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(54) Title: METHODS FOR TREATING CRANIOSYNOSTOSIS IN A PATIENT

(57) Abstract: The disclosure features methods for treating craniosynostosis in a patient (e.g., a patient having hypophosphatasia (HPP) and exhibiting or likely to have increased intracranial pressure (ICP)) by administering a soluble alkaline phosphatase (sALP) to the patient, e.g., in combination with a cranial surgery, e.g., a cranial vault remodeling procedure.

# METHODS FOR TREATING CRANIOSYNOSTOSIS IN A PATIENT

## FIELD

The disclosure concerns methods to treat craniosynostosis using a soluble alkaline phosphatase (sALP).

## 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. HPP is often fatal when observed at birth and has a high infant mortality rate of ~70%. Severely affected patients often die in infancy from respiratory insufficiency due to progressive chest deformity. The disorder results from loss-of-function mutations in the gene coding for tissue-nonspecific alkaline phosphatase (TNALP). TNALP activity plays an essential role in the development of the bone matrix. In particular, TNALP is an ectoenzyme present on the outer surface of osteoblast and chondrocyte cell membranes that hydrolyzes inorganic pyrophosphate (PPi), pyridoxal 5'-phosphate (PLP), and phosphoethanolamine (PEA). The primary role of TNALP *in vivo* is to regulate the extracellular PPi pool, as PPi is a potent inhibitor of bone mineralization. When there is a deficiency in TNALP activity, such as in HPP, PPi accumulates, which results in the inhibition of bone mineralization.

HPP leads to a remarkable range of symptoms and severity, from rickets (osteomalacia) to almost complete absence of bone mineralization *in utero*. Most patients exhibit the characteristics of skeletal changes, short stature, painful lower limbs, gait disturbance, and premature shedding of teeth. Bones of the cranium can also be affected, resulting in complex forms of craniosynostosis, particularly in cases of perinatal, childhood, and infantile HPP.

Craniosynostosis is a debilitating condition in which there is premature ossification of cranial sutures. Patients with craniosynostosis often suffer from high intracranial pressure, abnormal skull and facial shapes, airway impairments, obstructive sleep apnea, brain abnormalities, blindness, deafness, seizures, impairments in mental development, and death. The primary treatment available for craniosynostosis is surgical treatment with cranial vault remodeling in combination with genetic counseling, dental, and medical support.

Notably, surgically-corrected craniosynostosis may re-fuse necessitating multiple surgeries throughout infancy and childhood to relieve intracranial pressure, treat recurrent craniosynostosis, and normalize skull and facial shapes. Even with an early and accurate diagnosis, craniosynostosis has a high morbidity. Thus, there exists a need for methods that can be used to treat HPP patients with craniosynostosis.

## SUMMARY

A first aspect of one embodiment features a method of treating craniosynostosis in a patient having hypophosphatasia (HPP) (e.g., a human). For example, the patient can exhibit or is likely to have increased intracranial pressure (ICP). The method includes administering a soluble alkaline phosphatase (sALP) to the patient, e.g., in combination with a cranial vault remodeling procedure.

In an embodiment, the sALP is administered to the patient prior to a cranial vault remodeling procedure. Alternatively, the sALP is administered to the patient after a cranial vault remodeling procedure. In particular, the sALP is administered to the patient about two months to about 1 day, particularly six weeks, one month, three weeks, two weeks, one week, 6 days, 5 days, four days, or two days, prior to or after a cranial vault remodeling procedure. For example, the sALP is administered about three weeks prior to or after the cranial vault remodeling procedure. Additionally, the sALP may be administered to the patient prior to premature fusion of cranial sutures.

In an embodiment, the patient is diagnosed with craniosynostosis requiring surgical correction prior to administration of the sALP. Alternatively, the patient is diagnosed with craniosynostosis requiring surgical correction prior to a cranial vault remodeling procedure. For example, craniosynostosis may be diagnosed by clinical examination, radiography (three-dimensional (3D) computed tomography (CT)), and/or ultrasonography.

In several embodiments of the first aspect of the disclosure, the patient is a human. In particular, the patient has infantile HPP, childhood HPP, perinatal benign HPP, or perinatal lethal HPP.

In various embodiments of the first aspect of the disclosure, the patient exhibits one or more additional symptoms of craniosynostosis, e.g., headaches, irritability, nausea and emesis (vomiting), pulsatile tinnitus, hearing loss, abnormal skull and facial shapes, airway impairments, obstructive sleep apnea, Chiari Type I malformation, brain abnormalities, papilledema, optic nerve damage, blindness, vision impairment, double vision, decreased visual acuity, deafness, seizures, impairments in mental development, herniation of cerebellar tonsils, syringomyelia, bilateral papilledema, nystagmus, microcephaly, brachycephaly, dolichocephaly, ventilator dependence, and/or chronic optic nerve edema.

In an embodiment of the first aspect of the disclosure, the method further includes monitoring ICP in the patient. For example, the method further includes monitoring one or more additional symptoms of craniosynostosis in the patient, such as by one or more of radiography (e.g., CT scan), ultrasonography, clinical examination, and/or determination of sALP activity. In particular, the determination of sALP activity includes measuring at least one of phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and/or pyridoxal 5'-phosphate (PLP) in a serum and/or blood sample from the patient. In particular embodiments, the sALP activity is below the age-adjusted normal range.

In various embodiments of the first aspect of the disclosure, the sALP is administered in an amount that is therapeutically effective to treat increased ICP. Furthermore, the sALP may be administered to treat and/or ameliorate one or more additional symptoms of craniosynostosis, e.g., headaches, irritability, nausea and emesis (vomiting), pulsatile tinnitus, hearing loss, abnormal skull and facial shapes, airway impairments, obstructive sleep apnea, Chiari Type I malformation, brain abnormalities, blindness, vision impairment, double vision, decreased visual acuity, deafness, seizures, impairments in mental development, herniation of cerebellar tonsils, syringomyelia, bilateral papilledema, nystagmus, microcephaly, brachycephaly, dolichocephaly, ventilator dependence, and/or chronic optic nerve edema.

In some embodiments of the first aspect of the disclosure, the sALP is administered in an amount that is therapeutically effective to treat at least one HPP phenotype, e.g., including one or more of premature loss of deciduous teeth, incomplete bone mineralization, elevated blood and/or urine levels of

$PP_i$, elevated blood and/or urine levels of PEA, elevated blood and/or urine levels of PLP (particularly where PLP levels are at least twice the age-adjusted upper limit of normal), hypomineralization, rachitic ribs, hypercalciuria, short stature, skeletal deformity, waddling gait, bone pain, bone fracture, HPP-related seizure, inadequate weight gain, rickets, and/or calcium pyrophosphate dihydrate crystal deposition.

In some embodiments of the first aspect of the disclosure, the sALP is formulated in a pharmaceutical composition, with a pharmaceutically acceptable carrier (e.g., saline). In various embodiments, the pharmaceutical composition is formulated for intramuscular, subcutaneous, intravenous, oral, nasal, sublingual, intrathecal, or intradermal administration. For example, the pharmaceutical composition can be formulated for daily or weekly administration, e.g., in which the sALP is administered to the patient at a dosage of about 0.1 mg/kg to about 20 mg/kg, or at a weekly dosage of about 0.5 mg/kg to about 140 mg/kg.

In preferred embodiments of the first aspect of the disclosure, the sALP is physiologically active toward PEA, PPi, and PLP. For example, the sALP is catalytically competent to improve skeletal mineralization in bone.

In various embodiments of the first aspect of the disclosure, the sALP is the soluble extracellular domain of an alkaline phosphatase, e.g., in which the sALP is selected from the group consisting of tissue non-specific alkaline phosphatase (TNALP), placental alkaline phosphatase (PALP; e.g., SEQ ID NOs: 15 or 16), germ cell alkaline phosphatase (GCALP; e.g., SEQ ID NO: 17), and intestinal alkaline phosphatase (IALP; e.g., SEQ ID NO: 18). For example, the sALP is TNALP (e.g., the TNALP includes an amino acid sequence as set forth in SEQ ID NOs: 1-14).

In various embodiments of the first aspect of the disclosure, the sALP includes a polypeptide having the structure selected from the group consisting of Z-sALP-Y-spacer-X-$W_n$-V and Z-$W_n$-X-sALP-Y-spacer-V. For example, V, X, Y, and Z may each be absent or may be an amino acid sequence of at least one amino acid. In some embodiments, at least one of V, Z, and the spacer is absent. In particular embodiments, Y is two amino acid residues (e.g., Y is leucine-lysine) and/or X is two amino acid residues (e.g., X is aspartate-isoleucine). In certain embodiments, the structure is Z-sALP-Y-spacer-X-$W_n$-V.

