(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
22 February 2018 (22.02.2018)

WIPO | PCT

(10) International Publication Number
WO 2018/035420 A1

(51) International Patent Classification:
A61K 38/16 (2006.01) *C07K 19/00* (2006.01)
A61K 38/46 (2006.01) *C12N 9/16* (2006.01)
A61P 19/00 (2006.01) *C12N 15/55* (2006.01)

(21) International Application Number:
PCT/US2017/047527

(22) International Filing Date:
18 August 2017 (18.08.2017)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
62/376,650 18 August 2016 (18.08.2016) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DJ, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JO, JP, KE, KG, KH, KN, KP, KR, KW, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:
— *with international search report (Art. 21(3))*
— *with sequence listing part of description (Rule 5.2(a))*

(54) Title: METHODS FOR TREATING TRACHEOBRONCHOMALACIA

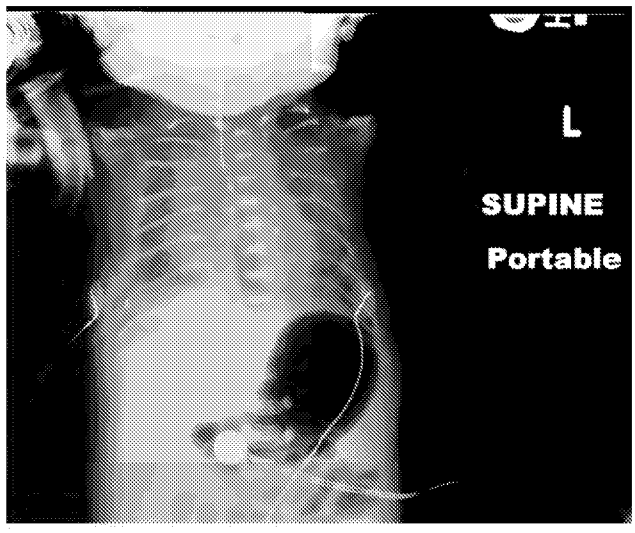

FIG. 2A

(57) Abstract: The disclosure features methods for treating tracheobronchomalacia (TBM) in a patient having hypophosphatasia (HPP), such as an infant, by administering a soluble alkaline phosphatase (sALP) to the patient.

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METHODS FOR TREATING TRACHEOBRONCHOMALACIA

INCORPORATION OF SEQUENCE LISTING

The instant application contains a Sequence Listing, which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. The ASCII copy, created on August 15, 2017, is named 50694-058WO2_Sequence_Listing_8.15.17_ST25.TXT and is 84KB in size.

FIELD

The disclosure relates to methods for treating tracheobronchomalacia.

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 encoding tissue-nonspecific alkaline phosphatase (TNSALP). HPP patients present a remarkable range of symptoms, from teeth loss or osteomalacia (rickets) to almost complete absence of bone mineralization *in utero*. Many patients with HPP present the characteristics of skeletal deformities, short stature, muscle and bone pain, impaired mobility, and premature loss of teeth. Perinatal-onset or infantile-onset HPP can also be characterized by the presence of rachitic chest deformity, vitamin B6-dependent seizures, and failure to thrive. In particular, HPP presenting at less than six months of age is often lethal due to respiratory insufficiency, with a low survival rate at one year of age.

Infants with HPP can exhibit respiratory compromise due to tracheobronchomalacia (TBM) and poorly mineralized ribs. TBM is a condition characterized by weakness of the tracheal and bronchial walls of the airway caused by hypotonia of myoelastic elements and softening of the supporting cartilage. The primary form of TBM is congenital. Severe TBM results in imminent risk of death from respiratory failure and complicated pulmonary infection. Thus, infants with TBM require aggressive therapy. In particular, HPP patients with TBM typically require assisted ventilation support to survive (Morrison, RJ et al., Mitigation of tracheobronchomalacia with 3D-printed personalized medical devices in pediatric patients. Sci Transl Med.;7(285):285ra264 (2015)).

There exists a need for methods to treat tracheobronchomalacia in patients with HPP, such as infants with HPP.

SUMMARY

Disclosed are methods to treat tracheobronchomalacia (TBM) in a patient (e.g. a human) having hypophosphatasia (HPP) by administering a soluble alkaline phosphatase (sALP), such as asfotase alfa (e.g., SEQ ID NO: 1). In particular, the sALP can be effective for the treatment of TBM and symptoms thereof in patients having HPP, such as infants having perinatal-onset HPP, when administered in a dosage regimen that provides greater than or equal to 6 mg/kg/week of the sALP to the patient in need thereof. Exemplary dosage regimens can include, but are not limited to, about 3 mg/kg of the sALP administered three times a week, about 2.5 mg/kg of the sALP administered three times a week, about 1.3 mg/kg of the sALP administered six times a week, or about 5 mg/kg of the sALP administered three

times a week. Additionally, the methods can include changing the dosage of and/or the frequency of administration of the sALP in order to determine the effective amount of the sALP to treat TBM and symptoms thereof in a patient having HPP. For instance, the dosage of an sALP can be increased to provide greater than or equal to 6 mg/kg/week, when the HPP patient does not exhibit an improvement in one or more symptoms of TBM after a treatment period of, e.g., at least two weeks, three weeks, one month, two months, three months, four months, five months, or six months.

A first aspect features a method of treating TBM in a patient having HPP (e.g., an infantile and perinatal-onset HPP patient) that includes administering an sALP to the patient in a dosage regimen that provides greater than or equal to 6 mg/kg/week of the sALP (e.g., asfotase alfa; SEQ ID NO: 1) to the patient. In particular, the sALP includes asfotase alfa (SEQ ID NO: 1) or an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1. Administration of the sALP can result in an improvement in TBM in the patient, such as an improvement in TBM following administration of the sALP for a treatment period of, e.g., about one month, about two months, about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, or longer.

For example, the sALP (e.g., SEQ ID NO: 1) can be administered twice a week, three times a week, four times a week, five times a week, six times a week, or seven times a week. In particular, the sALP can be administered on consecutive or alternating days. The dosage regimen can provide about 6.5 mg/kg/week to about 25 mg/kg/week of the sALP to the patient (e.g., the dosage regimen provides about 6.5 mg/kg/week of the sALP, about 7 mg/kg/week of the sALP, about 7.5 mg/kg/week of the sALP, about 7.8 mg/kg/week of the sALP, about 8 mg/kg/week of the sALP, about 8.5 mg/kg/week of the sALP, about 9 mg/kg/week of the sALP, about 10 mg/kg/week of the sALP, about 10.5 mg/kg/week of the sALP, about 11 mg/kg/week of the sALP, about 11.5 mg/kg/week of the sALP, about 12 mg/kg/week of the sALP, about 12.5 mg/kg/week of the sALP, about 13 mg/kg/week of the sALP, about 13.5 mg/kg/week of the sALP, about 14 mg/kg/week of the sALP, about 14.5 mg/kg/week of the sALP, about 15 mg/kg/week of the sALP, about 16 mg/kg/week of the sALP, about 17 mg/kg/week of the sALP, about 18 mg/kg/week of the sALP, about 19 mg/kg/week of the sALP, about 20 mg/kg/week of the sALP, about 21 mg/kg/week of the sALP, about 22 mg/kg/week of the sALP, about 23 mg/kg/week of the sALP, about 24 mg/kg/week of the sALP, or about 25 mg/kg/week of the sALP to the patient). In particular, the dosage regimen includes administering about 3 mg/kg of the sALP three times a week, about 2.5 mg/kg of the sALP three times a week, about 1.3 mg/kg of the sALP six times a week, or about 5 mg/kg of the sALP three times a week.

For instance, TBM in the patient having HPP can be characterized by one or more symptoms of TBM including, but not limited to, cardio-respiratory arrest, tracheostomy, cardiac arrest, respiratory distress, sputum retention, wheezing, coughing, anoxic spells, cyanosis, bradycardia, tachyarrhythmia, spontaneous hyperextension of the neck, prolonged expiratory breathing phase, failure to thrive, sternal retractions, substernal retractions, intercostal retractions, intermittent dyspnea, continuous dyspnea, recurrent bronchitis, and recurrent pneumonia. The patient (e.g., an infantile and perinatal-onset HPP patient) can exhibit an improvement in one or more of the symptoms of TBM following administration of the sALP (e.g., SEQ ID NO: 1).

The method can further include increasing the dosage of the sALP (e.g., SEQ ID NO: 1) if the patient (e.g., an infantile and perinatal-onset HPP patient) does not exhibit an improvement in one or more of the symptoms of TBM following administration of the sALP for a treatment period of at least two weeks, three weeks, one month, two months, three months, four months, five months, or six months. For instance, the patient exhibits an improvement in one or more of the symptoms of TBM (e.g., cardio-respiratory arrest, tracheostomy, cardiac arrest, respiratory distress, sputum retention, wheezing, coughing, anoxic spells, cyanosis, bradycardia, tachyarrhythmia, spontaneous hyperextension of the neck, prolonged expiratory breathing phase, failure to thrive, sternal retractions, substernal retractions, intercostal retractions, intermittent dyspnea, continuous dyspnea, recurrent bronchitis, and recurrent pneumonia) after receiving an increased dosage of the sALP. In particular, the patient exhibits an improvement in one or more of the symptoms of TBM after a treatment period of about one week, about two weeks, about three weeks, about one month, about two months, about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, about eleven months, or about one year.

In the above aspect, the symptoms of TBM (e.g., cardio-respiratory arrest, tracheostomy, cardiac arrest, respiratory distress, sputum retention, wheezing, coughing, anoxic spells, cyanosis, bradycardia, tachyarrhythmia, spontaneous hyperextension of the neck, prolonged expiratory breathing phase, failure to thrive, sternal retractions, substernal retractions, intercostal retractions, intermittent dyspnea, continuous dyspnea, recurrent bronchitis, and recurrent pneumonia) can be present in the patient (e.g., an infantile and perinatal-onset HPP patient) at birth or develop in the patient subsequent to birth. Additionally, the patient can be diagnosed with TBM prior to administration of the sALP (e.g., SEQ ID NO: 1).

The patient can require ventilator support prior to administration of the sALP (e.g., SEQ ID NO: 1). As a result of the methods, the patient (e.g., an infantile and perinatal-onset HPP patient) can exhibit decreased reliance on ventilator support, or no longer require ventilator support, after administration of the sALP. Moreover, the improvement in TBM can be sustained throughout administration of the sALP for a treatment period of 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. In particular, the improvement in TBM can be relative to an untreated HPP patient (e.g. an infant) having TBM.

The method can further include, prior to or after administration of the sALP to the patient (e.g., an infantile and perinatal-onset HPP patient), performing a tracheostomy on the patient. Additionally, the method can include, prior to or after administration of the sALP to the patient, performing a bronchoscopy (e.g., flexible bronchoscopy and/or microlaryngobronchoscopy) on the patient.

In the above aspect, the patient (e.g., an infantile and perinatal-onset HPP patient) can require at least one of high frequency oscillatory ventilation, positive end-expiratory pressure (PEEP), continuous positive airway pressure (CPAP), bilevel or biphasic positive airway pressure (BiPAP), and intermittent positive pressure ventilation (IPPV), prior to and/or concurrently with administration of the sALP. In particular, the patient can require a PEEP of about 5 cm H_2O to about 15 cm H_2O (e.g., the PEEP is about 5 cm H_2O, about 6 cm H_2O, about 7 cm H_2O, about 8 cm H_2O, about 9 cm H_2O, about 10 cm H_2O,

about 11 cm H_2O, about 12 cm H_2O, about 13 cm H_2O, about 14 cm H_2O, or about 15 cm H_2O). As a result of the methods, administration of the sALP can result in a decrease in the PEEP required by the patient, such as the PEEP required by the patient decreases by about 1 cm H_2O, about 2 cm H_2O, about 3 cm H_2O, about 4 cm H_2O, about 5 cm H_2O, about 6 cm H_2O, about 7 cm H_2O, about 8 cm H_2O, about 9 cm H_2O, or about 10 cm H_2O.

In the above aspect, the HPP patient can have at least one of perinatal-onset HPP and infantile-onset HPP. The HPP patient can be one that has not been previously administered the sALP (e.g., SEQ ID NO: 1). Additionally, administration of the sALP to the patient can occur about one month, about two months, about three months, about four months, about five months, or about six months after birth. In particular, the patient can be a human.

The patient (e.g., an infantile and perinatal-onset HPP patient) can exhibit one or more symptoms of HPP, which can include, but are not limited to, skeletal deformity, hypotonia, mobility impairments, bone deformity, joint pain, bone pain, muscle pain, bone fracture, muscle weakness, rickets, premature loss of deciduous teeth, incomplete bone mineralization, elevated blood and/or urine levels of phosphoethanolamine (PEA), elevated blood and/or urine levels of inorganic pyrophosphate (PPi), elevated blood and/or urine levels of pyridoxal 5'-phosphate (PLP), hypomineralization, rachitic ribs, hypercalciuria, short stature, waddling gait, HPP-related seizure, inadequate weight gain, craniosynostosis, and calcium pyrophosphate dihydrate crystal deposition. The one or more symptoms of HPP can be present in the patient at birth or develop in the patient subsequent to birth. As a result of the methods, the patient can exhibit an improvement in the one or more symptoms of HPP after administration of the sALP (e.g., SEQ ID NO: 1). Moreover, administration of the sALP can increase the survival of the patient.

The method can further include determining whether the patient (e.g., an infantile and perinatal-onset HPP patient) has a mutation in the patient's tissue non-specific alkaline phosphatase (TNALP) gene, in particular, the mutation in the TNALP gene is associated with HPP.

