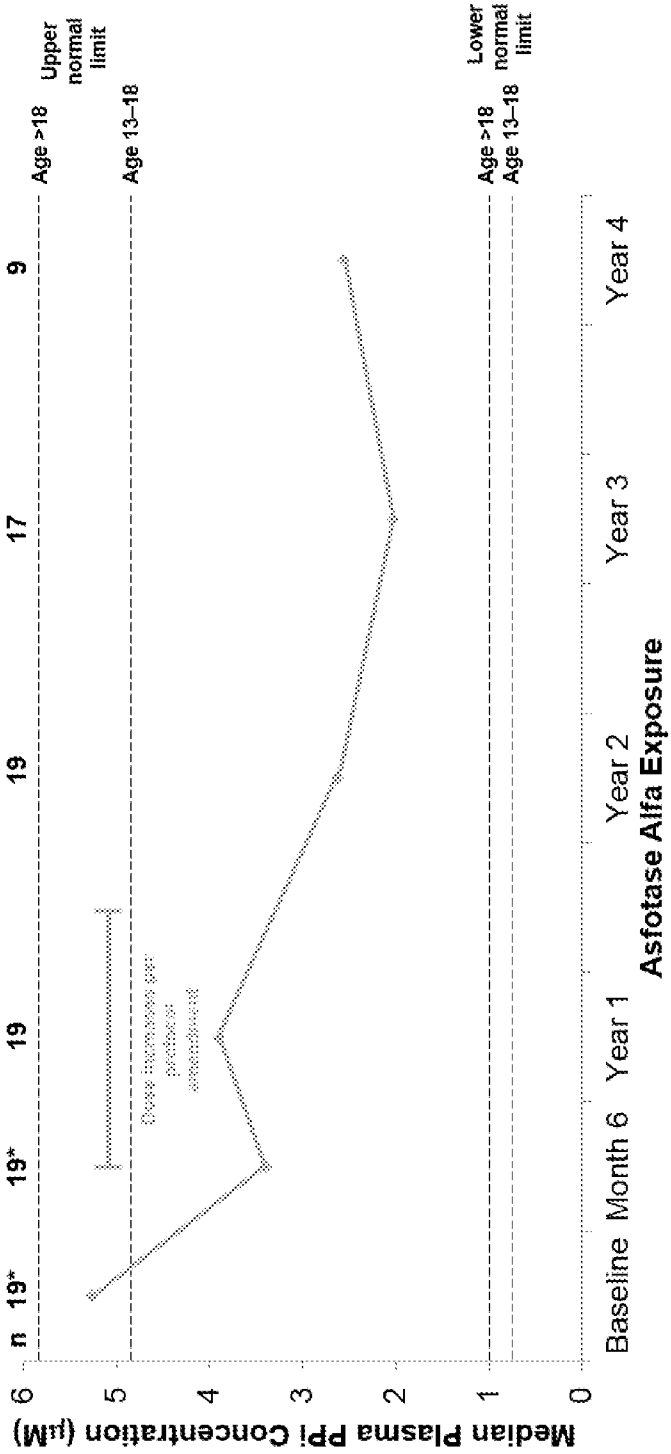
Median Plasma PPi Concentration (μM)
0
1
2
3
4
5
6
n 19* 19* 19 19 17 9
Age >18
Age 13–18
Upper normal limit
Lower normal limit
Baseline Month 6 Year 1 Year 2 Year 3 Year 4
Asfotase Alfa Exposure