$W_n$ can be a bone-targeting moiety, e.g., polyaspartic or polyglutamic region, 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. Alternatively, $W_n$ is absent. In some embodiments, the spacer includes a fragment crystallizable (Fc) region, e.g., a CH2 domain, a CH3 domain, and a hinge region. In particular, the Fc is a constant domain of an immunoglobulin selected from the group consisting of IgG-1, IgG-2, IgG-3, and IgG-4. For example, the Fc is a constant domain of an immunoglobulin IgG-1.

In some embodiments of the first aspect of the disclosure, the sALP includes an amino acid sequence as set forth in SEQ ID NO: 19, e.g., the sALP is the amino acid sequence of SEQ ID NO: 19.

For any of the above aspects, the patient can exhibit an improvement in one or more symptoms of craniosynostosis, e.g., in which the one or more symptoms includes increased ICP, abnormal skull and facial shapes, intracranial hypertension, airway impairments, obstructive sleep apnea, pulsatile tinnitus, Chiari Type I malformation, brain abnormalities, hearing loss, blindness, vision impairment, double vision, deafness, seizures, impairments in mental development, irritability, nausea, vomiting, emesis, herniation

of cerebellar tonsils, syringomyelia, headaches, bilateral papilledema, nystagmus, decreased visual acuity, microcephaly, brachycephaly, dolichocephaly, ventilator dependence, papilledema, and/or chronic optic nerve edema.

For any of the above aspects, the patient exhibits an improvement in one or more neurological symptoms.

For any of the above aspects, the sALP can be used in the manufacture of a medicament for treating or preventing craniosynostosis.

Another aspect of the disclosure features a method of diagnosing HPP in a patient, in which the method including determining the level of TNALP activity in a patient having craniosynostosis. In particular, the determination of TNALP activity can include measuring at least one of PEA, PPi, and/or PLP in a serum and/or blood sample from the patient (particularly where the PLP level is at least twice the upper limit of age-adjusted normal).

Another aspect of the disclosure features a method of diagnosing HPP in a patient, in which method including determining the presence of a mutation in TNALP in a patient having craniosynostosis. In various embodiments, the mutation in TNALP is associated with HPP.

**Definitions**

By “craniosynostosis” is meant a condition in which adjacent calvarial (skull cap) bones partially or completely fuse prematurely (including partial or complete mineralization), thereby deleteriously changing the growth pattern of the skull. Symptoms of craniosynostosis may include, but are not limited to, headaches, irritability, nausea and emesis (vomiting), pulsatile tinnitus, hearing loss, abnormal skull and facial shapes, airway impairments, obstructive sleep apnea, pulsatile tinnitus, Chiari Type I malformation, brain abnormalities, blindness, vision impairment, double vision, decreased visual acuity, deafness, seizures, impairments in mental development, herniation of cerebellar tonsils, syringomyelia, bilateral papilledema, nystagmus, microcephaly, brachycephaly, dolichocephaly, ventilator dependence, and/or chronic optic nerve edema.

The terms “intracranial pressure” and “ICP,” as used herein, refer to pressure within the skull that impinges on the brain tissue and cerebrospinal fluid. For example, increased ICP includes but is not limited to ICP greater than 10 mm Hg, in which 10-20 mm Hg is greater than typical ICP, and severe increased ICP is greater than 20 mm Hg. Symptoms associated with increased ICP may include headaches, irritability, nausea and emesis (vomiting), pulsatile tinnitus, hearing loss, abnormal skull and facial shapes, airway impairments, obstructive sleep apnea, pulsatile tinnitus, Chiari Type I malformation, brain abnormalities, blindness, vision impairment, double vision, decreased visual acuity, deafness, seizures, impairments in mental development, herniation of cerebellar tonsils, syringomyelia, bilateral papilledema, nystagmus, microcephaly, brachycephaly, dolichocephaly, ventilator dependence, and/or chronic optic nerve edema. Without being so limited, increased ICP may be diagnosed and/or monitored with one or more of radiography (e.g., computed tomography (CT) scan), ultrasonography, and clinical examination.

The terms “cranial vault remodeling procedure,” as used herein, refers to one or more surgeries involving repositioning and/or removal of bone of the skull, e.g., to relieve ICP and/or one or more other symptoms of craniosynostosis.

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 (TNALP). HPP may be further characterized as infantile HPP, childhood HPP, perinatal HPP (e.g., benign perinatal HPP or lethal perinatal HPP), adult HPP, or odontohypophosphatasia.

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

The terms “sALP,” “soluble alkaline phosphatase,” and “extracellular domain of an alkaline phosphatase” are used interchangeably and refer to a soluble, non-membrane-bound alkaline phosphatase or a domain, biologically active fragment, or biologically active variant thereof. sALPs include, for example, an alkaline phosphatase lacking a C-terminal glycolipid anchor (GPI signal sequence, e.g., polypeptides including or consisting of the amino acid residues 18-502 of a human TNALP (SEQ ID NOs: 1, 2, 3, 4, or 5)). In particular, a TNALP may include, e.g., a polypeptide including or consisting of amino acid residues 1-485 of SEQ ID NO: 19. sALPs further include, for example, mammalian orthologs of human TNALP, such as a rhesus TNALP (SEQ ID NO: 6), a rat TNALP (SEQ ID NO: 7), a canine TNALP (SEQ ID NO: 8), a porcine TNALP (SEQ ID NO: 9), a murine TNALP (SEQ ID NO: 10), a bovine TNALP (SEQ ID NOs: 11-13), or a feline TNALP (SEQ ID NO: 14). 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: 15 or 16), GCALP (e.g., polypeptides including or consisting of amino acid residues 18-502 of SEQ ID NO: 17), and IALP (e.g., polypeptides including or consisting of amino acid residues 18-502 of SEQ ID NO: 18), and additional variants and analogs thereof that retain alkaline phosphatase activity, e.g., the ability to hydrolyze $PP_i$. A sALP, in particular, lacks the N-terminal signal peptide (e.g., aa 1-17 of SEQ ID NOs: 1-5, 7, 10-12, or 14 or aa 1-25 of SEQ ID NO: 6).

By “sALP polypeptide” is meant a polypeptide having the structure A-sALP-B, wherein sALP is as defined herein and each of A and B is absent or is an amino acid sequence of at least one amino acid (e.g., any sALP fusion polypeptide described herein).

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 “bone-targeting moiety” is meant an amino acid sequence of between 1 and 50 amino acid residues in length having a sufficient affinity to the bone matrix, such that the bone-targeting moiety, singularly, has an *in vivo* binding affinity to the bone matrix that is about $10^{-6}$ M to about $10^{-15}$ M (e.g., $10^{-7}$ M, $10^{-8}$ M, $10^{-9}$ M, $10^{-10}$ M, $10^{-11}$ M, $10^{-12}$ M, $10^{-13}$ M, $10^{-14}$ M, or $10^{-15}$ M).

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

By “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: 1-5, 7, 10-12, or 14 or amino acid residues 1-25 of SEQ ID NO: 6.

By “fragment” is meant a portion of a polypeptide or nucleic acid molecule that contains, preferably, at least 10%, 20%, 30%, 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or more of the entire length of the reference nucleic acid molecule or polypeptide. A fragment may contain, e.g., 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 400, 500, 600, 700, or more amino acid residues, up to the entire length of the polypeptide. Exemplary sALP fragments have amino acid residues 18-498, 18-499, 18-500, 18-501, 18-502, 18-503, 18-504, 18-505, 18-506, 18-507, 18-508, 18-509, 18-510, 18-511, or 18-512 of ALP (e.g., SEQ ID NO: 1-5), and may include additional C-terminal and/or N-terminal portions.

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

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

By "nucleic acid molecule" is meant a molecule, e.g., RNA or DNA, having a sequence of two or more covalently bonded, naturally occurring or modified nucleotides. The nucleic acid molecule may be, e.g., single or double stranded, and may include modified or unmodified nucleotides, or mixtures or combinations thereof. Various salts, mixed salts, and free acid forms are also included.

The terms "peptide," "polypeptide," and "protein" are used interchangeably and refer to any chain of two or more natural or unnatural amino acid residues, regardless of post-translational modification (e.g., glycosylation or phosphorylation), constituting all or part of a naturally-occurring or non-naturally occurring polypeptide or peptide, as is described herein.

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

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

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

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

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

As used herein, "about" refers to an amount that is ± 10 % of the recited value.

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.

Other features and advantages of the present disclosure will be apparent from the following Detailed Description, the drawings, and the claims.

## BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

**Figures 1A-1B** are images showing a three-dimensional (3D) computed tomography (CT) scan of the head of a patient with craniosynostosis. Fig. 1A is a top view of the 3D head CT scan revealing left coronal and sagittal craniosynostosis with widening of the right coronal suture and persistent anterior fontanelle. Fig. 1B is an anterior view of the 3D head CT scan revealing left coronal craniosynostosis with facial scoliosis.