The sALP (e.g., SEQ ID NO: 1) can be administered to treat TBM and symptoms thereof in a patient having HPP (e.g., an infantile and perinatal-onset HPP patient) in a composition including a pharmaceutically acceptable excipient, carrier, or diluent, such as saline (e.g., sodium chloride and sodium phosphate). For example, the pharmaceutically acceptable excipient, carrier, or diluent includes 150 mM sodium chloride and 25 mM sodium phosphate. Moreover, the pharmaceutical composition can be administered to the patient parenterally (e.g., subcutaneously, intravenously, intramuscularly, intra-arterially, intrathecally, or intraperitoneally), enterally, or topically. In particular, the pharmaceutical composition can be administered to the patient subcutaneously.

In the above aspect, 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. For example, the sALP includes an amino acid sequence having at least 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as the sALP includes or consists of the amino acid sequence of SEQ ID NO: 1.

Additionally, the method can further include determining sALP activity in at least one of a serum sample and a blood sample from the patient (e.g., an infantile and perinatal-onset HPP patient). In

particular, the sALP activity includes measuring at least one of PEA, PPi, and/or PLP in the serum and/or blood sample from the patient.

A second aspect features the use of an sALP including an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, 97%, 98%, or 99% sequence identity) to the amino acid sequence of SEQ ID NO: 1 in the manufacture of a medicament for treating TBM in a patient (e.g., an infantile and perinatal-onset HPP patient) according to a dosage regimen. The dosage regimen provides greater than or equal to 6 mg/kg/week of the sALP (e.g., asfotase alfa; SEQ ID NO: 1) to the patient.

A third aspect features an sALP including an amino acid sequence having at least 95% sequence identity (e.g., at least 96%, 97%, 98%, or 99% sequence identity) to the amino acid sequence of SEQ ID NO: 1 for treating TBM in a patient having HPP (e.g., an infantile and perinatal-onset HPP patient). The sALP is administered to the patient in a dosage regimen that provides greater than or equal to 6 mg/kg/week of the sALP to the patient, in which the sALP (e.g., asfotase alfa; SEQ ID NO: 1) promotes an improvement in TBM in the patient.

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. For instance, the term "about" can be used to modify all dosages or ranges recited herein by ±10% of the recited values or range endpoints.

By "asfotase alfa" is meant a human TNSALP (hTNSALP) 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 hTNSALP, the human immunoglobulin G_1 Fc domain, and a deca-aspartate peptide used as a bone targeting domain (the structure hTNSALP-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.

The term "bronchoscopy," as used herein, refers to a method performed to visualize the airways of a patient, such as a patient having HPP (e.g., an infant with HPP, such as an infant having perinatal-onset HPP) to diagnose or treat lung diseases or conditions, such as tracheobronchomalacia (TBM). Bronchoscopy involves the insertion of a device, referred to as a bronchoscope, into the airways, usually through the nose or mouth, or occasionally through a tracheostomy. The bronchoscope can be a flexible or rigid tube that is typically less than about 2.5 cm in width and less than about 65 cm in length.

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 an affinity to bone matrix, such that the bone-targeting moiety, singularly, has an *in vivo* binding affinity to 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 of number "n," 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 term "catalytically competent," as used herein, refers to an sALP that hydrolyzes the bone mineralization inhibitor inorganic pyrophosphate (PPi) to provide inorganic phosphate (Pi), thereby decreasing the extracellular concentrations of PPi. A catalytically competent sALP improves skeletal mineralization in bone by regulating the concentration of PPi.

The term "dosage regimen" refers to the administration of a determined quantity of an active agent (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as asfotase alfa) calculated to produce a desired therapeutic effect (e.g., treatment of TBM, such as a reduction in one or many symptoms of TBM) at a particular frequency. An sALP, such as asfotase alfa, can be administered in a dosage regimen in association with any suitable pharmaceutical excipient, carrier, or diluent. For example, an sALP can be administered at a dosage regimen of greater than or equal to about 6 mg/kg/week to the patient, such as about 6.5 mg/kg/week of the sALP, about 7 mg/kg/week of the sALP, about 7.5 mg/kg/week of the sALP, about 7.8 mg/kg/week of the sALP, about 8 mg/kg/week of the sALP, about 8.5 mg/kg/week of the sALP, about 9 mg/kg/week of the sALP, about 10 mg/kg/week of the sALP, about 10.5 mg/kg/week of the sALP, about 11 mg/kg/week of the sALP, about 11.5 mg/kg/week of the sALP, about 12 mg/kg/week of the sALP, about 12.5 mg/kg/week of the sALP, about 13 mg/kg/week of the sALP, about 13.5 mg/kg/week of the sALP, about 14 mg/kg/week of the sALP, about 14.5 mg/kg/week of the sALP, about 15 mg/kg/week of the sALP, about 16 mg/kg/week of the sALP, about 17 mg/kg/week of the sALP, about 18 mg/kg/week of the sALP, about 19 mg/kg/week of the sALP, about 20 mg/kg/week of the sALP, about 21 mg/kg/week of the sALP, about 22 mg/kg/week of the sALP, about 23 mg/kg/week of the sALP, about 24 mg/kg/week of the sALP, or about 25 mg/kg/week of the sALP to the patient. In particular, the sALP can be administered multiple times per week (e.g., twice a week, three times a week, four times a week, five times a week, six times a week, or seven times a week) in the dosage regimen, such as on consecutive or alternating days.

By "extracellular domain" is meant any functional extracellular portion of a native protein, e.g., alkaline phosphatase. In particular, an extracellular domain lacks a 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, but less than the entire length of, a reference nucleic acid molecule or polypeptide. For example, a polypeptide fragment may contain 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 of the reference 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 can be tested in standard assays known in the art, e.g., by a non-compartmental analysis (NCA) to calculate pharmacokinetic parameters of the sALP fragment.

The terms "hypophosphatasia" and "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 (TNSALP). HPP can be further characterized as, e.g., infantile HPP or perinatal HPP (e.g., benign perinatal HPP or lethal perinatal HPP). For instance, "infantile HPP" describes a patient having HPP that is about three years of age or younger, whereas "perinatal HPP" describes a patient having HPP immediately before or after birth (e.g., one to four weeks after birth). The age of onset of HPP, such as when the subject exhibits symptoms of HPP, can also be categorized as, e.g., perinatal-onset HPP and infantile-onset HPP. Patients with HPP can exhibit symptoms of HPP including, but not limited to, skeletal deformity, hypotonia, mobility impairments, gait disturbance, bone deformity, joint pain, bone pain, bone fracture, muscle weakness, muscle pain, rickets (e.g., defects in growth plate cartilage), premature loss of deciduous teeth, incomplete bone mineralization, elevated blood and/or urine levels of phosphoethanolamine (PEA), PPi, pyridoxal 5'-phosphate (PLP), hypomineralization, rachitic ribs, hypercalciuria, short stature, HPP-related seizure, inadequate weight gain, craniosynostosis, and/or calcium pyrophosphate dihydrate crystal deposition (CPPD) in joints leading to, e.g., chondrocalcinosis and premature death. Symptoms of HPP can also include TBM and symptoms of TBM, such as cardio-respiratory arrest, tracheostomy, cardiac arrest, respiratory distress, sputum retention, wheezing, coughing, anoxic spells, cyanosis, bradycardia, tachyarrhythmia, spontaneous hyperextension of the neck, prolonged expiratory breathing phase, failure to thrive, sternal retractions, substernal retractions, intercostal retractions, intermittent or continuous dyspnea, and recurrent bronchitis or pneumonia.

The terms "patient" or "subject" refer to a mammal, including, but not limited to, a human (e.g., a human having HPP, such as an infant) or a non-human mammal.

"Parenteral administration," "administered parenterally," and other grammatically equivalent phrases, as used herein, refer to a mode 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 "pharmaceutically acceptable excipient, carrier, or diluent" is meant at least one excipient, carrier, or diluent, respectively, which is physiologically acceptable to the treated patient and which does not alter the therapeutic properties of an active agent (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as asfotase

alfa) 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 excipients, carriers, and diluents, and their formulations, are known to those skilled in the art and described, e.g., in *Remington: The Science and Practice of Pharmacy* (22nd Ed), Allen (2012). For instance, a pharmaceutically acceptable excipient, carrier, or diluent can include dibasic sodium phosphate, heptahydrate; monobasic sodium phosphate, monohydrate; and sodium chloride at a pH between 7.2 and 7.6.

By "pharmaceutical composition" is meant a composition containing an active agent, such as an sALP (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as asfotase alfa), as described herein, formulated with at least one pharmaceutically acceptable excipient, carrier, or diluent. The pharmaceutical composition can 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 (e.g., TBM) in a patient (e.g., an infant with HPP, such as an infant having perinatal-onset HPP, or an infant having infantile-onset HPP, or juvenile-onset HPP, or a patient having childhood-onset HPP). 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. For example, an sALP (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as asfotase alfa) can be formulated as a pharmaceutical composition including dibasic sodium phosphate, heptahydrate; monobasic sodium phosphate, monohydrate; and sodium chloride at a pH between about 7.2 and 7.6.

The term "physiologically active," as used herein, refers to an sALP (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as asfotase alfa) 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 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 TNSALP (SEQ ID NOs: 2, 3, 4, 5, or 6)). In particular, a TNSALP 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 TNSALP, such as a rhesus TNSALP (SEQ ID NO: 7), a rat TNSALP (SEQ ID NO: 8), a canine TNSALP (SEQ ID NO: 9), a porcine TNSALP (SEQ ID NO: 10), a murine TNSALP (SEQ ID NO: 11), a bovine TNSALP (SEQ ID NOs: 12-14), or a feline TNSALP (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, such as variants having at least 90, 95, 97, or 99% sequence identity to any one of SEQ ID NOs: 7-19. An 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 hTNSALP-Fc-D_{10} (e.g., asfotase alfa; 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.

As used herein, the term "sequence identity" refers to the percentage of amino acid (or nucleic acid) residues of a candidate sequence, e.g., an sALP, that are identical to the amino acid (or nucleic acid) residues of a reference sequence, e.g., the amino acid sequence of asfotase alfa (SEQ ID NO: 1), after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent identity (e.g., gaps can be introduced in one or both of the candidate and reference sequences for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). Alignment for purposes of determining percent identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software, such as BLAST, BLAST-2, BLAST-P, BLAST-N, BLAST-X, WU-BLAST-2, ALIGN, ALIGN-2, CLUSTAL, or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. For instance, the percent amino acid (or nucleic acid) sequence identity of a given candidate sequence to, with, or against a given reference sequence (which can alternatively be phrased as a given candidate sequence that has or includes a certain percent amino acid (or nucleic acid) sequence identity to, with, or against a given reference sequence) is calculated as follows:

$$100 \times (\text{fraction of } A/B)$$

where A is the number of amino acid (or nucleic acid) residues scored as identical in the alignment of the candidate sequence and the reference sequence, and where B is the total number of amino acid (or nucleic acid) residues in the reference sequence. In particular, a reference sequence aligned for comparison with a candidate sequence can show that the candidate sequence exhibits from, e.g., 50% to 100% identity across the full length of the candidate sequence or a selected portion of contiguous amino

acid (or nucleic acid) residues of the candidate sequence. The length of the candidate sequence aligned for comparison purpose is at least 30%, e.g., at least 40%, e.g., at least 50%, 60%, 70%, 80%, 90%, or 100% of the length of the reference sequence. When a position in the candidate sequence is occupied by the same amino acid (or nucleic acid) residue as the corresponding position in the reference sequence, then the molecules are identical at that position.

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.

The terms "tracheobronchomalacia" and "TBM" (which includes but is not limited to bronchomalacia, chondromalacia of the larynx, chondromalacia of the trachea, larangomalacia, laryngotracheobronchomalacia, and tracheomalacia), as used herein, refer to a rare condition characterized by softening or damage to the cartilaginous structures of the airway walls in the trachea and bronchi. In particular, TBM refers to the congenital form of the condition that can develop, e.g., during the perinatal period or infancy of a patient with HPP. TBM is characterized by symptoms including, but not limited to, cardio-respiratory arrest, tracheostomy, cardiac arrest, respiratory distress, sputum retention, wheezing, coughing, anoxic spells, cyanosis, bradycardia, tachyarrhythmia, spontaneous hyperextension of the neck, prolonged expiratory breathing phase, failure to thrive, sternal retractions, substernal retractions, intercostal retractions, intermittent or continuous dyspnea, and recurrent bronchitis or pneumonia.

The term "tracheostomy," as used herein, refers to a surgical procedure performed to create an opening through the neck into the trachea of a patient (e.g., an HPP patient having TBM). A tube is usually placed through this opening to provide an airway and to remove mucus from the lungs of the patient.

By "therapeutically effective amount" is meant an amount of an sALP (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as asfotase alfa) that is sufficient to substantially improve, treat, prevent, delay, suppress, or arrest at least one symptom of TBM in an HPP patient (e.g., an infant with HPP, such as an infant having perinatal-onset HPP), such as cardio-respiratory arrest, tracheostomy, cardiac arrest, respiratory distress, sputum retention, wheezing, coughing, anoxic spells, cyanosis, bradycardia, tachyarrhythmia, spontaneous hyperextension of the neck, prolonged expiratory breathing phase, failure to thrive, sternal retractions, substernal retractions, intercostal retractions, intermittent or continuous dyspnea, and recurrent bronchitis or pneumonia. A therapeutically effective amount of an sALP described herein can

depend on the severity of TBM 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 an sALP can be administered to an HPP patient having TBM in a dosage regimen as described herein over a period of time (e.g., at least one to six months, such as 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).

By "treating," "treat," or "treatment" is meant the medical management of an HPP patient (e.g., infantile and perinatal-onset HPP patients) with the intent to cure, ameliorate, stabilize, reduce the likelihood of, or prevent TBM, e.g., by administering an sALP (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as asfotase alfa). Treatment can occur for a treatment period, in which an sALP is administered for a period of time (e.g., days, months, years, or longer) to treat an HPP patient having TBM. This term includes active treatment directed toward the improvement of TBM in an HPP patient; symptomatic treatment directed toward symptoms of TBM in an HPP patient; preventative treatment directed to minimizing the development of TBM in an HPP patient, e.g., in an HPP patient who does not yet have TBM, but who is susceptible to or at risk of developing TBM; and supportive treatment employed to supplement another specific therapy directed toward the improvement of TBM in an HPP patient.