FIG. 2C

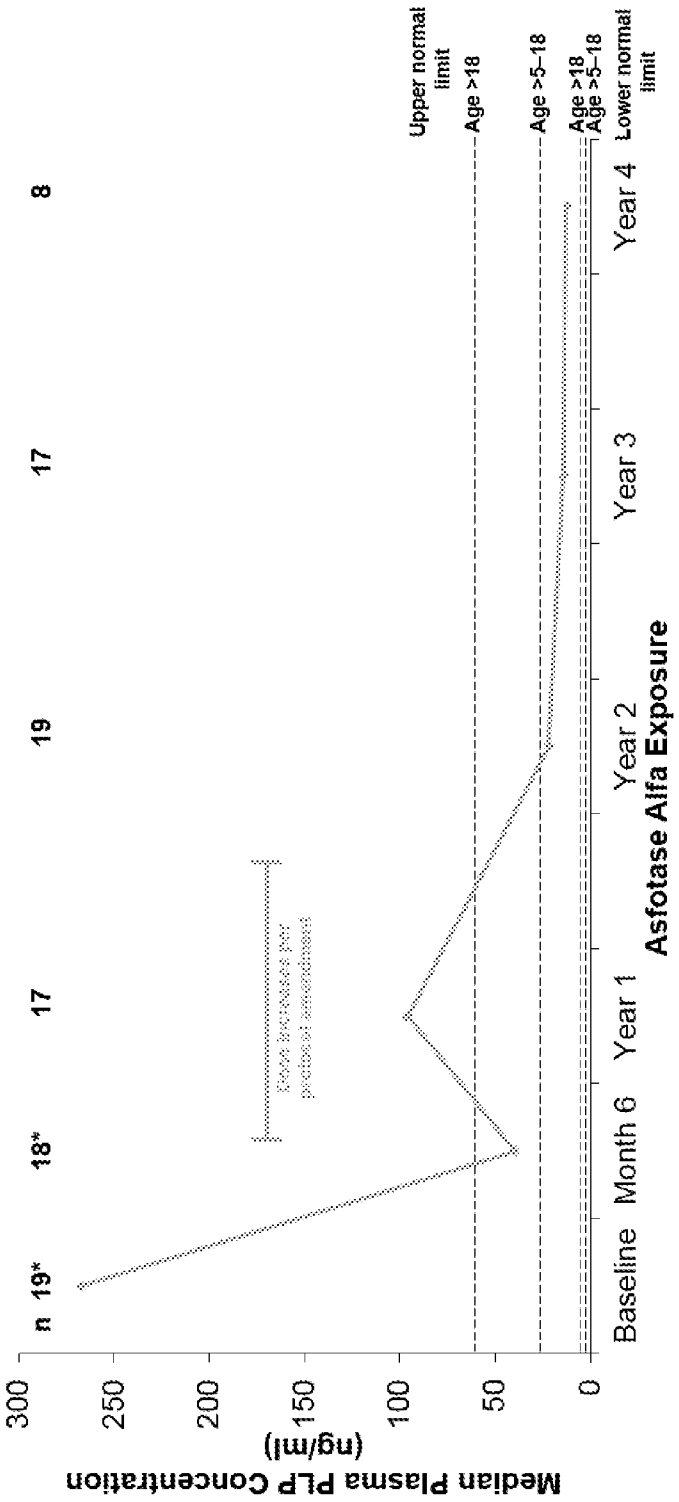
Median Plasma PLP Concentration (ng/ml)
300
250
200
150
100
50
0
n 19* 18* 17 19 17 8
Baseline
Month 6
Year 1
Year 2
Year 3
Year 4
Asfotase Alfa Exposure
Upper normal limit
Age >18
Age >5–18
Age >18
Age >5–18
Lower normal limit