**Figures 2A-2B** are images showing a CT scan of a patient (i.e., Patient 1) with craniosynostosis prior to and after surgery. Fig. 2A is a preoperative sagittal CT scan showing loss of sulci and gyri, scalloping of the inner table, absence of extraaxial spaces, and crowding at the foramen magnum suggestive of Chiari Type I malformation. Fig. 2B is a postoperative sagittal CT performed one year after surgery now exhibiting definition of sulci and gyri, increased space in the basal cisterns and extraaxial spaces, and less crowding at the craniovertebral junction.

**Figures 3A-3C** are images showing a CT scan of a patient (i.e., Patient 2) with craniosynostosis prior to and after surgery. Fig. 3A is a preoperative lateral view of a 3D head CT scan with an obliterated left coronal suture. Fig. 3B is a preoperative top view of a 3D head CT scan with an obliterated sagittal suture and bony prominence over the bregma with persistent anterior fontanelle. Fig. 3C is a seven-month postoperative lateral view of a 3D head CT scan demonstrating improved bone growth in the calvaria.

**Figures 4A-4B** are images showing a CT scan of a patient (i.e., Patient 2) with craniosynostosis prior to and after surgery. Fig. 4A is a preoperative axial CT scan demonstrating absence of extraaxial spaces, small ventricular spaces, and tight basilar cisterns. Fig. 4B is a postoperative axial CT scan performed 3 months after surgery revealing cranial expansion, open basal cisterns, reconstitution of the third ventricle, and presence of extraaxial spaces.

**Figures 5A-5C** are images of a lateral cranial X ray showing is the degree of cranial calcification of Patient 5 performed at ages 1 day (Fig. 5A), 6 months (Fig. 5B), and 18 months (Fig. 5C). There was significant improvement in cranial mineralization after initiation of treatment sALP at age 5 weeks.

**Figures 6A-6C** are images of a pre-surgical CT scan showing 3D reconstruction of Patient 5 performed at 12 months of age. The CT scan shows decreased mineralization of the cranium with partial synostosis of the right coronal suture (Fig. 6A), left coronal suture (Fig. 6B), and the metopic suture (Fig. 6C).

## DETAILED DESCRIPTION

We have discovered that a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) may be used effectively in combination with surgery, e.g., a cranial vault remodeling procedure, to treat craniosynostosis (e.g., the premature fusion of cranial bones) in a patient (e.g., a human, particularly an infant or a child) with hypophosphatasia (HPP). In particular, sALP may be administered to treat HPP patients exhibiting or likely to have increased intracranial pressure (ICP). The sALP may be a sALP polypeptide (e.g., a secreted soluble, extracellular domain of an ALP) or a sALP fusion polypeptide (e.g., a sALP fused to a fragment crystallizable (Fc) region and/or a bone-targeting moiety). Methods for administering a sALP in combination with a cranial surgery, e.g., a cranial vault remodeling procedure (e.g., in which the sALP is administered prior to, or after, a cranial vault remodeling procedure) to treat craniosynostosis, e.g., in HPP patients exhibiting or likely to have increased ICP, are described.

### Methods of Treatment

Provided herein are methods for treating craniosynostosis in a patient, such as a patient having hypophosphatasia (HPP) (e.g., a human). In particular, the patient may exhibit or may be likely to have increased intracranial pressure (ICP). The method involves administering a soluble alkaline phosphatase (sALP; such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) to the patient, e.g., in combination with a cranial surgery, e.g., a cranial vault remodeling procedure. In particular, a sALP can be administered to the patient prior to the cranial vault remodeling procedure to, e.g., allow for proper fusion of the cranial sutures or prevent immature fusion of the cranial sutures. Alternatively, if the patient exhibits symptoms of craniosynostosis (e.g., increased ICP) that require a cranial vault remodeling procedure prior to administration of a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™), the sALP can be administered after the cranial vault remodeling procedure to allow for, e.g., relief of increased ICP, proper fusion of the cranial sutures, and/or proper growth of the skull.

Patients may be diagnosed with craniosynostosis prior to administration of a sALP and/or cranial vault remodeling procedure, such as by clinical examination, radiography (e.g., computed tomography

(CT)), and/or ultrasonography. Symptoms of craniosynostosis can be monitored following treatment (e.g., following sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) administration in combination with a cranial vault remodeling procedure) to determine the effectiveness of the treatment and/or the timing of sALP administration relative to, e.g., the cranial vault remodeling procedure (e.g., administration of sALP prior to or after the cranial vault remodeling procedure).

Treatment with a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) combined with a cranial vault remodeling procedure, can result in an improvement in a symptom of craniosynostosis, such as a decrease in ICP. The methods of the present invention can be used to treat neurological symptoms associated with craniosynostosis, such that there is reversal of craniosynostosis or a reduction in the severity of symptoms of craniosynostosis, such as a decrease in ICP. In particular, the methods may result in an improvement in symptoms including, but not limited to, headaches, irritability, nausea and emesis (vomiting), pulsatile tinnitus, hearing loss, abnormal skull and facial shapes, airway impairments, obstructive sleep apnea, pulsatile tinnitus, Chiari Type I malformation, brain abnormalities, blindness, vision impairment, double vision, decreased visual acuity, deafness, seizures, impairments in mental development, herniation of cerebellar tonsils, syringomyelia, bilateral papilledema, nystagmus, microcephaly, brachycephaly, dolichocephaly, ventilator dependence, and/or chronic optic nerve edema.

The methods described herein may result in an improvement in any of the aforementioned symptoms. For example, treatment with a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) combined with a cranial vault remodeling procedure can result in a decrease the occurrence of headaches and irritability. Treatment can result in fewer incidents of nausea and emesis over, e.g., a 30 day period after receiving treatment with sALP combined with a cranial vault remodeling procedure (e.g., a 2 day period, a 4 day period, a 6 day period, a 8 day period, a 10 day period, a 15 day period, a 20 day period, a 25 day period, a 30 day period, a 40 day period, a 60 day period, a 60 day period after receiving treatment). Patients may experience an improvement in vision, such as the reversal of a vision impairment or increased visual acuity, following treatment with a sALP combined with a cranial vault remodeling procedure. The patient may exhibit improved neurological symptoms, such as a lack of or decrease in brain abnormalities, relative to the patient's condition prior to treatment. For example, following the methods of treatment, the head circumference of the patient may increase and approach the average head circumference according to the patient's age.

*Hypophosphatasia*

HPP is a matrix mineralization disorder that may be treated with a sALP, e.g., in combination with a cranial surgery, such as a cranial vault remodeling procedure. A sALP (such as TNALP, for example SEQ ID NO: 19, STRENSIQ™) can be administered, as described herein, to treat, e.g., perinatal HPP, infantile HPP, childhood HPP, adult HPP, and odontohypophosphatasia. In particular, patients having infantile HPP, childhood HPP, and perinatal HPP (e.g., perinatal benign or perinatal lethal HPP) can be treated with a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™), e.g., combined with a cranial vault remodeling procedure.

A phenotype associated with HPP, e.g., perinatal HPP, infantile HPP, childhood HPP, adult HPP, and odontohypophosphatasia, can be treated with a sALP, e.g., combined with a cranial vault remodeling procedure. For instance, the methods can be used to treat a perinatal HPP patient, such as a patient with increased respiratory compromise due to hypoplastic and rachitic disease of the chest; diminished ossification of the skull; diminished ossification and height of vertebral bodies; and/or absent ossification of the humeral, radial, and ulnar metaphyses with marked metaphyseal irregularity; fragmentation and fraying. The methods can also be used to treat patients exhibiting symptoms of infantile HPP, including, but not limited to, inadequate weight gain, the appearance of rickets, impaired skeletal mineralization, progressive skeletal demineralization, rib fractures, and chest deformity. A patient with childhood HPP may be treated with the methods, such as patients that exhibit symptoms including premature loss of deciduous teeth (e.g., as a result of aplasia, hypoplasia, or dysplasia of dental cementum) and rickets, which causes short stature and skeletal deformities, such as bowed legs and enlarged wrists, knees, and ankles as a result of flared metaphysis. Accordingly, the methods may be used to alleviate any of the symptoms of HPP described herein. Non-limiting examples of HPP symptoms that may be treated, e.g., with a sALP, include elevated blood and/or urine levels of inorganic pyrophosphate ($PP_i$), elevated blood and/or urine levels of phosphoethanolamine (PEA), elevated blood and/or urine levels of pyridoxal 5'-phosphate (PLP), hypomineralization, rachitic ribs, hypercalciuria, bone pain, bone fracture, HPP-related seizure, inadequate weight gain, and/or calcium pyrophosphate dihydrate crystal deposition.