The term "ventilator support," as used herein, refers to artificial ventilation of an HPP patient (e.g., infantile and perinatal-onset HPP patients) having TBM in which mechanical means, in particular, a ventilator, are used to assist or replace spontaneous breathing. For example, ventilator support of an HPP patient exhibiting symptoms of TBM or likely to have TBM can be required prior to treatment with an sALP (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as asfotase alfa), such as administration of an sALP in a dosage regimen providing greater than or equal to about 6 mg/kg/week of the sALP to the patient. For example, ventilator support of an HPP patient exhibiting symptoms of TBM or likely to have TBM can be required during treatment with an sALP, and the ventilator support may help maintain patency of the airways of the HPP patient. The HPP patient can exhibit decreased reliance on ventilator support, can maintain airway patency without ventilator support, or can no longer require ventilator support after administration of the sALP, such as after administration of the sALP for a treatment period of 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.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the amino acid sequence of asfotase alfa (STRENSIQ®, Alexion Pharmaceuticals, Inc., SEQ ID NO: 1).

Figures 2A-2D are radiograph images of the chest of a hypophosphatasia (HPP) patient with tracheobronchomalacia (TBM) at birth (Fig. 2A), 16 days (Fig. 2B), 9 months (Fig. 2C), and 12 months (Fig. 2D). Figures 2A-2B show radiographic images of the HPP patient's chest prior to treatment with asfotase alfa. Figure 2C shows a radiographic image of the HPP patient's chest after treatment with asfotase alfa at a dose of 1.3 mg/kg (7.8 mg/kg/week). Fig. 2D shows a radiographic image of the HPP

patient's chest after treatment with asfotase alfa at a dose of 2.5 mg/kg (7.5 mg/kg/week).

DETAILED DESCRIPTION

Patients with hypophosphatasia (HPP), particularly infants, can exhibit respiratory compromise due to tracheobronchomalacia (TBM), often requiring assisted ventilator support and positive airway pressure in order to survive. We have discovered that asfotase alfa (SEQ ID NO: 1, STRENSIQ®, Alexion Pharmaceuticals, Inc.) can be used effectively to treat and/or ameliorate TBM, its symptoms, and decreased respiratory function associated therewith in HPP patients (e.g., humans having HPP). In particular, asfotase alfa is effective for the treatment of TBM and symptoms thereof in infants having HPP, such as infants having perinatal-onset HPP and/or infantile-onset HPP, when administered in a dosage regimen that provides greater than or equal to about 6 mg/kg/week of asfotase alfa to the infant in need thereof.

Methods for administering asfotase alfa (SEQ ID NO: 1) to an HPP patient in need thereof (e.g., infantile-onset HPP patients and perinatal-onset HPP patients) that result in an improvement in TBM are described. For example, asfotase alfa can be administered to an HPP patient exhibiting one or more symptoms of TBM including, but not limited to, cardio-respiratory arrest, tracheostomy, cardiac arrest, respiratory distress, sputum retention, wheezing, coughing, anoxic spells, cyanosis, bradycardia, tachyarrhythmia, spontaneous hyperextension of the neck, prolonged expiratory breathing phase, failure to thrive, sternal retractions, substernal retractions, intercostal retractions, intermittent or continuous dyspnea, and recurrent bronchitis or pneumonia.

The HPP patient can exhibit an improvement in one or more of the symptoms of TBM following administration of asfotase alfa for a treatment period of, e.g., about one week, about two weeks, about three weeks, about one month, about two months, about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, about eleven months, or about one year. Accordingly, administration of asfotase alfa can decrease the reliance of the HPP patient on ventilator support, or can eliminate the need for ventilator support altogether. Moreover, treatment with asfotase alfa can increase survival of the HPP patient with TBM.

The methods of treatment can also include administering asfotase alfa to an HPP patient in combination with a medical procedure (e.g., a tracheostomy or a bronchoscopy). Asfotase alfa can be administered to the patient prior to, or after, the medical procedure (e.g., a tracheostomy or a bronchoscopy).

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) can be used to treat TBM or one or more symptoms thereof in HPP patients (e.g., infantile and perinatal-onset HPP patients).

The methods of treatment, alkaline phosphatases, and pharmaceutical compositions are described herein.

Methods of Treatment

The methods described herein can be used to treat TBM or one or more symptoms of TBM in HPP patients, such as infants having HPP (e.g., perinatal-onset HPP patients). Accordingly, an sALP can be administered to an HPP patient presenting one or more symptoms of TBM at birth or subsequent to birth. In particular, the HPP patient (e.g., infantile and perinatal-onset HPP patients) treated for TBM can be one that has not previously been treated with the sALP.

For example, TBM or one or more symptoms of TBM can be treated by administering an sALP to infants having HPP across a range of ages, e.g., about one day to about 1 month old, about 5 days to about 6 months old, about 10 days to about 8 months old, about 10 days to about 1 year old, about 1 month to about 6 months old, about 2 weeks to about 4 months old, about 3 months to about 9 months old, about 3 weeks to about 10 months old, about 3 months to about 16 months old, about 2 months to about 22 months old, about 7 weeks to about one year old, about 5 weeks to about 15 months old, about 5 months to about 17 months old, about 6 months to about 18 months old, or about 2 months to about 3 years old.

Symptoms of TBM

An HPP patient in need of treatment can exhibit one or more symptoms of TBM, e.g., relative to a healthy subject of about the same age and/or gender, prior to administration of an sALP. Symptoms that can be used to diagnose TBM prior to treatment with an sALP or that signify an HPP patient in need of treatment can include, but are not limited to, cardio-respiratory arrest, tracheostomy, cardiac arrest, respiratory distress (e.g., difficulty in breathing), sputum retention (e.g., mucus in the lower airways of the trachea and bronchi), wheezing, coughing, anoxic spells, cyanosis (e.g., the abnormal blue discoloration of the skin and mucous membranes), bradycardia (e.g., a slow heart rate), tachyarrhythmia (e.g., a heart rate that exceeds the resting rate of a healthy subject), spontaneous hyperextension of the neck, prolonged expiratory breathing phase, failure to thrive, sternal retractions (e.g., indrawing of the abdomen at the sternum or breastbone), substernal retractions (e.g., indrawing of the abdomen just below the sternum or breastbone), intercostal retractions (e.g., indrawing of the skin in between each rib), intermittent or continuous dyspnea (e.g., difficult or laboured breathing), and recurrent bronchitis or pneumonia. Following administration of an sALP, such as in a dosage regimen providing greater than or equal to about 6 mg/kg/week, an HPP patient can exhibit an improvement in one or more symptoms of TBM.

Administration of an sALP

The method of treatment involves administering an sALP to treat TBM or one or more symptoms thereof in an HPP patient (e.g., infantile and perinatal-onset HPP patients) in a dosage regimen providing greater than or equal to about 6 mg/kg/week of the sALP (e.g., about 7 mg/kg/week to about 25 mg/kg/week of the sALP) to the patient. In particular, a dosage regimen to treat TBM in an HPP patient can provide, e.g., about 6.5 mg/kg/week of the sALP, about 7 mg/kg/week of the sALP, about 7.5 mg/kg/week of the sALP, about 7.8 mg/kg/week of the sALP, about 8 mg/kg/week of the sALP, about 8.5 mg/kg/week of the sALP, about 9 mg/kg/week of the sALP, about 10 mg/kg/week of the sALP, about 10.5

mg/kg/week of the sALP, about 11 mg/kg/week of the sALP, about 11.5 mg/kg/week of the sALP, about 12 mg/kg/week of the sALP, about 12.5 mg/kg/week of the sALP, about 13 mg/kg/week of the sALP, about 13.5 mg/kg/week of the sALP, about 14 mg/kg/week of the sALP, about 14.5 mg/kg/week of the sALP, about 15 mg/kg/week of the sALP, about 16 mg/kg/week of the sALP, about 17 mg/kg/week of the sALP, about 18 mg/kg/week of the sALP, about 19 mg/kg/week of the sALP, about 20 mg/kg/week of the sALP, about 21 mg/kg/week of the sALP, about 22 mg/kg/week of the sALP, about 23 mg/kg/week of the sALP, about 24 mg/kg/week of the sALP, or about 25 mg/kg/week of the sALP to the patient.

For example, the dosage regimen can include administering about 3 mg/kg of the sALP three times a week, about 2.5 mg/kg of the sALP three times a week, about 1.3 mg/kg of the sALP six times a week, or about 5 mg/kg of the sALP three times a week. Moreover, an sALP can be administered to treat TBM or one or more symptoms of TBM in an HPP patient in any of the dosage regimens described herein for a treatment 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).

When administration of an sALP does not result in an improvement in one or more symptoms of TBM, the dosage regimen can be modified (e.g., increased or decreased) until an effective amount of the sALP to treat TBM or one or more symptoms of TBM in the HPP patient (e.g., infantile and perinatal-onset HPP patients) is identified. For instance, the dosage of an sALP can be increased to provide greater than or equal to about 6 mg/kg/week, as discussed above, when the HPP patient (e.g., infantile and perinatal-onset HPP patients) does not exhibit an improvement in one or more symptoms of TBM after a treatment period of, e.g., at least two weeks, three weeks, one month, two months, three months, four months, five months, or six months.

An sALP can also be administered prior to, simultaneously, or following administration of a second active agent. In particular, second active agents for use in the methods of treatment include, but are not limited to, opioids (e.g., methadone, codeine, lydrocodone, fentanyl, hydromorphone, morphine, and oxycodone), anti-anxiety drugs (e.g., alprazolam, midazolam, clobazam, clonazepam, clorazepate, diazepam, duloxetine, fluoxetine, escitalopram, lorazepam, nitrazepam, temazepam, and nimetazepam), anti-depressants (e.g., desipramine, amitriptyline, agomelatine, etoperidone, and phenelzine), anti-convulsant drugs (e.g., lithium carbonate, lithium citrate, topiramate, oxcarbazepine, and valproic acid), antipsychotics (e.g., aripiprazole, clozapine, risperidone, asenaphine, and olanzapine), non-steroidal anti-inflammatory drugs (e.g., aspirin, ibuprofen, ketoprofen, ketorolac tromethamine, and naproxen), corticosteroids (e.g., prednisolone, methylprednisolone, hydrocortisone, amcinonide, fluocinonide, flunisolide, prednicarbate, betamethasone, and triamcinolone acetonide), and muscle relaxants (e.g., carisoprodol, cyclobenzaprine, and diazepam). Administration of the second active agent can be discontinued or the dosage can be reduced once the HPP patient exhibits an improvement in one or more symptoms of TBM following administration of an sALP.

Additional medical procedures

An HPP patient in need of treatment can require ventilation prior to administration of an sALP (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid

sequence of SEQ ID NO: 1, such as asfotase alfa) to treat TBM. In particular, the HPP patient can require assisted ventilation, including but not limited to one or more of high-frequency oscillation, continuous positive airway pressure (CPAP) ventilation, positive end-expiratory pressure (PEEP) ventilation, bilevel or biphasic positive airway pressure (BiPAP), and intermittent positive pressure ventilation (IPPV). Assisted ventilation can be required to maintain patency of the airways during treatment with an sALP, and may be adjusted in strength and type in order to maintain sufficient airway patency during treatment. PEEP can be required in HPP patients exhibiting greater TBM severity. For example, an HPP patient with TBM can require PEEP ventilation of about 5 cm H_2O to about 15 cm H_2O, such as 5 cm H_2O, 6 cm H_2O, 7 cm H_2O, 8 cm H_2Or, 9 cm H_2O, 10 cm H_2O, 11 cm H_2O, 12 cm H_2O, 13 cm H_2O, 14 cm H_2O, or 15 cm H_2O.

Administration of an sALP to treat TBM in an HPP patient in a dosage regimen as described herein can result in the patient exhibiting decreased reliance on ventilator support. For example, administration of asfotase alfa in a dosage regimen providing greater than or equal to 6 mg/kg/week for a treatment period of about one month, about two months, about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, or longer can reduce or eliminate the need for support. For example, administration of an sALP can result in a decrease in the PEEP required by the HPP patient of, e.g., about 1 cm H_2O, about 2 cm H_2O, about 3 cm H_2O, about 4 cm H_2O, about 5 cm H_2O, about 6 cm H_2O, about 7 cm H_2O, about 8 cm H_2O, about 9 cm H_2O, about 10 cm H_2O, or more. Additionally, after administration of an sALP in a dosage regimen providing greater than or equal to 6 mg/kg/week, the HPP patient in need thereof can receive continuous positive airway pressure (CPAP) ventilation instead of positive end-expiratory pressure (PEEP) ventilation or bilevel or biphasic positive airway pressure (BiPAP), or may no longer require ventilator support at all.

HPP patients (e.g., infantile and perinatal-onset HPP patients) can be diagnosed with TBM prior to administration of an sALP using bronchoscopy (e.g., flexible bronchoscopy and/or microlaryngobronchoscopy). For example, bronchoscopy can be performed on an HPP patient at the age of one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, ten months, eleven months, one year, or older. In particular, bronchoscopy of an HPP patient can show, e.g., softened airways, collapse of the trachea and bronchi, dynamic bronchial collapse during restful breathing, narrowing of the subglottic space, cervical tracheomalacia, laryngotracheobronchomalacia, and/or suprastomal tracheal collapse. Bronchoscopy can also be performed on the patient after a treatment period of, e.g., about one week, about two weeks, about three weeks, about one month, about two months, about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, about eleven months, or about one year, to determine if the amount of the sALP administered is therapeutically effective.