FIG. 2D

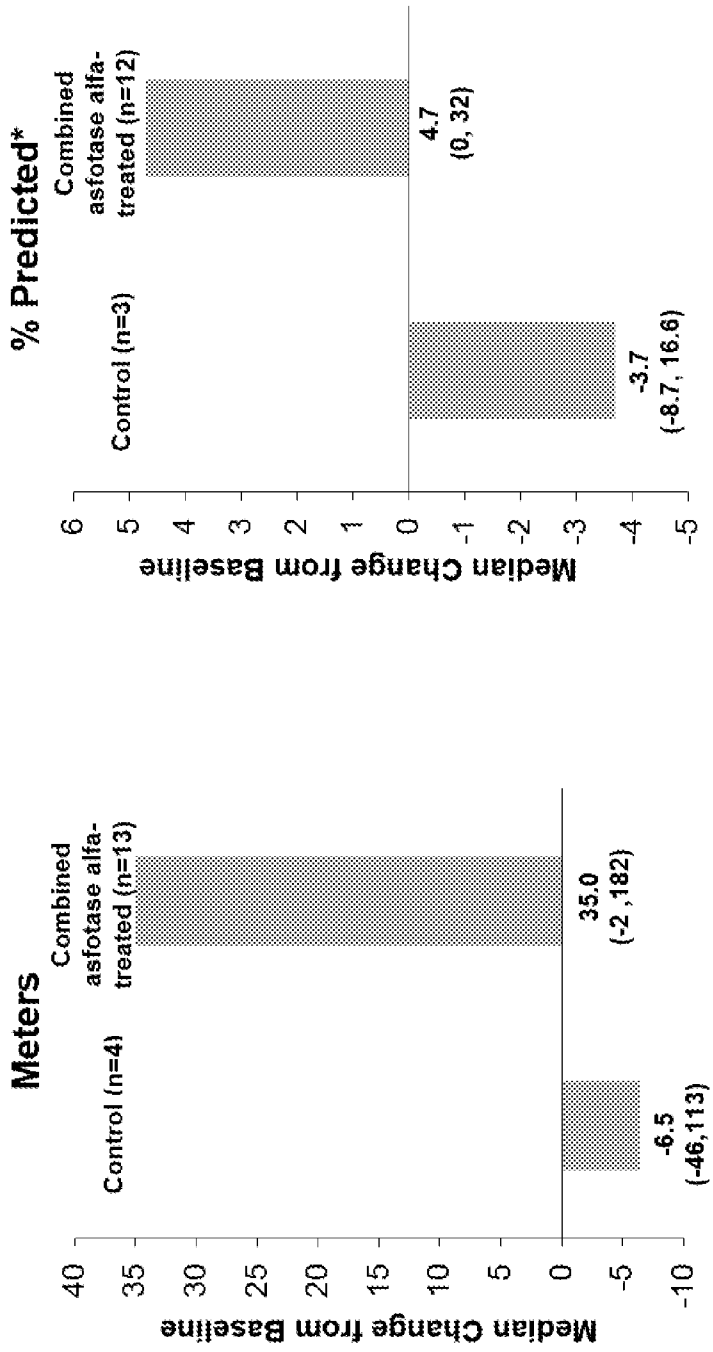
% Predicted*
Median Change from Baseline
6
5
4
3
2
1
0
-1
-2
-3
-4
-5
Control (n=3)
Combined asfotase alfa-treated (n=12)
-3.7
(-8.7, 16.6)
4.7
(0, 32)
Meters
Median Change from Baseline
40
35
30
25
20
15
10
5
0
-5
-10
Control (n=4)
Combined asfotase alfa-treated (n=13)
-6.5
(-46,113)
35.0
(-2 ,182)