A patient with a mutation in TNALP can also be treated with a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) combined with a cranial surgery, such a cranial vault remodeling procedure, e.g., to alleviate increased ICP or symptoms associated with craniosynostosis. Missense mutations at a variety of positions in TNALP, including the enzyme's active site vicinity, homodimer interface, crown domain, amino-terminal arm, and calcium-binding site, have all been found to affect its catalytic activity. In addition, missense, nonsense, frame-shift, and splice site mutations have also been shown to lead to aberrant mutant proteins or intracellular trafficking defects that lead to subnormal activity on the cell surface. Accordingly, the methods may be used to treat patients with different mutation in TNALP (e.g., missense mutations, frame-shift, nonsense, and splicing mutations). For instance, the presence of a mutation in TNALP may be detected in a sample from the patient prior to or after treatment (e.g., sALP administration in combination with cranial vault remodeling). Additionally, a parent of the patient and/or a fetal sample (e.g., fetal nucleic acid obtained from maternal blood, placental, and/or fetal samples) may be tested by methods known in the art for a mutation in TNALP. Traditional management of HPP has also included symptomatic treatment of the phenotypic manifestations of the disease, e.g., treating hypercalcemia with dietary restriction or calciuretics and orthopedic stabilization of fractures. Accordingly, these treatments (e.g., dietary restriction, calciuretics, and orthopedic stabilization of fractures) may be used with the administration of a sALP combined with a cranial surgery, such a cranial vault remodeling procedure, e.g., to alleviate increased ICP or symptoms associated with craniosynostosis. *Craniosynostosis*

Certain patients with HPP and craniosynostosis may be treated with a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) combined with a cranial surgery, such as cranial vault remodeling procedure. In particular, patients treated using the methods described herein may include,

e.g., infants, children, and perinatal patients with craniosynostosis (e.g., the premature fusion of cranial sutures), such as a patient exhibiting or likely to develop increased ICP. Treatment with a sALP, e.g., combined with a cranial vault remodeling procedure, may also be initiated in the neonatal period (e.g., within 1 hour, 8 hours, 12 hours, 1 day, 2 days, 3 days, 4 days, 5 days, 6 days, 1 week, 2 weeks, 3 weeks, or one month) of birth, or prior to birth. Such treatment may also be initiated in utero. These methods can also be used to treat craniosynostosis characterized by the suture or sutures that fuse. Fusion typically involves one or more of the sagittal, metopic, coronal, lambdoidal, and squamosal sutures. Accordingly, a patient exhibiting fusion of one or more cranial sutures (e.g., sagittal, metopic, coronal, lambdoidal, and squamosal) resulting in craniosynostosis may be treated with a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) in combination with surgery.

Patients with different forms of craniosynostosis can also be treated with a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) combined with a cranial vault remodeling procedure. In particular, known mutations associated with craniosynostosis occur in the fibroblast growth factor receptors (FGFRs) and are associated with over 20 different genetic disorders, including Pfeiffer, Saethre-Chotzen, Apert, Crouzon, Beare-Stevenson, Jackson-Weiss, Antley-Bixler, and Muenke syndromes. Thus, a patient treated with the methods disclosed herein may have, e.g., a mutation in a FGFR (e.g., FGFR1, FGFR2, or FGFR3) gene. Additionally, a mutation in a FGFR gene can be detected in a sample from the patient prior to or after treatment (e.g., a sALP combined with cranial vault remodeling). Additionally, the parents of the patient and/or fetal samples (e.g., fetal nucleic acid obtained from maternal blood, placental, or fetal samples) may be tested by methods known in the art for the mutation. Craniosynostosis may also develop in relation to an underlying disorder, which may include, but are not limited to HPP, hyperthyroidism, hypercalcemia, vitamin D deficiency, renal osteodystrophy, Hurler's Syndrome, sickle cell disease, and thalassemia. For instance, the methods described herein may resolve and/or prevent symptoms associated with craniosynostosis in a patient with any of the aforementioned disorders.

The methods may further include the diagnosis of patients (e.g., HPP patients) with craniosynostosis. Patients may be diagnosed with craniosynostosis prior to or after administration of a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) and/or cranial vault remodeling procedure. Craniosynostosis may be diagnosed, for example, by clinical examination, e.g., a physician may examine the head and suture lines of the patient. The presence of a ridge where the suture lines are located may be diagnostic of craniosynostosis, as this is not present with normal sutures. The symmetry in head shape, head size (e.g., microcephalic), location and symmetry of ears and eyes, shape and slope of the forehead, and size and shape of the sutures are also examined during diagnosis of craniosynostosis. Any of these clinical features can be used to diagnose craniosynostosis according to the methods described herein. Diagnostic methods of the present invention may further include radiography (e.g., an X-ray or computed tomography (CT)) and/or ultrasound. In particular, three-dimensional (3D) CT allows for determination of the severity and location of the fused sutures in addition to characterization of skull features and presence of deformities. Sonogram can also be used for diagnosis of craniosynostosis in a prenatal patient.

Symptoms of craniosynostosis in patients (e.g., HPP patients) may also be monitored prior to or after a patient is treated with a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) combined with a cranial vault remodeling procedure. For instance, symptoms of craniosynostosis may be monitored prior to treatment to assess the severity of craniosynostosis and condition of the patient prior to performing the methods. The methods of the present invention may include monitoring of ICP (e.g., direct ICP monitoring using a probe passed through the skull to allow continuous recording of ICP or indirect ICP monitoring via a lumbar cerebrospinal fluid (CSF) catheter) and symptoms associated with increased ICP. Symptoms associated with increased ICP may include, but are not limited to, headaches, irritability, nausea and emesis (vomiting), pulsatile tinnitus, hearing loss, abnormal skull and facial shapes, airway impairments, obstructive sleep apnea, Chiari Type I malformation, brain abnormalities, blindness, vision impairment, double vision, decreased visual acuity, deafness, seizures, impairments in mental development, herniation of cerebellar tonsils, syringomyelia, bilateral papilledema, nystagmus, microcephaly, brachycephaly, dolichocephaly, ventilator dependence, and/or chronic optic nerve edema. Monitoring may also include radiography, ultrasonography, clinical examination, and/or determination of sALP activity. In particular, sALP activity may be determined by measuring phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and/or pyridoxal 5'-phosphate (PLP) in a serum and/or blood sample from the patient.

**Alkaline Phosphatase**

The present disclosure is not limited to a particular alkaline phosphatase (ALP) or nucleic acid sequence encoding an ALP. Alkaline phosphatases encompass a group of enzymes that catalyze the cleavage of a phosphate moiety (e.g., hydrolysis of pyrophosphate, $PP_i$). There are four known mammalian alkaline phosphatase (ALP) isozymes: tissue nonspecific alkaline phosphatase (TNALP; described further below), placental alkaline phosphatase (PLALP) (e.g., Accession Nos. P05187, NP_112603, and NP_001623), germ cell alkaline phosphatase (GALP) (e.g., Accession No. P10696), and intestinal alkaline phosphatase (IALP) (e.g., Accession Nos. P09923 and NP_001622). In addition to the exemplary ALPs discussed above, this disclosure also provides any polypeptide comprising the identical or similar catalytic site structure and/or enzymatic activity of ALP for treating craniosynostosis in HPP patients. Bone delivery conjugates including sALP are further described in PCT publication Nos: WO 2005/103263 and WO 2008/138131, which are incorporated herein by reference in their entirety.

TNALPs that may be used according to the methods described herein include, e.g., human TNALP (Accession Nos. NP_000469, AAI10910, AAH90861, AAH66116, AAH21289, and AAI26166); rhesus TNALP (Accession No. XP_01109717); rat TNALP (Accession No. NP_037191); dog TNALP (Accession No. AAF64516); pig TNALP (Accession No. AAN64273), mouse (Accession No. NP_031457), cow TNALP (Accession Nos. NP_789828, NP_776412, AAM 8209, and AAC33858), and cat TNALP (Accession No. NP_001036028). In particular, TNALP may be a recombinant human TNALP (e.g., SEQ ID NO: 19, STRENSIQ™; see U.S. Patent Nos. 7,763,712 and 7,960,529, incorporated herein by reference in their entirety) used for the treatment of craniosynostosis, such as in HPP patients.

*Soluble Alkaline Phosphatase*

The ALPs of the present invention include soluble (e.g., extracellular or non-membrane-bound) forms of any of the alkaline phosphatases described herein. The sALP of the invention can be, for example, a soluble form of human tissue non-specific alkaline phosphatase (hTNALP). The present disclosure is not limited to a particular sALP and may include any sALP polypeptide that is physiologically active toward, e.g., phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and pyridoxal 5'-phosphate (PLP). In particular, a sALP of the present invention is catalytically competent to improve skeletal mineralization in bone. The present invention further includes nucleic acids encoding the sALPs described herein that may be used to treat the conditions described herein, e.g., craniosynostosis in HPP patients.