The method of treatment can further include performing tracheostomy in combination with administration of an sALP in a dosage regimen that provides greater than or equal to 6 mg/kg/week of the sALP to treat TBM or one or more symptoms of TBM in an HPP patient (e.g., infantile and perinatal-onset

HPP patients). Tracheostomy can be performed in combination with administration of an sALP to treat TBM in an HPP patient at, e.g., about one week, about two weeks, about three weeks, about one month, about two months, about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, about eleven months, or about one year of age.

The method can involve administering the sALP to the HPP patient prior or after performing a tracheostomy, e.g., to provide long-term ventilation to the airways and/or remove mucus from the lungs of the HPP patient. For example, the method of treatment can include increasing the dosage of an sALP after performing the tracheostomy if the patient does not exhibit an improvement in one or more of the symptoms of TBM following administration of the sALP for a treatment period of, e.g., at least two weeks, three weeks, one month, two months, three months, four months, five months, or six months.

Additionally, the method can include performing gastrostomy in combination with administration of an sALP in a dosage regimen that provides greater than or equal to 6 mg/kg/week of the sALP to treat TBM or one or more symptoms of TBM in an HPP patient (e.g., infantile and perinatal-onset HPP patients). Gastrostomy can be performed in combination with administration of an sALP to treat TBM in an HPP patient at, e.g., about one week, about two weeks, about three weeks, about one month, about two months, about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, about eleven months, or about one year of age.

Alkaline Phosphatase

Asfotase alfa is a human tissue non-specific alkaline phosphatase (TNSALP; SEQ ID NO: 1) fusion polypeptide formulated for the treatment of HPP. In particular, asfotase alfa can be used effectively to treat TBM and symptoms associated therewith in HPP patients (e.g., infantile and perinatal-onset HPP patients) in a dosage regimen that provides greater than or equal to 6 mg/kg/week of the sALP to the patient.

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 (TNSALP; 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). Any of these isozymes could potentially be used to treat TBM according to the methods described herein.

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 an sALP or an sALP fusion polypeptide as defined herein) for treating TBM or symptoms of TBM in HPP patients, such as infants with HPP. For example, sALP constructs that can be used to treat TBM in an HPP patient include,

e.g., the bone delivery conjugates described in PCT publication Nos. WO 2005/103263 and WO 2008/138131, incorporated herein by reference.

TNSALPs that can be administered according to the methods described herein include, e.g., human TNSALP (Accession Nos. NP_000469, AAI10910, AAH90861, AAH66116, AAH21289, and AAI26166); rhesus TNSALP (Accession No. XP_01109717); rat TNSALP (Accession No. NP_037191); dog TNSALP (Accession No. AAF64516); pig TNSALP (Accession No. AAN64273); mouse (Accession No. NP_031457); cow TNSALP (Accession Nos. NP_789828, NP_776412, AAM 8209, and AAC33858); cat TNSALP (Accession No. NP_001036028); and variants thereof having 90, 95, 97, or 99% sequence identity to any one of SEQ ID NOs: 7-19. In particular, TNSALP can be a recombinant human TNSALP (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 TBM or one or more symptoms of TBM in HPP patients (e.g., an infantile or perinatal-onset HPP patient). The TNSALP can also be one that exhibits at least about 95% sequence identity to the polypeptide or nucleic acid sequence of the above-noted TNSALPs.

Soluble Alkaline Phosphatases

ALPs that can be used in the methods described herein include soluble (e.g., extracellular or non-membrane-bound) forms of any of the ALPs described herein. 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, an sALP is one that is catalytically competent to improve skeletal mineralization in bone. Nucleic acids encoding the sALPs described herein can also be used in the methods for treating TBM or one or more symptoms of TBM in HPP patients (e.g., infantile or perinatal-onset HPP patients).

An example of an ALP that can be produced as an sALP is TNSALP (e.g., human TNSALP (hTNSALP)). TNSALP 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 TNSALP are extracellular.

In particular, TNSALP 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 hTNSALP that contains all amino acid residues of the native anchored form of TNSALP 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 sTNSALP 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, TNSALP 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 TNSALP 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/or at www.cbs.dtu.dk/services/SignalP/.

sALP consensus sequences derived from the extracellular domain of ALP isozymes (e.g., TNSALP, PALP, GCALP, or IALP) can also be used to produce an sALP for treatment of TBM according to the methods described herein. Thus, similar to sTNSALP discussed above, other soluble human ALP isozymes, i.e., those without the peptide signal and preferably comprising the extracellular domain of the ALPs, can be used in the methods. The sALPs also include polypeptide sequences satisfying a consensus sequence derived from the ALP extracellular domain of human ALP isozymes and of mammalian TNSALP orthologs (human, mouse, rat, cow, cat, and dog) or a consensus derived from the ALP extracellular domain of just mammalian TNSALP orthologs (human, mouse, rat, cow, cat, and dog). The sALPs also include those which satisfy similar consensus sequences derived from various combinations of these TNSALP orthologs or human ALP isozymes. Such consensus sequences are described, 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 Patent Application Publication No. 2013/0323244, hereby incorporated by reference in its entirety. An sALP can optionally be glycosylated at any appropriate one or more amino acid residues. An 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 TNSALP, 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, 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., TNSALP, 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 hTNSALP-Fc-D_{10} (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as 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 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 including a Fc region. For example, an 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) fragments. 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. Examples of engineered Fc regions are described in, e.g.,

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 an sALP fusion polypeptide. sALP fusion polypeptides can also lack a bone-targeting moiety.

The sALP fusion polypeptides (e.g., including a sALP variant having at least 95% sequence identity to the sequence of SEQ ID NO: 1, such as 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 can be glycosylated or PEGylated.

Production of Nucleic Acids and Polypeptides

The nucleic acids encoding an sALP (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as asfotase alfa) can be produced by any method known in the art. Typically, a nucleic acid encoding the desired polypeptide is generated using molecular cloning methods, and is generally placed within a vector, such as a plasmid or virus. The vector can be used, e.g., 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 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 (i.e., HYCLONE™, GE Healthcare; SFM4CHO, Sigma CHO DHFR$^-$; Cambrex POWER™ CHO CD supplemented with 2-4 mM L-glutamine, etc.). These media are desirably prepared without thymidine, hypoxanthine and L-glycine to maintain selective pressure, allowing stable protein-product expression.

Pharmaceutical compositions

A composition including an sALP that can be used in the methods (e.g., an sALP including an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1, such as asfotase alfa) can be administered by a variety of methods known in the art. For example, asfotase alfa (SEQ ID NO: 1) can be administered at a range of dosages, in a variety of formulations, and in combination with pharmaceutically acceptable excipients, carriers, or vehicles. In particular, asfotase alfa is a sterile, preservative-free, nonpyrogenic, clear, slightly opalescent or opalescent, colorless to slightly yellow, with few small translucent or white particles, aqueous solution that is formulated for, e.g., subcutaneous administration. Asfotase alfa can be supplied in glass single-use vials containing asfotase alfa in combination with dibasic sodium phosphate, heptahydrate; monobasic sodium phosphate, monohydrate; and sodium chloride at a pH between 7.2 and 7.6.

Dosage

A pharmaceutical composition including an sALP can be formulated for administration to HPP patients (e.g., infantile and perinatal-onset HPP patients) having TBM or one or more symptoms of TBM at a range of dosages. The dosages will depend on many factors including the mode of administration and the age of the patient (e.g., three years old or younger). Typically, the amount of the composition including an sALP contained within a single dose will be an amount that is effective to treat TBM or symptoms of TBM as described herein without inducing significant toxicity.

For example, an sALP can be formulated for administration to HPP patients having TBM or one or more symptoms of TBM, 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 an sALP include, but are not limited to, 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. Dosages of compositions including sALPs 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 formulated for administration, 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 having TBM or one or more symptoms of TBM (e.g., an infantile or perinatal-onset HPP patient).

An sALP can be formulated as a solution for injection, which is a clear, colorless to slightly yellow, aqueous solution, pH 7.4. The sALP may be formulated at a concentration of 12mg/0.3mL,18mg/0.45mL, 28mg/0.7mL, 40mg/1ml, or 80mg/0.8mL. For example, the composition can be formulated as a 40 mg/ml solution for injection, in which each ml of solution contains 40 mg of sALP (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)). Additionally, an sALP 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 (e.g., each vial contains 0.8 ml solution and 80 mg of asfotase alfa (100 mg/ml)).

For example, a dosage of an sALP can be 2 mg/kg of body weight administered subcutaneously three times per week, or 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

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

The sALP compositions 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.

Preparations containing an sALP can be provided to an HPP patient having TBM or one or more symptoms of TBM (e.g., an infant with HPP, such as an infant having perinatal-onset HPP), in combination with pharmaceutically acceptable excipients, carriers, or diluents. Examples of non-aqueous excipients, carriers, or diluents are propylene glycol, polyethylene glycol, vegetable oil, fish oil, and injectable organic esters. Aqueous excipients, carriers, or diluents 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.

Pharmaceutically acceptable salts can also be included in the sALP compositions, such as mineral acid salts including hydrochlorides, hydrobromides, phosphates, sulfates, and the salts of organic acids (e.g., acetates, propionates, malonates, and benzoates). 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.

Compositions including an sALP can also be formulated with pharmaceutically acceptable excipients, carriers, or diluents that will protect the sALP composition 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.

The following examples are intended to illustrate, rather than limit, the disclosure. These studies feature the administration of asfotase alfa (SEQ ID NO: 1) at, e.g., a dosage regimen of greater than or equal to 6 mg/kg/week, to treat tracheobronchomalacia (TBM) and symptoms thereof in patients with HPP.

EXAMPLES

Example 1. Overview of Tracheobronchomalacia (TBM) Cases

Treatment of tracheobronchomalacia (TBM) in 3 infants having hypophosphatasia (HPP) with asfotase alfa was initiated as part of a clinical trial and one under a compassionate use program. Four HPP patients with TBM (2 female and 2 male) were identified and treated with a soluble alkaline phosphatase (sALP) composition (STRENSIQ™ (asfotase alfa); SEQ ID NO: 1 as shown in Figure 1). Key inclusion criteria for the clinical trial included a patient age of 5 years old or younger with onset of HPP symptoms at less than 6 months old and low alkaline phosphatase (ALP) levels, high pyridoxal 5'-phosphate (PLP) levels, and radiographic evidence of HPP.

Additional inclusion criteria for the clinical trial included two or more of the following: non-traumatic post-natal fracture or delayed fracture healing, nephrocalcinosis or history of elevated serum calcium, functional craniosynostosis, respiratory compromise or rachitic chest deformity, pyridoxine (vitamin B6)-responsive seizures, and failure to thrive. Key exclusion criteria included serum calcium or phosphate levels below the normal range, serum 25-hydroxy vitamin D levels less than 20 ng/mL, and previous treatment with bisphosphonates. HPP diagnosis in each of the patients was confirmed by serum biochemistry analysis (e.g., ALP levels), supplemented by physical examination and skeletal survey. Mutations in the *ALPL* gene were found in all four patients as described herein.

Initial medical problems associated with HPP in these four patients included respiratory distress requiring respiratory support. TBM was identified by direct laryngotracheobronchoscopy or flexible bronchoscopy between two and five months of age. Respiratory support requirements were defined as mechanical ventilation via intubation or tracheostomy and ventilation by continuous positive airway pressure (CPAP) or intermittent positive pressure (IPP) ventilation. Requirements for positive-end expiratory pressure (PEEP) were also noted. Peak inspiratory pressure (PIP) was also measured.

All patients were treated with asfotase alfa at two months old or younger at a dosage of 6 mg/kg/week to 15 mg/kg/week. At birth, all four patients required ventilation with subsequent tracheostomy for long-term ventilation with PEEP. All patients experienced frequent episodes of profound desaturations and bradycardia, and three patients experienced cardio-respiratory arrests. When the patients were 15 to 24 months old, the TBM had resolved in two patients (off ventilator support), partially resolved in one patient (27 months old, ventilator support) and remained significant in one patient (23 months old, tracheostomy *in situ*). Without asfotase alfa treatment, these infants would not likely have survived to one year of age and there is little chance that their airways would have matured. Of the four patients treated, two patients experienced complete resolution of TBM, and one patient experienced

partial resolution of TBM, within two years of birth. An overview of treatment and patient outcomes based on these studies featuring treatment with asfotase alfa is shown in Table 2.

Table 2. Overview of treatment and patient outcomes during.