FIG. 3A

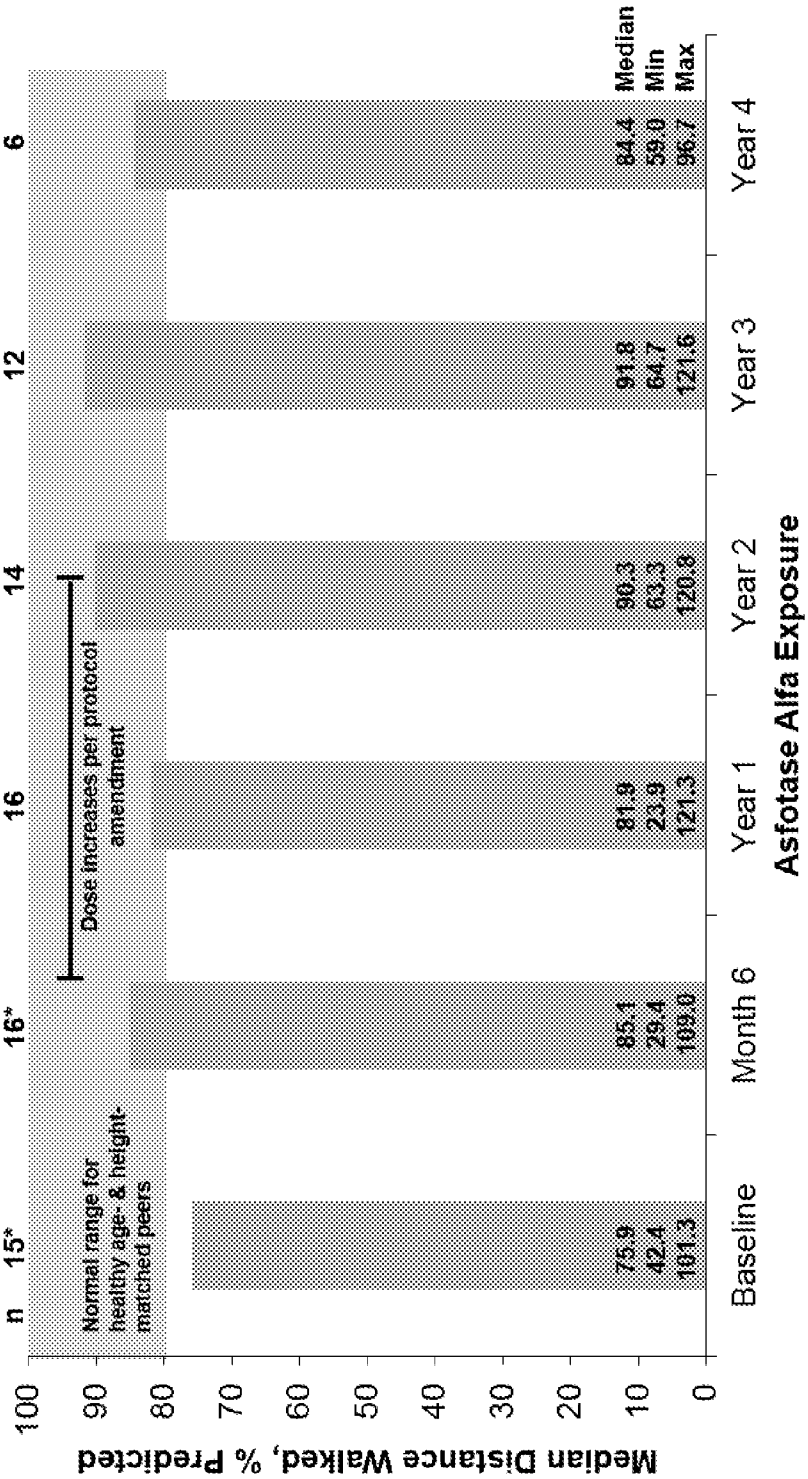
Median Distance Walked, % Predicted
100
90
80
70
60
50
40
30
20
10
0
n 15* 16* 16 14 12 6
Normal range for healthy age- & height-matched peers
Dose increases per protocol amendment
75.9
42.4
101.3
85.1
29.4
109.0
81.9
23.9
121.3
90.3
63.3
120.8
91.8
64.7
121.6
84.4
59.0
96.7
Median
Min
Max
Baseline
Month 6
Year 1
Year 2
Year 3
Year 4
Asfotase Alfa Exposure