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

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

The present invention also includes sALP consensus sequences derived from the extracellular domain of ALP isozymes (e.g., TNALP, PALP, GCALP, IALP, etc.). Thus, similar to sTNALP discussed above, the present disclosure also provides other soluble human ALP isozymes, i.e., without the peptide signal, preferably comprising the extracellular domain of the ALPs. The sALPs of the invention also include polypeptide sequences satisfying a consensus sequence derived from the ALP extracellular domain of human ALP isozymes and of mammalian TNALP orthologs (human, mouse, rat, cow, cat, and dog) or a consensus derived from the ALP extracellular domain of just mammalian TNALP orthologs

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

sALPs of the present invention may 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). Examples of mutations that may be introduced into an ALP sequence are described in US Publication No. 2013/0323244, hereby incorporated by reference in its entirety. A sALP may optionally be glycosylated at any appropriate one or more amino acid residues. In addition, an sALP may have at least 50% (e.g., 55%, 60%, 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more) sequence identity to any of the sALPs described herein. A sALP may 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.

*sALP Fusion Polypeptides*

Any of the sALPs and linkers described herein may be combined in a sALP polypeptide, e.g., a sALP polypeptide of A-sALP-B, wherein each of A and B is absent or is an amino acid sequence of at least one amino acid. When present, A and/or B can be any linker described herein. In some sALP polypeptides, A is absent, B is absent, or A and B are both absent. The sALP polypeptides of the invention can optionally include an Fc region to provide an sALP fusion polypeptide, as described herein. The sALP polypeptide can optionally include a bone-targeting moiety, as described herein. In some sALP polypeptides, a linker, e.g., a flexible linker, may be included between the bone-targeting moiety and the sALP, such as a dipeptide sequence (e.g., leucine-lysine or aspartic acid-isoleucine). Further exemplary Fc regions, linkers, and bone-targeting moieties are described below.

Any of the sALPs, linkers, and Fc regions described herein may be combined in a fusion polypeptide, e.g., a recombinant fusion polypeptide, which includes 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 structure may be Z-sALP-Y-spacer-X-$W_n$-V or Z-$W_n$-X-spacer-Y-sALP-V. The sALP may be the full-length or functional fragments of ALPs, such as the soluble, extracellular domain of the ALP, as is described herein (e.g., TNALP, PALP, GCALP and IALP). Any one of X, Y, Z, and V and/or the spacer may be absent or an amino acid sequence of at least one amino acid. $W_n$ may be a bone-targeting moiety, e.g., having a series of consecutive Asp or Glu 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, may 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 is at the C-terminal end. sALP polypeptides and fusion polypeptides may not include a bone-targeting moiety.

sALP fusion polypeptides of the present invention may be of the structure hTNALP-Fc-$D_{10}$. In particular, sALP fusion polypeptides may include an amino acid sequence of SEQ ID NO: 19.

Useful spacers include, but are not limited to, polypeptides comprising a Fc, and hydrophilic and flexible polypeptides able to alleviate the repulsive forces caused by the presence of the terminal highly negatively charged peptide (e.g., $W_n$). For example, an sALP of the invention can be a fusion polypeptide including an Fc region of an immunoglobulin at the N-terminal or C-terminal domain. An immunoglobulin molecule has a structure that is well known in the art. It includes two light chains (~23 kD each) and two heavy chains (~50-70 kD each) joined by inter-chain disulfide bonds. Immunoglobulins are readily cleaved proteolytically (e.g., by papain cleavage) into Fab (containing the light chain and the VH and CH1 domains of the heavy chain) and Fc (containing the CH2 and CH3 domains of the heavy chain, along with adjoining sequences). Useful Fc fragments as described herein include the Fc fragment of any immunoglobulin molecule, including IgG, IgM, IgA, IgD, or IgE, and their various subclasses (e.g., IgG-1, IgG-2, IgG-3, IgG-4, IgA-1, IgA-2), from any mammal (e.g., human). For instance, the Fc fragment is human IgG-1. The Fc fragments of the invention may include, for example, the CH2 and CH3 domains of the heavy chain and any portion of the hinge region. The Fc region may 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: 25, 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: 25. Engineered, e.g., non-naturally occurring, Fc regions may be utilized in the methods of the invention, e.g., as described in International Application Pub. No. WO2005/007809, which is hereby incorporated by reference. An Fc fragment as described herein may 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.

The sALP fusion polypeptides described herein may include a peptide linker region between the Fc fragment. In addition, a peptide linker region may be included between the Fc fragment and the optional bone-targeting moiety. The linker region may 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 may also contain, e.g., serine residues. In some cases, the amino acid sequence of linkers consists only of glycine and serine residues. A linker may optionally be glycosylated at any appropriate one or more amino acid residues. Additionally, a linker as described herein may include any other sequence or moiety, attached covalently or non-covalently. The linker may also be absent, in which the Fc fragment and the sALP are fused together directly, with no intervening residues. Certain Fc-sALP or sALP-Fc fusion polypeptides may be viewed, according to the present disclosure, either as 1) having no linker, or as 2) having a linker which corresponds to a portion of the sALP. For example, Fc fused directly to hsTNALP (1-502) may be

viewed, e.g., either as having no linker, in which the hsTNALP is amino acids 1-502, or as having a 17-amino acid linker, in which the hsTNALP (18-502).

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

The sALPs and sALP fusion polypeptides of the invention may 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 polypeptide or fusion polypeptide of the invention (e.g., a sALP polypeptide or fusion polypeptide) may be glycosylated or PEGylated.

*Production of Nucleic Acids and Polypeptides*

The nucleic acids encoding sALPs and sALP fusion polypeptides of the invention can be produced by any method known in the art. Typically, a nucleic acid encoding the desired fusion polypeptide is generated using molecular cloning methods, and is generally placed within a vector, such as a plasmid or virus. The vector is used to transform the nucleic acid into a host cell appropriate for the expression of the fusion polypeptide. Representative methods are disclosed, for example, in Maniatis et al. (Cold Springs Harbor Laboratory, 1989). Many cell types can be used as appropriate host cells, although mammalian cells are preferable because they are able to confer appropriate post-translational modifications. Host cells of the present invention may include, e.g., Chinese Hamster Ovary (CHO) cell, L cell, C127 cell, 3T3 cell, BHK cell, COS-7 cell or any other suitable host cell known in the art. For example, the host cell is a Chinese Hamster Ovary (CHO) cell (e.g., a CHO-DG44 cell).

The sALPs and sALP fusion polypeptides can be produced under any conditions suitable to effect expression of the sALP polypeptide in the host cell. Such conditions include appropriate selection of a media prepared with components such as a buffer, bicarbonate and/or HEPES, ions like chloride, phosphate, calcium, sodium, potassium, magnesium, iron, carbon sources like simple sugars, amino acids, potentially lipids, nucleotides, vitamins and growth factors like insulin; regular commercially available media like alpha-MEM, DMEM, Ham's-F12, and IMDM supplemented with 2-4 mM L-glutamine and 5% Fetal bovine serum; regular commercially available animal protein free media like Hyclone™ SFM4CHO, Sigma CHO DHFR$^-$, Cambrex POWER™ CHO CD supplemented with 2-4 mM L-glutamine. These media are desirably prepared without thymidine, hypoxanthine and L-glycine to maintain selective pressure, allowing stable protein-product expression.

**Pharmaceutical compositions and formulations**

A composition of the present invention (e.g., including a sALP or sALP fusion polypeptide, such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) can be administered by a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. The route of

administration can depend on a variety of factors, such as the environment and therapeutic goals. In particular, the polypeptides and fusion polypeptides described herein can be administration by any route known in the art, e.g., subcutaneous (e.g., by subcutaneous injection), intravenously, orally, nasally, intramuscularly, sublingually, intrathecally, or intradermally. By way of example, pharmaceutical compositions of the invention can be in the form of a liquid, solution, suspension, pill, capsule, tablet, gelcap, powder, gel, ointment, cream, nebulae, mist, atomized vapor, aerosol, or phytosome.

*Timing of Treatment*

The compositions described herein, including a sALP or sALP fusion polypeptide (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™), can be administered prior to a cranial surgery, e.g., a cranial vault remodeling procedure (e.g., four months or more prior, 3 months prior, 2 months prior, 1 month prior, 4 weeks prior, 3 weeks prior, 2 weeks prior, 1 week prior, 6 days prior, 5 days prior, 4 days prior, 3 days prior, 2 days prior, 1 day prior, within less than 24 hours prior to the cranial surgery, e.g., the cranial vault remodeling procedure). Furthermore, the compositions can be administered after a cranial surgery, e.g., a cranial vault remodeling procedure (e.g., four months or more after, 3 months after, 2 months after, 1 month after, 4 weeks after, 3 weeks after, 2 weeks after, 1 week after, 6 days after, 5 days after, 4 days after, 3 days after, 2 days after, 1 day after, within less than 24 hours after the cranial surgery, e.g., the cranial vault remodeling procedure). A sALP composition can be administered prior to a cranial surgery, e.g., a cranial vault remodeling procedure, for instance, if symptoms of craniosynostosis are considered manageable. Administration of a sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) composition prior to a cranial vault remodeling procedure can also be performed, e.g., to allow for proper fusion of the cranial sutures or prevent immature fusion of the cranial sutures. Alternatively, if the patient exhibits symptoms of craniosynostosis (e.g., increased ICP) that require a cranial vault remodeling procedure prior to administration of a sALP, the sALP can be administered after the cranial vault remodeling procedure to allow for, e.g., relief of increased ICP, proper fusion of the cranial sutures, and/or proper growth of the skull.