	Patient 1	Patient 2	Patient 3	Patient 4
Pharmaceutical treatments	• 1 mo 1 d: Asfotase alfa 6 mg/kg/wk • 2 mo 24 d: Asfotase alfa 9 mg/kg/wk	• 5 wk: Asfotase alfa 6 mg/kg/wk • 4 mo: Lorazepam and morphine for sedation • 6 mo: Asfotase alfa 7.8 mg/kg/wk • 9 mo: Asfotase alfa 7.5 mg/kg/wk • 9.5 mo: 9 mg/kg/wk	• 7 wk 4 d: Asfotase alfa 6 mg/kg/wk • 5 wk: Lorazepam 0.05 mg/kg every 6 h (for agitation) • 4 mo: Methadone (for sedation and pain) and continued lorazepam • 6 mo: Methadone weaned, midazolam added for agitation • 3yr, 4 mo. 12 mg/kg/wk	• 1 mo: Asfotase alfa 6 mg/kg/wk • 3 mo 14 d (post–cardiac arrest): Asfotase alfa 15 mg/kg/wk • 3 yr, 11 mo.: Asfotase alfa 2.5 mg/kg/wk
Surgical treatments	• 1 mo 8 d: Tracheostomy	• 6 wk: Tracheostomy • 4 mo: Gastrostomy	• 7 wk: Tracheostomy • 3 mo: Gastrostomy	• 1 mo 15 d: Tracheostomy • 1 yr Gastrostomy
Age at TBM diagnosis	2 mo	5 mo	6 mo (suspected at 8 wk)	5 mo
Additional findings	• 1 mo: CPAP with PEEP (5 cm H_2O) • 1 mo 2 d: Change from CPAP to IPPV PEEP and central venous line insertion; PEEP (8 cm H_2O) • 1 mo 5 d: PEEP (10 cm H_2O) • 2 mo: Severe TBM identified; PEEP (12 cm H_2O) • 9 mo: Flexible DLTB revealed normal patency of larynx, trachea, and left main bronchus; residual left bronchomalacia required PEEP (6 cm H_2O) • 12 mo: Repeat DLTB revealed resolution of TBM, with mild left bronchomalacia; weaned to CPAP; PEEP (5 cm H_2O)	• 3 mo: Respiratory arrest requiring bag and mask positive pressure ventilation • 4 and 6 mo: Cardio-respiratory arrest • 5 mo: MLB revealed Grade 3 stenosis of subglottis; flexible bronchoscopy revealed narrowed subglottic space and severe cervical tracheomalacia • 10 mo: TBM improved with mild dynamic collapse during restful breathing • 12 mo: Significant	• 7 wk: PEEP maintained at 6 cm H_2O • 8 wk: TBM suspected; PEEP increased to 10 cm H_2O • 2 mo: Cardio-respiratory arrest necessitating chest compressions with recovery • 3 mo: Severe cardio-respiratory episodes requiring major intervention began • 6 mo: Moderate/severe TBM confirmed (complete loss of airway lumen with coughing/heavy breathing) • 12 mo: Improvement to	• 1 mo: PIP (30 cm H_2O); PEEP (6 cm water) • 3 mo 10 d: Cardio-respiratory arrest • 3 mo, 14 d: Asfotase alfa dose increased to 15 mg/kg/wk; improvement seen within 2 wk and patient placed back into conventional ventilation • 5 mo: Significant TBM identified; PEEP (12 cm H_2O) • 8 mo: Significant improvement in respiratory function; PEEP (9 cm H_2O) • 11 mo: Complete resolution of TBM; PIP (10 cm H_2O); PEEP (5 cm water) • 12–22 mo: CPAP ventilation (with decreasing frequency); PEEP (4–5 cm H_2O)

	Patient 1	Patient 2	Patient 3	Patient 4
		improvement to mild TBM; respiratory arrest resolved; lorazepam discontinued • 13 mo: Discharged to home	moderate TBM; severe respiratory episodes resolved • 15 mo: Discharged to home	• 23 mo: Discharged to home; CPAP 1 night/wk; PEEP (5 cm H_2O)
Current status	• 15 mo: Complete clinical resolution of TBM and breathing room air	• 17 mo: Normal appearing lower airways, but TBM only when coughing or bearing down • 27 mo: Remained on ventilator; respiratory issues	• 18 mo: Significant TBM remained • 23 mo: Tracheostomy *in situ* with ventilator support	• 2 y: Complete clinical resolution of TBM, breathing room air

Example 2. TBM Patient 1

Patient 1 was a term male infant born to non-consanguineous parents (birth weight of 2,890 g). Immediately after birth, the patient presented poor feeding, significant hypotonia, and respiratory distress. Antenatal scans had shown shortening of long bones. He required CPAP ventilation on the first day after birth for respiratory distress. He received intravenous (IV) antibiotics (ampicillin and gentamicin) for 5 days because his mother was Group B Streptococcus positive. A skeletal survey of the patient at nine days old revealed skeletal manifestations of HPP with severe rickets and shortened long bones of the upper and lower limbs with bony spurs at the ends. There was absence of an ossification center in the patient's skull bones and the short bones of the patient's hands and feet. His biochemistry after birth was also suggestive of HPP, with elevated serum calcium of 3.04 mmol/L relative to the reference range of 2.25–2.74 mmol/L, and with undetectably low serum alkaline phosphatase (ALP) activity.

Patient 1 was transferred to a tertiary hospital and enrolled in the ENB-010-10 clinical trial at four weeks old. Consistent with the diagnosis of HPP, his pyridoxal 5'-phosphate (PLP) and inorganic pyrophosphate levels (PPi) were elevated prior to treatment. The PLP concentration was 4,740 ng/mL in comparison to the reference range of 11.8 ng/mL to 68.3 ng/mL, and the PPi concentration was 9.47 µM in comparison to the reference range of 1.33 µM to 5.7 µM.

Treatment of patient 1 with asfotase alfa at a dose of 2 mg/kg (6 mg/kg/week) commenced at one month and one day old. The patient was intubated and ventilated for general anaesthesia, and a central venous catheter was inserted for venous access. The patient could not be weaned off the ventilator as his respiratory function progressively worsened. He continued to have episodes of desaturations requiring an increase in PEEP from 5 cm to 8 cm H_2O.

A direct laryngotracheobronchoscopy (DLTB) performed at two months old (one month after commencing asfotase alfa) revealed severe laryngotracheobronchomalacia, which required PEEP of 12 cm H_2O to keep the airways patent. The dose of asfotase alfa was then increased to 3 mg/kg/dose (9 mg/kg/week). The patient's respiratory function progressively improved with a reduction in ventilator

pressures to PEEP of 6 cm H_2O. A flexible DLTB performed at nine months of age (eight months after commencing asfotase alfa) showed normal patency of the larynx, trachea, and left main bronchus with residual left bronchomalacia requiring PEEP of 6 cm H_2O to keep the airways patent.

DLTB was repeated under anaesthesia at 12 months of age, which revealed resolution of laryngotracheobronchomalacia with mild left bronchomalacia. The patient was changed from PEEP to CPAP. His respiratory function progressively improved, and he was self-ventilating in room air with complete clinical resolution of the laryngotracheobronchomalacia by 15 months of age. Genetic analysis revealed that the patient was positive for an autosomal recessive mutation in the *ALPL* gene, which was found to be secondary to uniparental disomy (see Hancarova *et al. Bone* 81:765-6, 2015, hereby incorporated by reference in its entirety). The timeline of key events for patient 1 is shown in Table 3.

Table 3: Timeline of key events for TBM patient 1

Age	Event
Pre-birth	Antenatal scans showed shortening of long bones
0	Poor feeding, significant hypotonia and respiratory distress. CPAP required at birth.
9 day	Skeletal radiographs revealed characteristics of HPP including severe rickets; long bones of upper and lower limbs were short, with bony spurs at their ends; and absence of ossification center of skull bones and of short bones of hand and feet.
1 month	Transferred to trial center. On CPAP with PEEP of 5 cm H_2O.
1 month 1 day	First dose of asfotase alfa administered at 2 mg/kg (6 mg/kg/week).
1 month 2 days	Central venous line insertion and change from CPAP to intermittent positive pressure ventilation (IPPV) or PEEP.
1 month 5 days	PEEP of 10 cm H_2O.
1 month 8 days	Tracheostomy for long term ventilation.
2 months 3 days	Diagnosed with laryngotracheobronchomalacia. PEEP increased to 12 cm H_2O.
2 months 24 days	Dose of asfotase alfa increased to 3 mg/kg (9 mg/kg/week) due to insufficient skeletal mineralisation.
3 months	Discharged to hospital.
9 months	DLTB performed. Resolution of tracheobronchomalacia with residual malacia of left bronchus. PEEP reduced to 6 cm H_2O.
12 months	DLTB under anaesthesia. Significant improvement with mild left bronchomalacia. Weaned to CPAP with PEEP of 5 cm H_2O.
15 months	Complete clinical resolution of TBM, and breathing room air.

Example 3. TBM Patient 2

Patient 2 was a male infant born by spontaneous vaginal delivery at 37 weeks and 5 days of gestation to non-consanguineous parents (birth weight of 3,460 g) with Apgar scores were 2 at 1 and 5

minutes and 5 at 10 minutes. The patient was intubated and placed on a ventilator. Radiographs at one day of age revealed that the patient had remarkably diminished ossification of the skull with almost no cranial calcification. He also had diminished ossification and height of vertebral bodies and absent ossification of the humeral, radial, and ulnar metaphyses with marked metaphyseal irregularity, fragmentation, and fraying. The patient's chest was small, and the patient's bones were abnormal with absent ossification of medial ribs and gracile appearance of the ribs (Figure 2A). His PLP (vitamin B_6) level was >2000 ng/mL. Symptoms of HPP in the patient's airways were also evident at 16 days of age in chest radiographs (Figure 2B). The patient was transferred to a tertiary care children's hospital at 27 days of age and enrolled in a clinical trial. His ALP was 14 U/L. He required a PEEP of 8 cm H_2O.

Treatment with asfotase alfa was initiated at 5 weeks of age at a dose of 1 mg/kg six times per week, during which the patient required PEEP of 8 cm H_2O. A tracheostomy was performed at 6 weeks of age. The patient required constant ventilator support with a rate initially maintained at 40 bpm. He had significant chest compliance and was maintained on high PEEP up to 12 cm H_2O.

Gastrostomy was performed at 4 months of age. The need for continuous ventilator support and events of respiratory and cardiac arrest led to microlaryngobronchoscopy (MLB) and flexible bronchoscopy being performed at 5 months of age. MLB showed normal supraglottis, a type II laryngeal cleft, a normal appearing glottis, and Grade III stenosis of subglottis. Flexible bronchoscopy showed normal bronchial branching pattern, the subglottic space was significantly narrowed, severe cervical tracheomalacia, mild suprastomal tracheal collapse, and mild dynamic bronchial collapse during restful breathing, which was predicted to be more severe during agitation or heavy breathing. The patient experienced cardiac arrest at four and six months of age.

The dose of asfotase alfa administered was increased to 1.3 mg/kg (7.8 mg/kg/week) at 6 months of age. The dose of asfotase alfa was then changed to 2.5 mg/kg (7.5 mg/kg/week) at 9 months of age. Following treatment with asfotase alfa, improvements in HPP were visible in chest radiographs of the patient at 9 months and 12 months of age (Figures 2C-2D). The patient's TBM improved with mild dynamic collapse during restful breathing at 10 months of age. Bronchoscopy showed significant improvement with mild TBM at 12 months of age. The episodes of cardio-respiratory arrest resolved, and lorazepam was able to be weaned and then discontinued. Transferral to a tertiary care center occurred at 13 months of age, and the patient subsequently was discharged to home. The patient remained on a ventilator and has experienced respiratory issues with viral infections and hospital readmission.

The lower airways appeared normal at 17 months of age, with no appreciable dynamic collapse during breathing at rest; however, TBM manifested only when the patient was coughing or bearing down.

Genetic analysis of the *ALPL* gene revealed the patient to be a compound heterozygote with the following mutations: c.668 G>A and c.1171 C>T. The timeline of key events is shown in Table 4.

Table 4: Timeline of key events for TBM patient 2

Age	Event
0	Intubated and placed on ventilator at birth for respiratory distress.
1 day	Chest radiographs revealed characteristics of HPP including diminished ossification of skull; almost no cranial calcification;

	diminished ossification and height of vertebral bodies; absent ossification of humeral, radial, and ulnar metaphyses; marked metaphyseal irregularity, fragmentation, and fraying; small chest; abnormal bones; absent ossification of medial ribs; and gracile appearance of ribs (Figure 2A).
16 days	Chest radiographs show progression of HPP (Figure 2B).
27 days	Transferred to study center hospital.
5 weeks	Started treatment with asfotase alfa of 1 mg/kg (6 mg/kg/week). PEEP of 8 cm H_2O required.
6 weeks	Tracheostomy.
3 months	Respiratory arrest, required positive pressure ventilation, and reattached to ventilator.
4 months	Gastrostomy. Intermittent oxygen desaturations not related to tracheal secretions. Treated with lorazepam and morphine for sedation. Episodes continued and responded to positive pressure ventilation. Cardio-respiratory arrest.
5 months	Laryngotracheoscopy and bronchoscopy revealed severe cervical tracheomalacia.
6 months	Cardio-respiratory arrest. Dose of asfotase alfa increased to 1.3 mg/kg (7.8 mg/kg/week).
9 months	Dose of asfotase alfa changed to 2.5 mg/kg (7.5 mg/kg/week). Improvement in HPP as evidenced by radiographs (Figure 2C).
10 months	TBM improved with mild dynamic collapse during restful breathing.
12 months	Significant improvement with mild TBM. Continued improvements in HPP as evidenced by radiographs (Figure 2D).
13 months	Patient transferred to a tertiary care center and subsequently discharged to home.
17 months	Normal appearing lower airways, TBM evidenced only when the patient was coughing or bearing down, hospital readmission only sporadic. TBM improved in Patient 2; the need for ventilator support persisted, although the patient is weaning from PEEP and has brief ventilator-free periods during the day.

Example 4. TBM Patient 3

Patient 3 was a female infant born to non-consanguineous parents. Her birth weight was 3.06 kg, length 45 cm, and head circumference 32 cm. She was delivered by a planned repeat caesarean section after spontaneous rupture of membranes. An abnormal fetal ultrasound was suggestive of a skeletal dysplasia, either HPP or osteogenesis imperfecta. A fetal echocardiogram was normal. Her Apgar scores were 6 at 1 minute, 7 at 5 minutes, and 8 at 10 minutes.

The patient initially required positive pressure ventilation (bagging) for the first 5 minutes, then intermittently for the next 5 minutes. She was intubated and placed on a ventilator. Initial examination showed very large anterior fontanelle, cranial moulding, small chest with subcostal retractions, and short

deformed extremities with bowing (equinovarus). Her feet were angulated and clubbed with dimpling below knees. She was transferred to a tertiary care children's hospital for diagnosis and management. Radiographs revealed severely decreased mineralization, diffuse osteopenia, poorly ossified ribs, irregularity of the right proximal humerus consistent with fracture and atelectasis, and bilateral humeral fractures. Renal ultrasound at one day of age was normal; however, there were focal areas of cortical echogenicity in both kidneys consistent with nephrocalcinosis at one month of age. Ionized calcium was 1.31 mmol/L and phosphorus was 6.8 mmol/L at eight days of age. Ventilator settings had a PEEP of 6 cm H_2O and a respirator rate of 20 bpm. The patient was transferred to the study center primary care hospital. Her ALP level was 18 U/L at five weeks. She required assisted ventilation and was treated with lorazepam (0.05 mg/kg) every 6 hours for agitation.