FIG. 3B

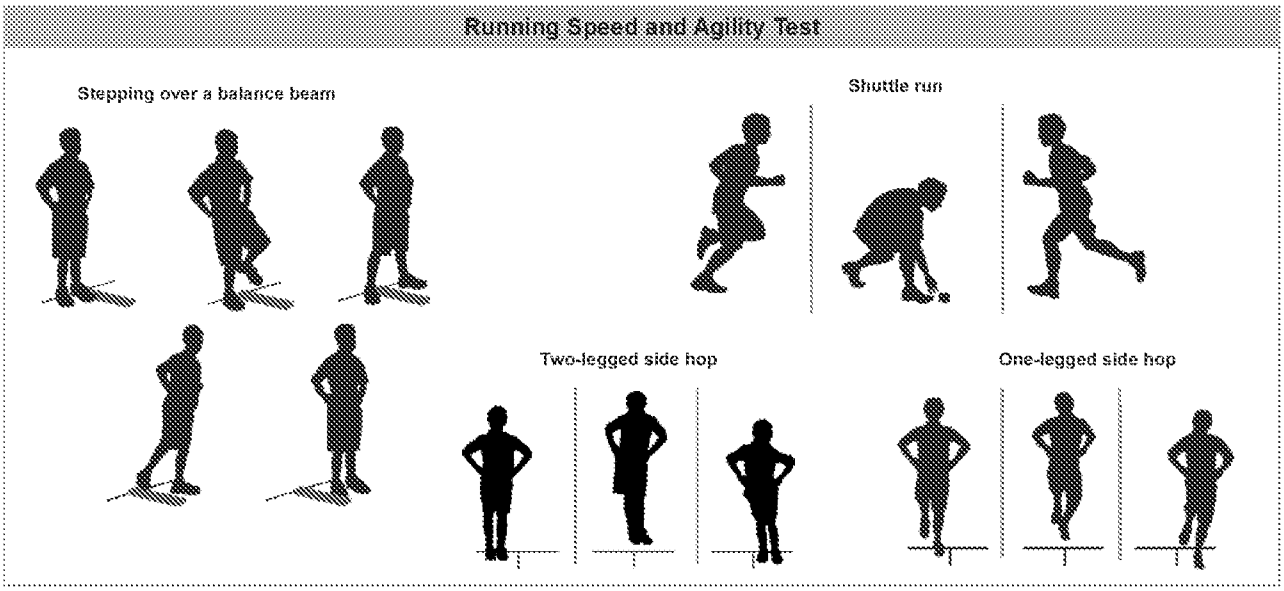
Running Speed and Agility Test
Stepping over a balance beam
Shuttle run
Two-legged side hop
One-legged side hop

FIG. 4A

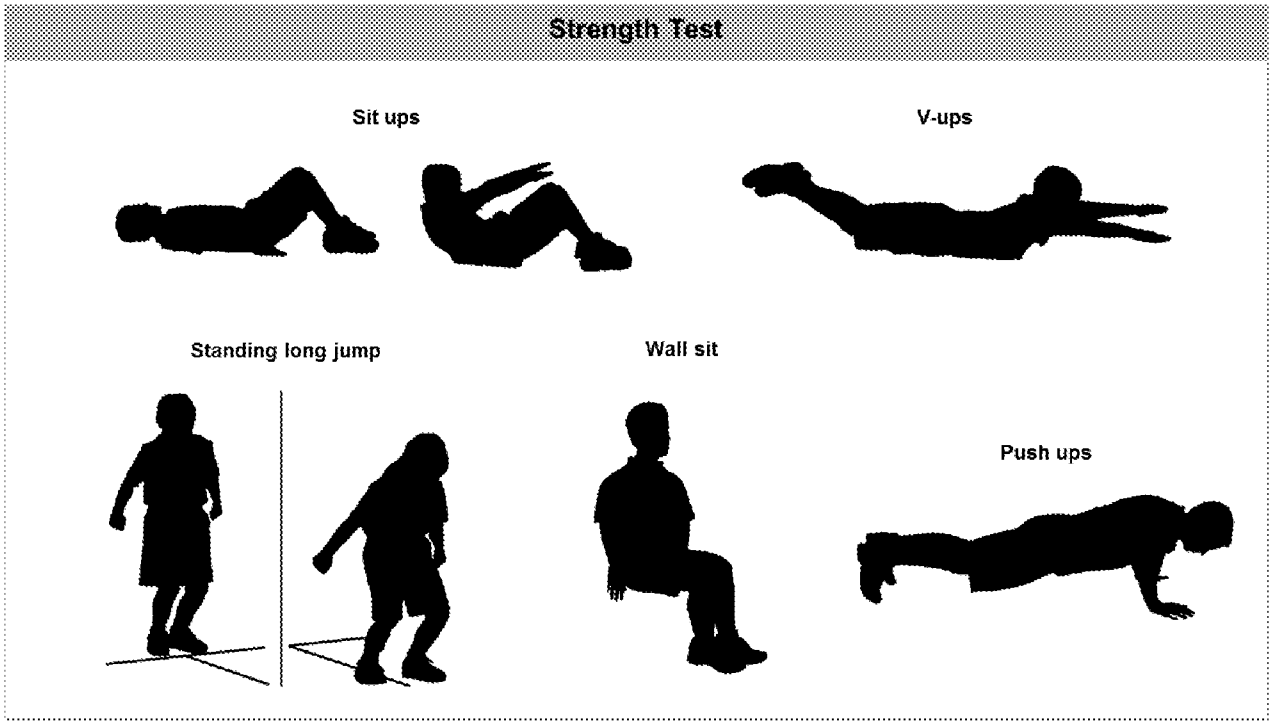
Strength Test
Sit ups
V-ups
Standing long jump
Wall sit
Push ups