*Dosage*

Any amount of a pharmaceutical composition (e.g., including a sALP or sALP fusion polypeptide(such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™)) can be administered to a patient, such as patient (e.g., a HPP patient) with craniosynostosis. The dosages will depend on many factors including the mode of administration and the age of the patient. Typically, the amount of the composition (e.g., a sALP or sALP fusion polypeptide) contained within a single dose will be an amount that is effective to treat a condition (e.g., craniosynostosis) as described herein without inducing significant toxicity.

For example, the sALP polypeptides (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) described herein can be administered to patients in individual doses ranging, e.g., from 0.01 mg/kg to 500 mg/kg (e.g., from 0.05 mg/kg to 500 mg/kg, from 0.1 mg/kg to 20 mg/kg, from 5 mg/kg to 500 mg/kg, from 0.1 mg/kg to 100 mg/kg, from 10 mg/kg to 100 mg/kg, from 0.1 mg/kg to 50 mg/kg, 0.5 mg/kg to 25 mg/kg, 1.0 mg/kg to 10 mg/kg, 1.5 mg/kg to 5 mg/kg, or 2.0 mg/kg to 3.0 mg/kg) or from 1

μg/kg to 1,000 μg/kg (e.g., from 5 μg/kg to 1,000 μg/kg, from 1 μg/kg to 750 μg/kg, from 5 μg/kg to 750 μg/kg, from 10 μg/kg to 750 μg/kg, from 1 μg/kg to 500 μg/kg, from 5 μg/kg to 500 μg/kg, from 10 μg/kg to 500 μg/kg, from 1 μg/kg to 100 μg/kg, from 5 μg/kg to 100 μg/kg, from 10 μg/kg to 100 μg/kg, from 1 μg/kg to 50 μg/kg, from 5 μg/kg to 50 μg/kg, or from 10 μg/kg to 50 μg/kg).

Exemplary doses of a sALP include, e.g., 0.01, 0.05, 0.1, 0.5, 1, 2, 2.5, 5, 10, 20, 25, 50, 100, 125, 150, 200, 250, or 500 mg/kg; or 1, 2, 2.5, 5, 10, 20, 25, 50, 100, 125, 150, 200, 250, 500, 750, 900, or 1,000 μg/kg. For all dosages or ranges recited herein, the term "about" may be used to modify these dosages by ±10% of the recited values or range endpoints. In particular, compositions (e.g., including sALP (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™)) in accordance with the present disclosure can be administered to patients in doses ranging from about 0.001 mg/kg/day to about 500 mg/kg/day, about 0.01 mg/kg/day to about 100 mg/kg/day, or about 0.01 mg/kg/day to about 20 mg/kg/day. For example, the sALP compositions (such as TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) can be administered to patients in a weekly dosage ranging, e.g., from about 0.5 mg/kg/week to about 140 mg/kg/week, e.g., about 0.8 mg/kg/week to about 50 mg/kg/week, or about 1 mg/kg/week to about 10 mg/kg/week (e.g., 6 mg/kg/week). The dosage will be adapted by the clinician in accordance with conventional factors such as the extent of the disease and different parameters from the patient.

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

Nucleic acids encoding sALPs and sALP fusion polypeptides can be administered according the formulations described herein to a patient in dosages suitable for gene therapy. The amount of the nucleic acids administered will depend on a number of factors known to those skilled in the art, including: the length and nature of the nucleic acid, the vector (e.g., viral or non-viral) used, the activity of the polypeptide encoded, the presence of excipients, the route and method of administration, and the general condition and fitness of the patient. Exemplary dosages and routes of administration are described, e.g., in Melman et al. (*Isr. Med. Assoc. J.* 9:143-146, 2007; describing the intrapenile injection of 0.5 mg to 7.5 mg of a human cDNA in a plasmid for treating erectile dysfunction), Powell et al. (*Circulation* 118:58-65, 2008; describing the intramuscular injection of 0.4 mg to 4.0 mg of a hepatocyte growth factor plasmid to treat critical limb ischemia, Waddill et al. (*AJR Am. J. Roentgenol.* 169:63-67, 1997; describing the CT-guided intra-tumoral injection of 0.01 mg to 0.25 mg of plasmid DNA encoding an MHC antigen to treat melanoma), Kastrup et al. (*J. Am. Coll. Cardiol.* 45:982-988, 2005; describing the intramyocardial

injection of 0.5 mg of a VEGF plasmid to treat severe angina pectoris), and Romero et al. (*Hum. Gene. Ther.* 15:1065-1076, 2004; describing the intramuscular injection of 0.2 mg to 0.6 mg of a plasmid to treat Duchenne/Becker muscular dystrophy), each of which is hereby incorporated by reference.

Nucleic acids encoding sALPs and sALP fusion polypeptides can be administered to the patient at a dose in the range from, e.g., 0.01 mg to 100 mg (e.g., from 0.05 mg to 50 mg, 0.1 mg to 10 mg, 0.3 mg to 3 mg, or about 1 mg) of nucleic acid. The total volume at which the nucleic acid can be administered will depend on its concentration, and can range from, e.g., 1 µL to 10 mL (e.g. from 10 µL to 1 mL, 50 µL to 500 µL, 70 µL to 200 µL, 90 µL to 150 µL, or 100 µL to 120 µL). The nucleic acids 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, the nucleic acids can be administered, e.g., twice, three times, four times, five times, six times, seven times, eight times, nine times, 10 times, 11 times, or 12 times per day. 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, weeks, or months, or even for the remaining lifespan of the patient.

These are guidelines, since the actual dose should be carefully selected and titrated by an attending physician or nutritionist based upon clinical factors unique to each patient. The optimal periodic dose will be determined by methods known in the art and will be influenced by factors such as the age of the patient, as indicated above, and other clinically relevant factors. In addition, patients may be taking medications for other diseases or conditions. The other medications may be continued during the time that a polypeptide or nucleic acid of the invention is given to the patient, but it is advisable in such cases to begin with low doses to determine if adverse side effects are experienced.

For example, a sALP or sALP fusion polypeptide (such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) may be formulated as a solution for injection, which is a clear, colorless to slightly yellow, aqueous solution, pH 7.4. The sALP or sALP polypeptide may be formulated at a concentration of 12mg/0.3mL,18mg/0.45mL, 28mg/0.7mL, 40mg/1ml, or 80mg/0.8mL. In particular, the composition may be formulated as a 40 mg/ml solution for injection, in which each ml of solution contains 40 mg of sALP or sALP polypeptide (e.g., each vial contains 0.3 ml solution and 12 mg of sALP (40 mg/ml), each vial contains 0.45 ml solution and 18 mg of sALP (40 mg/ml), each vial contains 0.7 ml solution and 28 mg of sALP(40 mg/ml), or each vial contains 1.0 ml solution and 40 mg of asfotase alfa (40 mg/ml)). A sALP or sALP polypeptide (such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) may be formulated as a solution for injection at a concentration of100 mg/ml, in which each 1 ml of solution contains 100 mg of sALP or sALP polypeptide (e.g., each vial contains 0.8 ml solution and 80 mg of asfotase alfa (100 mg/ml)).

For example, the recommended dosage of a sALP or sALP fusion polypeptide (such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) is 2 mg/kg of body weight administered subcutaneously three times per week, or a dosage regimen of 1 mg/kg of body weight administered subcutaneously six times per week. Additional dosage information is provided below (Table 1).