The patient had a tracheostomy at seven weeks of age. Bronchoscopy was also performed and revealed profound dynamic collapse of the trachea and bronchi during coughing or heavy breathing, even while intubated and receiving positive pressure. There was moderate collapse of the posterior tracheal wall with very light, intermittent suction, which was considered to be most likely due to a lack of outward elastic chest wall recoil. PEEP was maintained at 6 cm H_2O. The patient had a poor result at eight weeks of age on the non-invasive partial carbon dioxide rebreathing system (NICO) due to excessive chest compliance, lack of elastic recoil, and severe airway malacia. It was recommended to increase PEEP to 10 cm H_2O.

The patient was enrolled in a clinical trial at 7 weeks and 4 days of age to receive 1 mg/kg (6 mg/kg/week) of asfotase alfa. She had a respiratory arrest at two months of age with a drop in heart rate and oxygen desaturation, which necessitated chest compressions with recovery. The patient underwent microlaryngoscopy, bronchoscopy, and flexible tracheoscopy at 2.5 months of age. Significant findings were narrowed subglottis with lateral shelves, dynamic collapse, and moderate to severe tracheomalacia.

The patient had gastrostomy at three months of age. She was also noted to have respiratory episodes with severe oxygen desaturations requiring increased positive pressure ventilations (bagging). One episode was associated with a drop in heart rate necessitating chest compressions. Respiratory failure persisted and CO_2 levels remained elevated. Treatment with methadone was initiated to address sedation and pain issues.

Severe respiratory arrests requiring major intervention occurred on an almost weekly basis. Treatment with lorazepam was initiated along with methadone at four months of age. Bronchoscopy at five months of age showed profound bronchomalacia with complete loss of the airway lumen with coughing or heavy breathing. Methadone was weaned at six months of age. The patient also received midazolam as needed for agitation.

The severe cardio-respiratory episodes decreased in frequency. Flexible bronchoscopy at 12 months of age revealed moderate tracheobronchomalacia, which appeared to improve from her previous evaluation (six months), although still significant. The severe cardio-respiratory episodes resolved at 12 months of age. The patient was transferred to a primary care hospital at 15 months of age and was subsequently able to be discharged to home care.

Flexible bronchoscopy revealed significant TBM at 18 months of age. The severity of the tracheomalacia was difficult to assess as the long custom tracheostomy tube was very well positioned in the distal trachea.

Genetic analysis of the *ALPL* gene revealed the patient was a compound heterozygote with the following mutations: c.876_872delAGGGGACinsT and c.650T>C(p.V217A). The timeline of key events for patient 3 is shown in Table 5.

Table 5: Timeline of key events for TBM patient 3

Age	Event
Pre-birth	Fetal ultrasound suggestive of HPP or osteogenesis imperfecta.
0	Intubated and placed on ventilator for respiratory distress at birth. Transferred to tertiary care children's hospital.
1 day	Renal ultrasound normal.
4 weeks	Renal ultrasound consistent with nephrocalcinosis.
5 weeks	Patient transferred to study center primary care hospital.
7 weeks	Tracheostomy.
7 weeks 4 days	Enrolled in clinical trial, receiving asfotase alfa at 1 mg/kg (6 mg/kg/week).
3 months	Gastrostomy. Respiratory episodes with severe oxygen desaturation requiring bagging.
4 months	Lorazepam and methadone initiated.
5 months	Bronchomalacia with complete loss of airway lumen identified on bronchoscopy.
6 months	Methadone weaned. Midazolam as needed for agitation.
12 months	Moderate TBM with improvements from 6 months. Severe respiratory episodes resolved.
15 months	Transferred to primary care hospital and subsequently discharged to home care.
18 months	While significant TBM remained, patient remained at home without additional cardio-respiratory episodes.

Example 5. TBM Patient 4

Patient 4 was a 34-week preterm female infant born by normal vaginal delivery following premature rupture of membranes to consanguineous parents (birth weight was 1.69 kg). Immediately after birth, she was floppy with marked chest recessions. The patient required immediate intubation and ventilation to support breathing. Respiratory distress syndrome was suspected and surfactant therapy was administered. Despite low ventilator requirements, she continued to have significant respiratory distress and ventilation was continued.

The patient was found to have dysmorphic features with short limbs, craniotabes, and significant hypotonia. She was discussed with the tertiary metabolic bone disease team because she had hypercalcaemia with undetectably low ALP activity levels. Her skeletal survey revealed characteristic features of HPP. She was transferred to a tertiary neonatal unit for further management with asfotase alfa, under a compassionate use program. Her biochemistry results improved within seven days of commencing asfotase alfa, with a reduction in serum calcium levels. She continued to have increased ventilator requirements with significant episodes of bradycardia and desaturations, which required an increase in ventilator settings to peak inspiratory pressure (PIP) of 30 cm H_2O and PEEP of 6 cm H_2O. Despite adequate ventilation, she continued to have episodes of desaturations and bradycardia, which culminated in an acute deterioration at two months and nine days of age with profound desaturations, bradycardia requiring chest compression, and inotropes for cardiac arrest. Subsequently, the patient required high frequency oscillatory ventilation, nitric oxide, 100% oxygen requirement, and inotropes for support of cardiac function. Concerns that she might not survive the acute episode led to discussion of withdrawal of cardio-pulmonary-resuscitation with the parents.

Following discussion with a clinical research team, the dose of asfotase alfa was increased to 5 mg/kg three times a week (15 mg/kg/week) at 3 months and 13 days of age. Within 2 weeks of treatment with 15 mg/kg/week of asfotase alfa, she showed improvement and was able to receive conventional ventilation. However, she continued to have episodes of bradycardia and desaturations and a thorough review by the ENT and respiratory team was planned. A DLTB performed at 5 months of age demonstrated significant laryngotracheobronchomalacia, which required PEEP of 12 cm H_2O to keep the airways patent. Her respiratory function significantly improved with increase in PEEP and by 8 months of age, her PEEP was reduced to 9 cm H_2O.

A second DLTB was performed at 11 months of age, which showed complete resolution of laryngotracheobronchomalacia with opening pressure reduced to 4 cm H_2O. Her ventilator pressures were then weaned to PIP of 10 cm H_2O and PEEP of 5 cm H_2O. Intermittent CPAP ventilation was introduced by 12 months of age with PEEP of 4 to 5 cm of water. By 16 months of age, she was self-ventilating in room air for 12 hours of the day. She was discharged home at 23 months of age on CPAP for 1 night a week. Her ventilation was completely discontinued at two years of age.

Genetic analysis showed a mutation in the *ALPL* gene, homozygous for C.1336G7A (PA446T) mutation, with both parents' carriers for the mutation. The timeline of key events s shown in Table 6.

Table 6: Timeline of key events for TBM patient 4

Age	Event
0	Intubated and ventilated at birth.
3 days	Skeletal survey revealed characteristics of HPP including dysmorphic features (short limbs), craniotabes, and significant hypotonia.
5 days	HPP confirmed by biochemistry and skeletal survey images.
8 days	Discussed with clinical research team for compassionate

	use.
1 month	Transferred to neonatal unit.
1 months 15 days	Tracheostomy insertion for long-term ventilation.
2 months 13 days	Discharge from neonatal unit to paediatric intensive care unit. Pressures of PIP of 30 cm H_2O and PEEP of 6 cm H_2O.
3 months 10 days	Cardiac arrest requiring high frequency ventilation.
3 months 14 days	Increased dose to 5 mg/kg/dose (15 mg/kg/week). Indications of increased ventilator requirements.
5 months	DLTB and pressures increased to PEEP of 12 cm H_2O.
9 months	Transferred to high dependency unit (HDU). PEEP of 9 cm H_2O.
9.5 months	Transferred from HDU to long term ventilation unit.
10 months	PIP of 13 cm and PEEP of 8 cm H_2O.
11 months	DLTB improved PIP of 10 cm and PEEP of 5 cm H_2O.
13 months	CPAP mode of ventilation.
15 months	Off CPAP for 6 hours each day.
16 months	CPAP only at night.
22 months	CPAP only 2 nights a week. Pressure of 5 cm H_2O.
23 months	Discharged home on CPAP ventilation for 1 night per week. PEEP of 5 cm H_2O.
2 years	Complete clinical resolution of TBM, and breathing room air.

Example 6. Sustained improvements in respiratory function in infants and children treated with asfotase alfa

An open-label, multinational study was conducted to assess the efficacy and safety of asfotase alfa in a large cohort of patients with perinatal-onset HPP or infantile-onset HPP over approximately 168 weeks of treatment. Participants included 59 patients of five years of age or younger (27 male and 32 female patients) with first signs and symptoms of HPP prior to six months of age. These infants and children with perinatal-onset HPP or infantile-onset HPP received 6 to 9 mg/kg/week of asfotase alfa subcutaneously either as a dosage regimen of 1 mg/kg six times per week or 2 to 3 mg/kg three times per week.

Treatment of these infants and children having perinatal-onset HPP or infantile-onset HPP for three years or more with asfotase alfa resulted in significantly improved respiratory status, as indicated by reduced use of ventilation or supplemental oxygen. Of 19 HPP patients that required baseline respiratory support, this respiratory support was eliminated or reduced over the course of treatment for 11 patients (58%). Of the eight patients whose respiratory support was not reduced, five were on supplemental oxygen and three were on non-invasive ventilator support. Of the 40 patients who were free of baseline

respiratory support, most patients (35 of 40; 88%) remained so throughout the study. A total of five patients developed the need for respiratory support after baseline, and two of these patients were subsequently weaned off support, while one patient required invasive support until the last assessment. A total of two patients required ventilator support until their deaths from HPP; however, their TBM status was unknown. These patients still responded to asfotase alfa treatment, as evidenced by improved respiratory status (e.g., reduced use of ventilation or supplemental oxygen) and improvements in skeletal manifestations of HPP.

These results demonstrate that improvements in respiratory status of infants and children with perinatal-onset HPP, juvenile-onset, or infantile-onset HPP due to treatment with asfotase alfa were sustained over an extended treatment period (e.g., three or more years).

Example 7. Respiratory status of patients with an inferred post-diagnosis of tracheobronchomalacia

The following example provides information regarding the respiratory status of patients enrolled in a treatment study with STRENSIQ with an inferred post-diagnosis of TBM upon review of ventilator pressures recorded at the time of mechanical ventilation.

Study design

The study was a multicenter, open-label, multinational study of the safety, efficacy, and pharmacokinetics (PK) of asfotase alfa (STRENSIQ®) in infants and children up to and including 5 years of age with perinatal- or infantile-onset HPP. Perinatal- and infantile-onset HPP was defined as onset of first signs/symptoms from *in utero* to before 6 months of age.

The study included patients with a documented diagnosis of HPP with onset of symptoms prior to 6 months of age and age ≤5 years at study entry. This interim analysis includes 59 patients enrolled at 19 sites. Patients received a total of 6 mg/kg/week of asfotase alfa administered by subcutaneous (SC) injection, either as 1 mg/kg asfotase alfa 6 times per week or 2 mg/kg asfotase alfa 3 times per week per investigator discretion. Dose adjustments could be made for changes in weight and/or to improve safety and/or efficacy.

Baseline

There were 3 of 59 patients assessed in the interim analysis as presenting with TBM in the study (patients 11-13). Additionally, there were a total of 10 of 59 patients (patients 1-10) with an inferred post-diagnosis of TBM upon review of ventilator pressures recorded at the time of mechanical ventilation.

All 13 patients (7 male, 6 female) ranged in age from 0.1 weeks to 269.9 weeks old, and all displayed an abnormally shaped chest. At baseline, patient 1 required no respiratory support, patients 2-5, and 12 required endotracheal mechanical ventilation, patient 6 required supplemental oxygen, patients 7-10, and 13 required mechanical ventilation by tracheostomy, and patient 11 required continuous positive airway pressure (CPAP).\

Results

Patient 1 did not use respiratory support at study entry, but did require intermittent support (oxygen via facemask, bilevel or biphasic positive airway pressure (BiPAP), and continuous positive airway pressure (CPAP) due to respiratory distress shortly after the 12 weeks and up to week 26. Inspiratory pressure and expiratory pressure during BiPAP was 18 and 8 cm H_2O, respectively. She was free of respiratory support from week 36 through week 216.

Patient 2 required continuous mechanical ventilation via endotracheal tube for respiratory support from study entry until week 24, when he was weaned from the ventilator and placed on supplemental O_2 via nasal prongs. By week 36, he was free of respiratory support and remained free of support through week 192. At baseline, the mechanical ventilation inspiratory pressure was 0.35 cm H_2O and expiratory pressure was 10 cm H_2O. At week 3, mechanical ventilation inspiratory pressure was 18 cm H_2O and expiratory pressure was 13 cm H_2O. At week 6, mechanical ventilation inspiratory pressure was 33 cm H_2O and expiratory pressure was 12 cm H_2O. At week 12, mechanical ventilation inspiratory pressure was 24 cm H_2O and expiratory pressure was 7 cm H_2O.

Patient 3 required intubation at birth, with continuous mechanical ventilation respiratory support at baseline and throughout the time period evaluated in this study. The endotracheal tube was exchanged with a tracheostomy at week 24, which remained in place at the patient's last recorded visit at week 60. The patient died after experiencing acute respiratory failure on several occasions, with a background of atelectasis and pulmonary hypertension. Inspiratory pressure / expiratory pressure at baseline and weeks 3, 6, 12, 24, 36, 48, and 60 were - / 10 cm H_2O, 29 / 12 cm H_2O, 24 / 9 cm H_2O, 26 / 10 cm H_2O, 32 / 11 cm H_2O, 40 / 10 cm H_2O, 29 / 12 cm H_2O, and 33 / 14 cm H_2O, respectively.