FIG. 4B

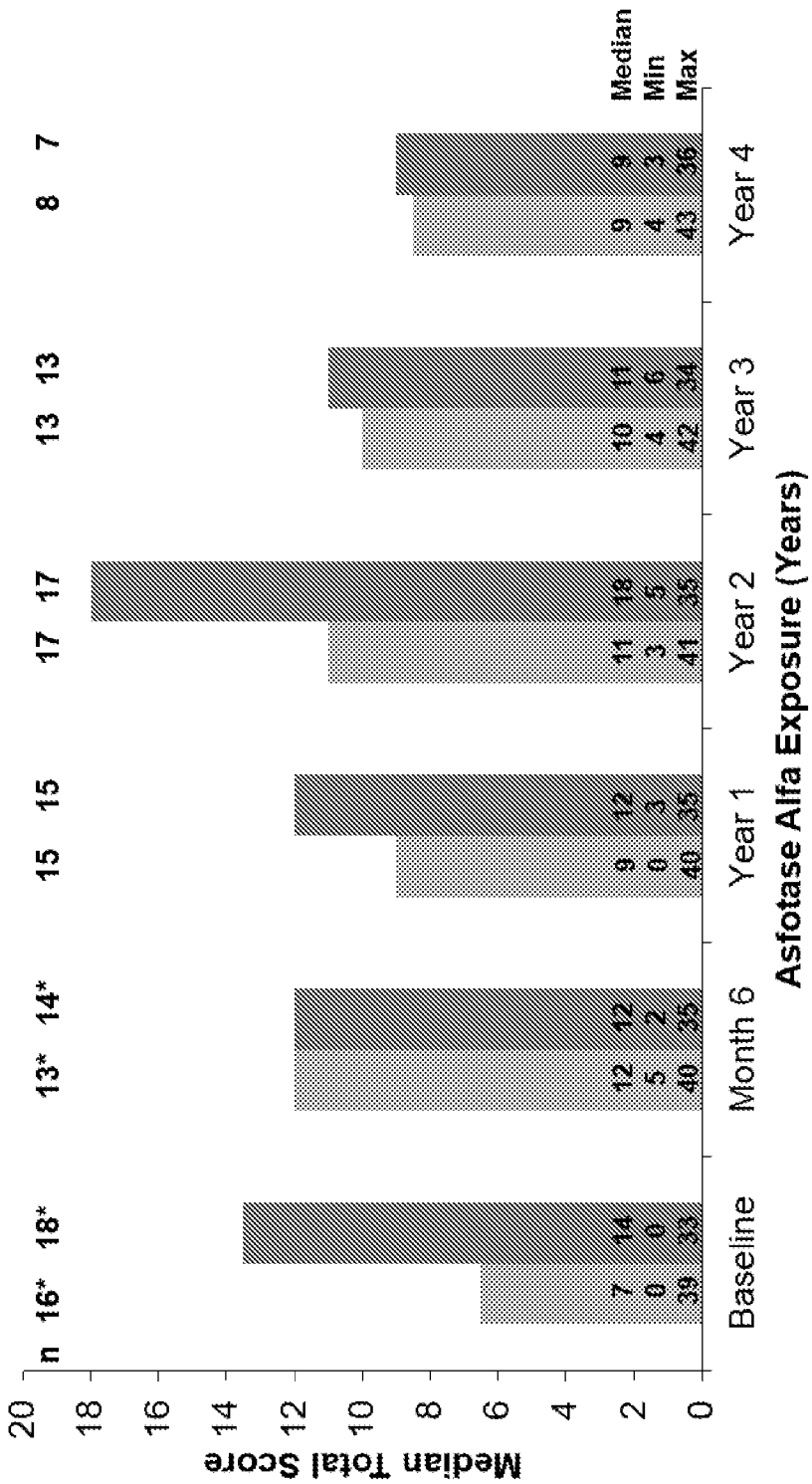
Running Speed/Agility
Strength
Median Total Score
20
18
16
14
12
10
8
6
4
2
0
n 16* 18* 13* 14* 15 15 17 17 13 13 8 7
Median
Min
Max
7 0 39
14 0 33
12 5 40
12 2 35
9 0 40
12 3 35
11 3 41
18 5 35
10 4 42
11 6 34
9 4 43
9 3 36
Baseline
Month 6
Year 1
Year 2
Year 3
Year 4
Asfotase Alfa Exposure (Years)