**Table 1. Dosing chart for a sALP or sALP fusion polypeptide (SEQ ID NO: 19, such as STRENSIQ™).**

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

*Formulations*

The compositions including sALPs and sALP fusion polypeptides (such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) can be formulated according to standard methods. Pharmaceutical formulation is a well-established art, and is further described in, e.g., Gennaro (2000) "Remington: The Science and Practice of Pharmacy," 20th Edition, Lippincott, Williams & Wilkins (ISBN: 0683306472); Ansel et al. (1999) "Pharmaceutical Dosage Forms and Drug Delivery Systems," 7th Edition, Lippincott Williams & Wilkins Publishers (ISBN: 0683305727); and Kibbe (2000) "Handbook of Pharmaceutical Excipients American Pharmaceutical Association," 3rd Edition (ISBN: 091733096X). For instance, a sALP composition (e.g., TNALP, for example SEQ ID NO: 19, such as STRENSIQ™) can be formulated, for example, as a buffered solution at a suitable concentration and

suitable for storage at 2-8°C (e.g., 4°C). A composition can also be formulated for storage at a temperature below 0°C (e.g., -20°C or -80°C). A composition can further be formulated for storage for up to 2 years (e.g., one month, two months, three months, four months, five months, six months, seven months, eight months, nine months, 10 months, 11 months, 1 year, 1½ years, or 2 years) at 2-8°C (e.g., 4°C). Thus, the compositions described herein may be stable in storage for at least 1 year at 2-8°C (e.g., 4°C).

The compositions including sALPs and sALP fusion polypeptides (such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) can be in a variety of forms. These forms include, e.g., liquid, semi-solid and solid dosage forms, such as liquid solutions (e.g., injectable and infusible solutions), dispersions or suspensions, tablets, pills, powders, liposomes and suppositories. The preferred form depends, in part, on the intended mode of administration and therapeutic application.

For example, compositions intended for systemic or local delivery can be in the form of injectable or infusible solutions. Accordingly, the compositions (e.g., a sALP polypeptide or sALP fusion polypeptide, such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) can be formulated for administration by a parenteral mode (e.g., subcutaneous, intravenous, intraperitoneal, or intramuscular injection). "Parenteral administration," "administered parenterally," and other grammatically equivalent phrases, as used herein, refer to modes of administration other than enteral and topical administration, usually by injection, and include, without limitation, subcutaneous, intradermal, intravenous, intranasal, intraocular, pulmonary, intramuscular, intra-arterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intrapulmonary, intraperitoneal, transtracheal, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural, intracerebral, intracranial, intracarotid, and intrasternal injection and infusion.

The compositions including sALPs and sALP fusion polypeptides (such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) can be formulated as a solution, microemulsion, dispersion, liposome, or other ordered structure suitable for stable storage at high concentration. Sterile injectable solutions can be prepared by incorporating a composition described herein in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filter sterilization. Generally, dispersions are prepared by incorporating a composition described herein into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods for preparation include vacuum drying and freeze-drying that yield a powder of a composition described herein plus any additional desired ingredient (see below) from a previously sterile-filtered solution thereof. The proper fluidity of a solution can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prolonged absorption of injectable compositions can be brought about by including in the composition a reagent that delays absorption, for example, monostearate salts, and gelatin.

The compositions described herein can also be formulated in immunoliposome compositions. Such formulations can be prepared by methods known in the art such as, e.g., the methods described in

Epstein et al. (1985) *Proc Natl Acad Sci USA* 82:3688; Hwang et al. (1980) *Proc Natl Acad Sci USA* 77:4030; and U.S. Patent Nos. 4,485,045 and 4,544,545. Liposomes with enhanced circulation time are disclosed in, e.g., U.S. Patent No. 5,013,556.

Compositions including sALPs and sALP fusion polypeptides (such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) may also be formulated with a carrier that will protect the composition (e.g., a sALP polypeptide or sALP fusion polypeptide) against rapid release, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Many methods for the preparation of such formulations are known in the art. See, e.g., J.R. Robinson (1978) "Sustained and Controlled Release Drug Delivery Systems," Marcel Dekker, Inc., New York.

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

Compositions including sALPs and sALP fusion polypeptides (such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) may be formulated for administration to a patient or, if administered to a fetus, to a female carrying such fetus, along with intravenous gamma globulin therapy (IVIG), plasmapheresis, plasma replacement, or plasma exchange.

*Carriers/vehicles*

Preparations containing a sALP or sALP fusion polypeptide (such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) may be provided to patients in combination with pharmaceutically acceptable sterile aqueous or non-aqueous solvents, suspensions or emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oil, fish oil, and injectable organic esters. Aqueous carriers include water, water-alcohol solutions, emulsions or suspensions, including saline and buffered medical parenteral vehicles including sodium chloride solution, Ringer's dextrose solution, dextrose plus sodium chloride solution, Ringer's solution containing lactose, or fixed oils. For example, the pharmaceutically acceptable carrier may include sodium chloride and/or sodium phosphate, in which the composition includes, e.g., about 150 mM sodium chloride and/or about 25 mM sodium phosphate, pH 7.4.

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

*Gene Therapy*

The sALP and sALP fusion polypeptides (such as TNALP or TNALP fusion polypeptides, for example SEQ ID NO: 19, such as STRENSIQ™) could also be delivered through gene therapy, where an exogenous nucleic acid encoding the proteins is delivered to tissues of interest and expressed *in vivo*. Gene therapy methods are discussed, e.g., in Verme et al. (*Nature* 389:239-242, 1997), Yamamoto et al. (*Molecular Therapy* 17:S67-S68, 2009), and Yamamoto et al., (*J. Bone Miner. Res.* 26:135-142, 2011), each of which is hereby incorporated by reference. Both viral and non-viral vector systems can be used. The vectors may be, for example, plasmids, artificial chromosomes (e.g., bacterial, mammalian, or yeast artificial chromosomes), virus or phage vectors provided with an origin of replication, and optionally, a promoter for the expression of the nucleic acid encoding the viral polypeptide and optionally, a regulator of the promoter. The vectors may contain one or more selectable marker genes, for example, an ampicillin or kanamycin resistance gene in the case of a bacterial plasmid or a resistance gene for a fungal vector. Vectors may be used in *in vitro*, for example, for the production of DNA, RNA, or the viral polypeptide, or may be used to transfect or transform a host cell, for example, a mammalian host cell, e.g., for the production of the viral polypeptide encoded by the vector. The vectors may also be adapted to be used *in vivo*, for example, in a method of vaccination or gene therapy.

Examples of suitable viral vectors include, retroviral, lentiviral, adenoviral, adeno-associated viral, herpes viral, including herpes simplex viral, alpha-viral, pox viral, such as Canarypox and vaccinia-viral based systems. Gene transfer techniques using these viruses are known in the art. Retrovirus vectors, for example, may be used to stably integrate the nucleic acids of the invention into the host genome. Replication-defective adenovirus vectors by contrast remain episomal and therefore allow transient expression. Vectors capable of driving expression in insect cells (e.g., baculovirus vectors), in human cells, yeast, or in bacteria may be employed in order to produce quantities of the viral polypeptide(s) encoded by the nucleic acids of the invention, for example, for use in subunit vaccines or in immunoassays. Useful gene therapy methods include those described in WO 06/060641, U.S. Pat. No. 7,179,903 and WO 01/36620 (each of which is hereby incorporated by reference), which use an adenovirus vector to target a nucleic acid of interest to hepatocytes as protein producing cells.

In an additional example, a replication-deficient simian adenovirus vector may be used as a live vector. These viruses contain an E1 deletion and can be grown on cell lines that are transformed with an E1 gene. Examples of these replication-deficient simian adenovirus vectors are described in U.S. Patent No. 6,083,716 and WO 03/046124 (each of which is hereby incorporated by reference). These vectors can be manipulated to insert a nucleic acid of the invention, such that the encoded viral polypeptide(s) may be expressed.

Promoters and other expression regulatory signals may be selected to be compatible with the host cell for which expression is designed. For example, mammalian promoters include the metallothionein promoter, which can be induced in response to heavy metals such as cadmium, and the β-actin promoter. Viral promoters, such as the SV40 large T antigen promoter, human cytomegalovirus (CMV) immediate early (1E) promoter, rous sarcoma virus LTR promoter, adenovirus promoter, or a HPV promoter, particularly the HPV upstream regulatory region (URR) may also be used. All these promoters, as well as additional promoters, are well-described in the art.

The nucleic acid molecules described herein may also be administered using non-viral based systems. For example, these administration systems include microsphere encapsulation, poly(lactide-co-glycolide), nanoparticle, and liposome-based systems. Non-viral based systems also include techniques facilitating the delivery of "naked" polynucleotides (such as electroporation, "gene gun" delivery and various other techniques used for the introduction of polynucleotides).

The introduced polynucleotide can be stably or transiently maintained in the host cell. Stable maintenance typically requires that the introduced polynucleotide either contains an origin of replication compatible with the host cell or integrates into a replicon of the host cell such as an extrachromosomal replicon (e.g., a plasmid) or a nuclear or mitochondrial chromosome.

The following examples are intended to illustrate, rather than limit, the claimed invention.