Patient 4 required mechanical ventilation via endotracheal tube/tracheostomy tube for respiratory support at baseline through week 48. At week 60, the patient was receiving continuous positive airway pressure (CPAP) and from week 72 through week 168 the patient was once again receiving mechanical ventilation via tracheostomy. Mechanical ventilation inspiratory pressure / expiratory pressure at baseline and weeks 3, 6, 12, 24, 36, 48, 60, 72, 96, 120, 144, and 168 were 26 / 7 cm H_2O, 30 / 7.5 cm H_2O, 30 / 9 cm H_2O, 29 / 8.7 cm H_2O, 27 / 6 cm H_2O, 13 / 4 cm H_2O, 14 / 5 cm H_2O, 5 / 5 cm H_2O (week 60, as measured by CPAP), 24 / 5 cm H_2O, 12 / 5 cm H_2O, 12 / 5 cm H_2O, 13 / 5 cm H_2O, and 15 / 5 cm H_2O, respectively.

Patient 5 was placed on mechanical ventilation via endotracheal tube prior to starting the study; he was reported to have had multiple seizures and severe hypoxic episodes requiring increased oxygenation and positive pressure ventilation. The patient continued to require respiratory support at Baseline and at the last milestone visit at week 3, at which time the patient was discontinued from the study. After 2 doses of study drug this patient was withdrawn from the study by family/medical consensus when a brain magnetic resonance imaging (MRI) showed hypoxia induced lesions and encephalopathy (assessed as an unlikely related serious adverse event (SAE)). The patient died less than a week later of "respiratory failure and cerebral death", also assessed as unlikely related to the study drug and more likely due to the complications of the seizures. Mechanical ventilation inspiratory pressure / expiratory at baseline and at week 3 were 17 / 7 cm H_2O and 22 / 7 cm H_2O, respectively.

Patient 6 required 24-hour supplementary oxygen at baseline due to difficulty breathing, and received mechanical ventilation (endotracheal or CPAP) from week 6 (just prior to a dose increase at week 7) through week 36 due to pneumonia, difficulty breathing, and/or respiratory worsening. At Week 48, the patient was reported to receive only intermittent supplementary oxygen; no respiratory support was received week 60 through week 96. Mechanical inspiratory pressure / expiratory pressure was 16 / 6 cm H_2O at baseline. Endotracheal inspiratory pressure / expiratory pressure at week 6 was 16 / 6 cm H_2O. CPAP inspiratory pressure / expiratory pressure at weeks 12, 24, and 36 were 17 / 6 cm H_2O, 8 / 6 cm H_2O, and - / 6, respectively.

Patient 7 was intubated prior to enrollment and removed from endotracheal mechanical ventilation support 1 day prior to the baseline visit. She was on nasal CPAP (non-invasive ventilation) for 1 day prior and at the start of study treatment. The patient used 24-hour oxygen support (30 L/Min) at baseline. Her need for oxygen support varied throughout the study, including BiPAP at week 6, manual resuscitation via bag mask at approximately week 9, mechanical ventilation via tracheostomy at week 12 and week 48 through week 72. Patient was on no ventilation support at week 96. Inspiratory pressure / expiratory pressure during ventilation support at baseline and weeks 3, 6, 12, 24, 36, and 48, were 31 / 7 cm H_2O, 25 / 7 cm H_2O, 18 / 10 cm H_2O, 21 / 8 cm H_2O, 25 / 5 cm H_2O, 25 / 5 cm H_2O, and 25 / 5 cm H_2O, respectively.

Patient 8 used mechanical ventilation with intermittent mandatory ventilation 10 times per minute at night via tracheostomy at baseline (inspiratory pressure 20.0 cm H_2O, expiratory pressure 5.0 cm H_2O). This level of mechanical ventilation continued until week 24. From Week 36 onward, no respiratory suppor" was noted.

Patient 9 required 24-hour mechanical ventilation via tracheostomy at baseline (inspiratory pressure 32.0 cm H_2O, expiratory pressure 6.0 cm H_2O, FiO_2 35.0%, 35.0 L O_2/min), which continued through week 72 with some improvement observed in lower inspiratory and expiratory pressures, ventilator rates, and FiO_2. Mechanical ventilation inspiratory / expiratory pressure at baseline and weeks 36, 48, and 60-72 were 32 / 6 cm H_2O, 26 / 6 cm H_2O, 22 / 5 cm H_2O, and 22 / 5 cm H_2O, respectively.

Patient 10 required 24-hour mechanical ventilation via tracheostomy at baseline. Inspiratory pressure of the mechanical ventilation was reduced over time, and from week 24 onward (through week 72) the patient required no respiratory support. Mechanical ventilation inspiratory / expiratory pressure at baseline and weeks 3-6 and 12 were 25 / - cm H_2O, 17 / - cm H_2O, and 11 / - cm H_2O, respectively.

Patient 11 was receiving ventilation via CPAP (FiO_2 30%, 24 hours a day) at baseline. At weeks 3, 6, and 12, the patient was receiving mechanical ventilation via tracheostomy. At week 24, the patient continued to receive ventilation support. CPAP inspiratory pressure / expiratory pressure was - / 5 cm H_2O at baseline. Mechanical ventilation inspiratory / expiratory pressure at weeks 3, 6, 12, and 24 were 18 / 10 cm H_2O, 20 / 12 cm H_2O, 1 / 3.4 cm H_2O, and 15 / 10 cm H_2O, respectively.

Patient 12 received 24-hour mechanical respiratory support (endotracheal and tracheostomy) during the study observation period from baseline to week 36, the last study visit prior to data cut-off. Mechanical ventilation inspiratory pressure / expiratory at baseline and weeks 3, 6, 12, 24, and 36 were 32 / 8 cm H_2O, 35 / 11 cm H_2O, 28 / 12 cm H_2O, 31 / 10 cm H_2O, 30 / 12 cm H_2O, and 34 / 13 cm H_2O, respectively.

Patient 13 was on mechanical ventilation (tracheostomy) from baseline to week 12. Mechanical ventilation inspiratory pressure / expiratory pressure at baseline, weeks 3-6, and week 12 were 24 / 6 cm H_2O, 30 / 8 cm H_2O, and 32 / 12 cm H_2O, respectively.

Of the 13 patients studied, the inspiratory / expiratory ratio decreased during the course of asfotase alfa treatment in 8 patients and increased in 3 patients. Insufficient data was collected for the remaining 2 patients. Inspiratory pressure increased in 6 of the 11 patients and expiratory pressure decreased or stayed the same in 7 of the patients, indicating an increase in lung capacity. Furthermore, 6 of the 13 patients required no ventilation support by the end of the testing period, which is indicative of a significant improvement in respiratory function following asfotase alfa treatment in patients with TBM.

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 tracheobronchomalacia (TBM) in a patient having hypophosphatasia (HPP) comprising administering a soluble alkaline phosphatase (sALP) to the patient in a dosage regimen that provides greater than or equal to 6 mg/kg/week of the sALP to the patient, wherein the sALP comprises asfotase alfa (SEQ ID NO: 1) or 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 improvement in TBM in the patient.

2. The method of claim 1, wherein the sALP is administered twice a week, three times a week, four times a week, five times a week, six times a week, or seven times a week.

3. The method of claim 1 or 2, wherein the dosage regimen provides about 6.5 mg/kg/week to about 25 mg/kg/week of the sALP to the patient.

4. The method of any one of claims 1 to 3, wherein the dosage regimen provides about 6.5 mg/kg/week of the sALP, about 7 mg/kg/week of the sALP, about 7.5 mg/kg/week of the sALP, about 7.8 mg/kg/week of the sALP, about 8 mg/kg/week of the sALP, about 8.5 mg/kg/week of the sALP, about 9 mg/kg/week of the sALP, about 10 mg/kg/week of the sALP, about 10.5 mg/kg/week of the sALP, about 11 mg/kg/week of the sALP, about 11.5 mg/kg/week of the sALP, about 12 mg/kg/week of the sALP, about 12.5 mg/kg/week of the sALP, about 13 mg/kg/week of the sALP, about 13.5 mg/kg/week of the sALP, about 14 mg/kg/week of the sALP, about 14.5 mg/kg/week of the sALP, about 15 mg/kg/week of the sALP, about 16 mg/kg/week of the sALP, about 17 mg/kg/week of the sALP, about 18 mg/kg/week of the sALP, about 19 mg/kg/week of the sALP, about 20 mg/kg/week of the sALP, about 21 mg/kg/week of the sALP, about 22 mg/kg/week of the sALP, about 23 mg/kg/week of the sALP, about 24 mg/kg/week of the sALP, or about 25 mg/kg/week of the sALP to the patient.

5. The method of any one of claims 1 to 4, wherein the dosage regimen comprises administering about 3 mg/kg of the sALP three times a week, about 2.5 mg/kg of the sALP three times a week, about 1.3 mg/kg of the sALP six times a week, or about 5 mg/kg of the sALP three times a week.

6. The method of any one of claims 1 to 5, wherein said TBM comprises one or more symptoms of TBM selected from the group comprising cardio-respiratory arrest, tracheostomy, cardiac arrest, respiratory distress, sputum retention, wheezing, coughing, anoxic spells, cyanosis, bradycardia, tachyarrhythmia, spontaneous hyperextension of the neck, prolonged expiratory breathing phase, failure to thrive, sternal retractions, substernal retractions, intercostal retractions, intermittent dyspnea, continuous dyspnea, recurrent bronchitis, and recurrent pneumonia.

7. The method of claim 6, wherein the patient exhibits an improvement in one or more of the symptoms of TBM following administration of the sALP.

8. The method of claim 6, wherein the method further comprises increasing the dosage of the sALP if the patient does not exhibit an improvement in one or more of the symptoms of TBM following administration of the sALP for a treatment period of at least two weeks, three weeks, one month, two months, three months, four months, five months, or six months.

9. The method of claim 8, wherein the patient exhibits an improvement in one or more of the symptoms of TBM after receiving an increased dosage of the sALP.

10. The method of claim 9, wherein the patient exhibits an improvement in one or more of the symptoms of TBM after a treatment period of about one week, about two weeks, about three weeks, about one month, about two months, about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, about eleven months, or about one year.

11. The method of any one of claims 6 to 10, wherein one or more of the symptoms of TBM are present in the patient at birth.

12. The method of any one of claims 6 to 10, wherein one or more of the symptoms of TBM develop in the patient subsequent to birth.

13. The method of any one of claims 1 to 12, wherein the patient requires ventilator support prior to administration of the sALP.

14. The method of claim 13, wherein the patient exhibits decreased reliance on ventilator support, or no longer requires ventilator support, after administration of the sALP.

15. The method of any one of claims 1 to 14, wherein the patient is diagnosed with TBM prior to administration of the sALP.

16. The method of any one of claims 1 to 15, wherein the improvement in TBM is sustained throughout administration of the sALP for a treatment period of 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.

17. The method of any one of claims 1 to 16, wherein, prior to or after administration of the sALP to the patient, the method further comprises performing a tracheostomy on the patient.

18. The method of any one of claims 1 to 17, wherein the improvement in TBM is relative to an untreated HPP patient having TBM.

19. The method of any one of claims 1 to 18, wherein, prior to or after administration of the sALP to the patient, the method further comprises performing a bronchoscopy on the patient.

20. The method of any one of claims 1 to 19, wherein the patient requires at least one of high frequency oscillatory ventilation, positive end-expiratory pressure (PEEP), continuous positive airway pressure (CPAP), bilevel or biphasic positive airway pressure (BiPAP), and intermittent positive pressure ventilation (IPPV), prior to and/or concurrently with administration of the sALP.

21. The method of claim 20, wherein the PEEP is about 5 cm H_2O to about 15 cm H_2O.

22. The method of claim 21, wherein the PEEP is about 5 cm H_2O, about 6 cm H_2O, about 7 cm H_2O, about 8 cm H_2O, about 9 cm H_2O, about 10 cm H_2O, about 11 cm H_2O, about 12 cm H_2O, about 13 cm H_2O, about 14 cm H_2O, or about 15 cm H_2O.

23. The method of any one of claims 20 to 22, wherein administration of the sALP results in a decrease in the PEEP required by the patient.

24. The method of claim 23, wherein the PEEP required by the patient decreases by about 1 cm H_2O, about 2 cm H_2O, about 3 cm H_2O, about 4 cm H_2O, about 5 cm H_2O, about 6 cm H_2O, about 7 cm H_2O, about 8 cm H_2O, about 9 cm H_2O, or about 10 cm H_2O.

25. The method of claim 23 or 24, wherein the sALP is administered for a treatment period of about one month, about two months, about three months, about four months, about five months, about six months, about seven months, about eight months, about nine months, about ten months, or longer.

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

27. The method of any one of claims 1 to 26, wherein the patient is an infant.

28. The method of any one of claims 1 to 27, wherein administration of the sALP occurs about one month, about two months, about three months, about four months, about five months, or about six months after birth.

29. The method of any one of claims 1 to 28, wherein the patient has at least one of perinatal-onset HPP and infantile-onset HPP.

30. The method of any one of claims 1 to 29, wherein the patient is a human.

31. The method of any one of claims 1 to 30, wherein the patient exhibits one or more symptoms of HPP selected from the group consisting of skeletal deformity, hypotonia, mobility impairments, bone deformity, joint pain, bone pain, muscle pain, bone fracture, muscle weakness, rickets, premature loss of deciduous teeth, incomplete bone mineralization, elevated blood and/or urine levels of phosphoethanolamine (PEA), elevated blood and/or urine levels of inorganic pyrophosphate (PPi), elevated blood and/or urine levels of pyridoxal 5'-phosphate (PLP), hypomineralization, rachitic ribs, hypercalciuria, short stature, waddling gait, HPP-related seizure, inadequate weight gain, craniosynostosis, and calcium pyrophosphate dihydrate crystal deposition.