FIG. 4C

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/025590

A. CLASSIFICATION OF SUBJECT MATTER

IPC(8) - A61K 31/53; A61K 38/46; A61K 39/395; A61P 19/08; A61P 19/10; C07K 19/00 (2017.01)
CPC - A61K 38/46; C07K 2319/33; C12N 9/16; C12N 2799/025; C12Y 301/03001 (2017.02)

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
See Search History document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC - 424/134.1; 514/245; 514/263.34; 514/368; 514/384; 514/563; 544/209; 544/267; 548/155; 548/264.4; 562/561 (keyword delimited)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
See Search History document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X -- Y	PHILLIPS et al. "OR29-4: Significantly Improved Muscle Strength, Running Speed, and Agility in Children with Hypophosphatasia Treated with Asfotase Alfa" Endocrine Society's 97th Annual Meeting and Expo, 07 March 2015 (07.03.2015), Pgs. 1-2. Retrieved from the Internet: <http://press.endocrine.org/doi/abs/10.1210/endo-meetings.2015.BCHVD.8.OR29-4> on 26 May 2017 (26.05.2017). entire document	13-15, 17-19, 61, 62 ---------- 64
Y	US 2010/0297119 A1 (CRINE et al) 25 November 2010 (25.11.2010) entire document	1-4, 53-60, 63-94
Y	WHYTE et al. "Enzyme-Replacement Therapy in Life-Threatening Hypophosphatasia," New England Journal of Medicine, 08 March 2012 (08.03.2012), Vol. 366, Pgs. 904-913. entire document	1-4, 53-60, 63-94
Y	US 2015/0353633 A1 (ULTRAGENYX PHARMACEUTICAL INC. et al.) 10 December 2015 (10.12.2015) entire document	2-4
Y	US 2010/0093678 A1 (DELLA-FERA et al) 15 April 2010 (15.04.2010) entire document	53, 54
Y	MILLAN et al. "Alkaline Phosphatase and Hypophosphatasia," Calcified Tissue International, 21 November 2015 (21.11.2015), Vol. 98, Pgs. 398-416. entire document	75, 81
P, X	WO 2016/123342 A2 (ALEXION PHARMACEUTICALS, INC.) 04 August 2016 (04.08.2016) entire document	1-4, 13-15, 17-19, 53-94

☒ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
26 May 2017	29 JUN 2017
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, VA 22313-1450 Facsimile No. 571-273-8300	Authorized officer Blaine R. Copenheaver PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/025590

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	KISHNANI et al. "OR26-3: Biochemical and Physical Function Outcomes in Adolescents and Adults with Hypophosphatasia Treated with Asfotase Alfa for up to 4 Years: Interim Results from a Phase II Study," Endocrine Society's 98th Annual Meeting and Expo, 03 April 2016 (03.04.2016), Pgs. 1-2. Retrieved from the Internet: <http://press.endocrine.org/doi/abs/10.1210/endo-meetings.2016.BCHVD.9.OR26-3> on 26 May 2017 (26.05.2017). entire document	1-4, 13-15, 17-19, 53-94
A	US 2012/0088771 A1 (MILLAN) 12 April 2012 (12.04.2012) entire document	1-4, 13-15, 17-19, 53-94

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/025590

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 5-12, 16, 20-52
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.