32. The method of claim 31, wherein one or more of the symptoms of HPP are present in the patient at birth.

33. The method of claim 31, wherein one or more of the symptoms of HPP develop in the patient subsequent to birth.

34. The method of any one of claims 31 to 33, wherein the patient exhibits an improvement in one or more of the symptoms of HPP after administration of the sALP.

35. The method of any one of claims 1 to 34, wherein administration of the sALP increases survival of the patient.

36. The method of any one of claims 1 to 35, wherein the method further comprises determining whether the patient has a mutation in the patient's tissue non-specific alkaline phosphatase (TNALP) gene.

37. The method of claim 36, wherein the mutation in the TNALP gene is associated with HPP.

38. The method of any one of claims 1 to 37, wherein the sALP is administered to the patient in a composition comprising a pharmaceutically acceptable excipient, carrier, or diluent.

39. The method of claim 38, wherein the pharmaceutically acceptable excipient, carrier, or diluent comprises saline.

40. The method of claim 39, wherein the pharmaceutically acceptable excipient, carrier, or diluent comprises sodium chloride and sodium phosphate.

41. The method of claim 40, wherein the pharmaceutically acceptable excipient, carrier, or diluent comprises 150 mM sodium chloride and 25 mM sodium phosphate.

42. The method of any one of claims 38 to 41, wherein the composition is administered to the patient parenterally, enterally, or topically.

43. The method of claim 42, wherein the composition is administered to the patient subcutaneously, intravenously, intramuscularly, intra-arterially, intrathecally, or intraperitoneally.

44. The method of claim 43, wherein the composition is administered to the patient by subcutaneous injection.

45. The method of any one of claims 1 to 44, wherein the sALP is administered on consecutive or alternating days.

46. The method of any one of claims 1 to 45, wherein the sALP is physiologically active toward PEA, PPi, and PLP.

47. The method of any one of claims 1 to 46, wherein the sALP is catalytically competent to improve skeletal mineralization in bone.

48. The method of any one of claims 1 to 47, wherein the sALP is the soluble extracellular domain of an ALP.

49. The method of any one of claims 1 to 48, wherein the method further comprises determining sALP activity in a serum sample and/or blood sample from the patient.

50. The method of claim 49, wherein the determining sALP activity comprises measuring the concentration of at least one of phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and pyridoxal 5'-phosphate (PLP) in the serum sample and/or blood sample.

51. The method of any one of claims 1 to 50, wherein the sALP comprises an amino acid sequence having at least 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of SEQ ID NO: 1.

52. The method of any one of claims 1 to 51, wherein the sALP comprises or consists of the amino acid sequence of SEQ ID NO: 1.

53. Use of a soluble alkaline phosphatase (sALP) comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1 in the manufacture of a medicament for treating tracheobronchomalacia (TBM) in a patient according to a dosage regimen, wherein the dosage regimen provides greater than or equal to 6 mg/kg/week of the sALP to the patient.

54. A soluble alkaline phosphatase (sALP) comprising an amino acid sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 1 for treating tracheobronchomalacia (TBM) in a patient having hypophosphatasia (HPP), wherein the sALP is administered to the patient in a dosage

regimen that provides greater than or equal to 6 mg/kg/week of the sALP to the patient, and wherein the sALP promotes an improvement in TBM in the patient.

LVPEKEKDPK YWRDQAQETL KYALELQKLN TNVAKNVIMF LGDGMGVSTV TAARILKGQL
HHNPGEETRL EMDKFPFVAL SKTYNTNAQV PDSAGTATAY LCGVKANEGT VGVSAATERS
RCNTTQGNEV TSILRWAKDA GKSVGIVTTT RVNHATPSAA YAHSADRDWY SDNEMPPEAL
SQGCKDIAYQ LMHNIRDIDV IMGGGRKYMY PKNKTDVEYE SDEKARGTRL DGLDLVDTWK
SFKPRYKHSH FIWNRTELLT LDPHNVDYLL GLFEPGDMQY ELNRNNVTDP SLSEMVVVAI
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DHSHVFTFGG YTPRGNSIFG LAPMLSDTDK KPFTAILYGN GPGYKVVGGE RENVSMVDYA
HNNYQAQSAV PLRHETHGGE DVAVFSKGPM AHLLHGVHEQ NYVPHVMAYA ACIGANLGHC
APASSLKDKT HTCPPCPAPE LLGGPSVFLF PPKPKDTLMI SRTPEVTCVV VDVSHEDPEV
KFNWYVDGVE VHNAKTKPRE EQYNSTYRVV SVLTVLHQDW LNGKEYKCKV SNKALPAPIE
KTISKAKGQP REPQVYTLPP SREEMTKNQV SLTCLVKGFY PSDIAVEWES NGQPENNYKT
TPPVLDSDGS FFLYSKLTVD KSRWQQGNVF SCSVMHEALH NHYTQKSLSL SPGKDIDDDD
DDDDDD **(SEQ ID NO: 1)**

FIG. 1

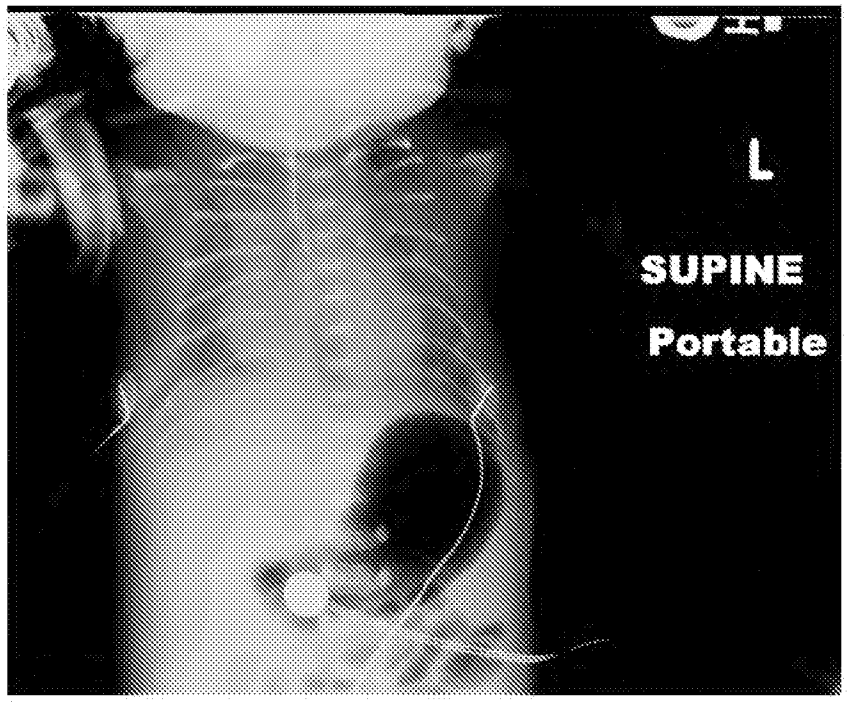

FIG. 2A

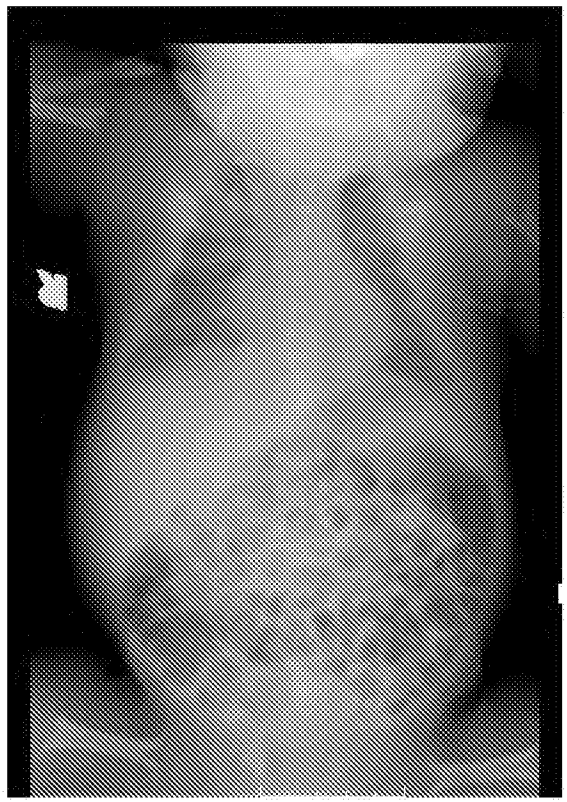

FIG. 2B

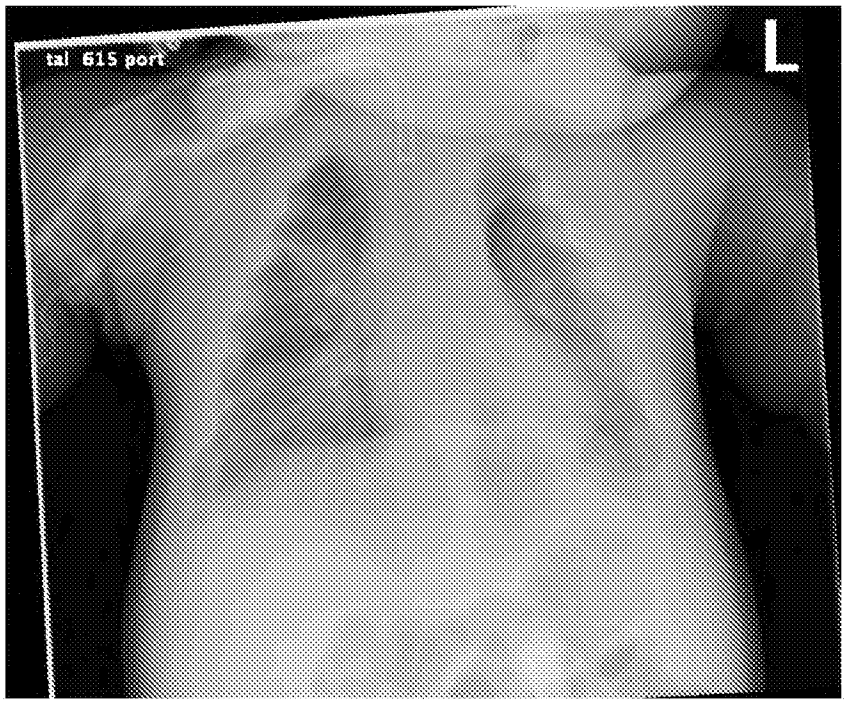

FIG. 2C

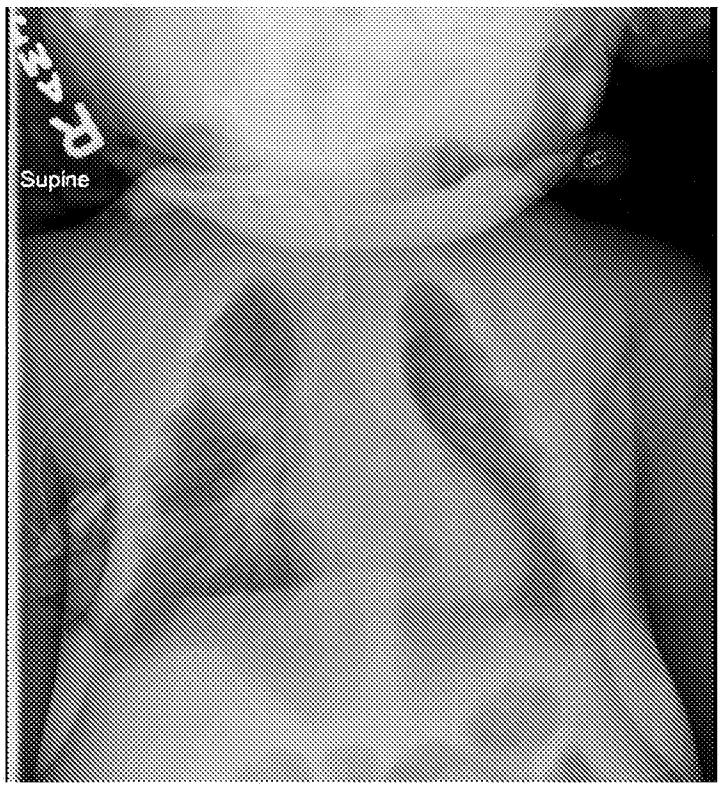

FIG. 2D

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/047527

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☒ forming part of the international application as filed:

      ☒ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:

      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

SEQ ID NOs: 1-20 were searched.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/047527

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.: 4-52
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.

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2017/047527

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/16; A61K 38/46; A61P 19/00; C07K 19/00; C12N 9/16; C12N 15/55 (2017.01)
CPC - A61K 38/00; A61K 38/465; C12N 9/16; C12Y 301/03001 (2017.08)

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 - 424/94.6; 435/196; 514/2; 536/23.2 (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	WO 2016/007873 A1 (THE REGENTS OF THE UNIVERSITY OF MICHIGAN) 14 January 2016 (14.01.2016) entire document	53
A	WO 2016/090251 A1 (ALEXION PHARMACEUTICALS, INC.) 09 June 2016 (09.06.2016) entire document	1-3, 53, 54
A	WO 2013/058833 A1 (ENOBIA CANADA LIMITED PARTNERSHIP et al) 25 April 2013 (25.04.2013) entire document	1-3, 53, 54
A	MILLÁN et al. "Hypophosphatasia - pathophysiology and treatment," Actual osteol, 01 September 2012 (01.09.2012), Vol. 8, Pgs. 164-182. entire document	1-3, 53, 54
P, X	PADIDELA et al. "P1-118: Management of Tracheobronchomalacia During Asfotase Alfa Treatment in Infants With Perinatal-Onset Hypophosphatasia: A Case Series," 10 September 2016 (10.09.2016), European Society for Paediatric Endocrinology (ESPE) 2016, Pg. 1 of 1. entire document	1-3, 53, 54

☐ 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
16 October 2017	06 NOV 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