## EXAMPLES

### *Example 1. Overview of Case Series*

Four pediatric patients with hypophosphatasia (HPP) and craniosynostosis were treated initially. The average age at presentation to our craniofacial team was 38.2 months. Each patient was treated with a soluble alkaline phosphatase (sALP) composition according to an ongoing, approved clinical trial with agent ENB-0040 (STRENSIQ™ (asfotase alfa); SEQ ID NO: 19). One patient received the sALP composition preoperatively, and three patients received the sALP composition postoperatively. All four patients presented symptoms of altered neurological function and underwent neurodiagnostic imaging to confirm the presence of craniosynostosis. Two patients were from the same family, both patients having infantile HPP associated with craniosynostosis and a familial history of HPP, with four male cousins affected by HPP.

### *Example 2. Craniosynostosis Patient 1*

Patient 1 was a 3 year and 9 month old male that was initially presented to an endocrinology medical service at the age of 2 years and 3 months. His medical history included HPP, craniosynostosis, restrictive lung disease, respiratory infections, failure to thrive, and multiple fractures. He was admitted to the hospital for management of acute exacerbation of a chronic respiratory illness. Consultation with our craniofacial team was requested to evaluate the clinical findings of dolichocephaly and ridging over the left coronal suture.

Upon clinical evaluation, the patient also presented symptoms of irritability, intermittent emesis, headaches, an asymmetrical cranial base, facial scoliosis, and bilateral papilledema. As part of a clinical trial, the patient was awaiting treatment with the sALP composition, ENB-0040 (asfotase alfa; SEQ ID NO: 19). Diagnostic radiographic imaging with three dimensional (3D) head computerized tomography (CT) scan revealed left coronal and sagittal craniosynostosis, calvarial thinning, dysplasia of the right frontal lobe, and venous anomalies including congenital absence of the right sigmoid sinus (Figs. 1A and 1B).

Due to these symptoms, a fronto-orbital advancement with cranial vault reconstruction was performed to reduce suspected intracranial hypertension and to correct the anatomical deformity.

Allograft material (Grafton DBM in Flex and Putty forms, BioHorizons IPH, Inc.) was used to augment bone formation. The patient tolerated the procedure well with no complications noted.

Three weeks after surgery, the patient started treatment with the sALP composition ENB-0040 (asfotase alfa; SEQ ID NO: 19) at a dosage of 1 mg/kg given subcutaneously six times a week. One year after the surgery, the patient was well with resolution of his papilledema and symptoms attributed to increased ICP including headache, irritability, and vomiting. This patient showed improvement in his body bone mineralization, tooth eruption, increased weight gain, and overall health. The cranial vault remodeling procedure resulted in increased space in the basal cisterns and foramen magnum with less crowding at the craniovertebral junction (Fig. 2A, pre-operative sagittal CT scan).

The lambdoid suture tends to close early in HPP, thus preventing normal growth of the posterior fossa and potentially causing herniation of the cerebellar tonsils with a resulting Chiari Type I malformation. As seen in Patient 1, the Chiari Type I malformation can resolve following cranial vault remodeling and expansion, a phenomenon that is described in other forms of craniosynostosis (Fig. 2B, sagittal CT scan, taken one year post-operatively).

***Example 3. Craniosynostosis Patient 2***

Patient 2 was the 5 year and 6 month old sister of Patient 1. Patient 2 exhibited the symptoms of malformed head shape, headaches, nystagmus, chronic optic nerveedema, and decreased visual acuity. She was previously evaluated in her home country with concerns regarding her genetic predisposition to HPP and the presence of a complex craniosynostosis.

During clinical evaluation, the patient's head circumference was 47 cm (microcephalic for her age), with a significant bony prominence over the bregma with persistence of an open anterior fontanelle that was tense on palpation. Radiography evaluation by 3D CT scanning revealed abnormal calvarial morphology with marked scalloping of the inner table with absence of the extraaxial spaces and complete obliteration of the sagittal and left coronal suture lines. These results were concerning for chronically elevated ICP (Figs. 3A and 3B). Given the chronic changes secondary to untreated HPP, healing of bone postoperatively was a concern.

The patient underwent an open cranial vault reconstruction with identification of multiple areas of calcified dura. In particular, the patient underwent multiple barrel stave osteotomies for cranial vault expansion without any perioperative complications, such as cerebrospinal fluid (CSF) leaks, associated with the calcified dura. Seven months following surgery, a 3D CT scan of the patient's head demonstrated improved bone growth of the calvaria (Fig. 3C). Although radiographically left coronal craniosynostosis was evident, clinically the patient did not exhibit asymmetrical orbital findings of unilateral coronal craniosynostosis, such as harlequin deformity. Therefore, barrel stave osteotomies were completed rather than modification of the frontoorbital complex. Due to the complexity of the patient and high risk of CSF leak because of the calcified dura, this course of cranial vault remodeling was chosen instead of a frontoorbital cranioplasty with the osteotomies.

After cranial vault reconstruction, the patient's head circumference increased to 49 cm, approaching the normal curve for the patient's age. Symptoms associated with elevated intracranial

pressure resolved, with improvement in her headaches, stabilization of chronic optic nerve edema, and improvement in visual acuity (Figs. 4A and 4B, taken one year post-operatively).

***Example 4. Craniosynostosis Patient 3***

Patient 3 was a female infant born with perinatal hypophosphatasia (birth weight was 3060 grams, length was 45 cm, and head circumference was 32 cm). She was prenatally diagnosed by fetal ultrasound with skeletal dysplasia, osteogenesis imperfect (e.g., brittle bone disease), and HPP. After delivery by repeat cesarean section, Patient 3 rapidly developed respiratory distress after delivery requiring positive pressure ventilation for marked subcostal retractions and apnea. The patient was also intubated for oxygen desaturations and remained intubated with assisted ventilation for abnormal chest compliance. The patient was also diagnosed as dysmorphic with a soft cranium and having a large anterior fontanel with widely split sagittal and metopic sutures, short limbs with deformities, talipes equinovarus, brachydactyly, narrow chest, and HPP.

The patient was then evaluated for HPP biomarkers. The initial ALP level was <20 iU/L. Genetic testing revealed two genomic variants of unknown significance (i.e., deletion 1p31.1 and duplication 6q21). *ALPL* gene testing showed compound heterozygosity with pathogenic variant 876_delAGGGGACinsT and 650T>C of unknown significance. Given the clinical presentation, the genotype was supportive of the diagnosis of HPP. Further testing of ALP activity demonstrated that pyridoxal phosphate was elevated (e.g., >250 mcg/L) and urine phosphoethanolamine was also elevated (e.g., 6025 nmol/mg).

The patient started treatment at 2 months of age with the sALP composition ENB-0040 (asfotase alfa; SEQ ID NO: 19) at a dosage of 1 mg/kg administered subcutaneously six times a week (prior to cranial vault remodeling). CT scan of the cranium at 7 months of age showed poor ossification of the bony structures with diffuse thickening of the calvarium and facial structures. The patient also had brachycephaly with bilateral coronal synostosis. She required open cranial vault remodeling with multiple osteotomies and bilateral parietooccipital remodeling at 8 months of age. Pre-surgical treatment with the sALP composition combined with cranial vault remodeling surgery resulted in an improvement of signs of craniosynostosis, and the patient was transferred to her original managing institution at 16 months, with a body weight of 8.025 kg and body length of 61.5 cm.

***Example 5. Craniosynostosis Patient 4***

Patient 4 was a female infant born with perinatal hypophosphatasia. Patient 4 presented with bilateral optic nerve edema. CT scan of the cranium showed poor ossification, left coronal craniosynostosis, sagittal craniosynostosis, and metopic synostosis. She required cranial vault remodeling surgery at 22 months of age. Post-operative treatment with the sALP composition combined with cranial vault remodeling surgery resulted in an improvement of signs of craniosynostosis.

***Example 6. Craniosynostosis Perinatal Patient 5***

A perinatal patient was treated by administration of a sALP combined with a cranial vault remodeling surgery. At birth, the male patient weighed 3460 grams. The patient was intubated and

placed on ventilator, then transferred from a community hospital to tertiary care children's hospital. The patient was treated with genetic counseling, and sequencing of the ALPL gene revealed that the patient was a compound heterozygote (c.668 G>A; c.1171 C>T). The patient's Vitamin B6 (pyridoxal phosphate) level was >2000. The patient also exhibited remarkably diminished ossification of the skull, diminished ossification and height of vertebral bodies, absence of humeral ossification, radial and ulnar metaphyses with marked metaphyseal irregularity, fragmentation and fraying, a small chest, and bones abnormal with absent ossification of medial ribs and gracile appearance of the ribs.

The patient began treatment with a sALP composition ENB-0040 (asfotase alfa; SEQ ID NO: 19) at five weeks of age. The patient then exhibited good weight gain (all nutrition by gastrostomy), normal psychosocial development, and increased bone size, while the symptoms of short stature, poor growth of limbs with continued poor mineralization, and gross motor and fine motor delays remained. The growth parameters at 12 months were as follows: weight of 8.585 kg, length of 66 cm, and head circumference of 47.5 cm. While there was almost no cranial calcification at birth, cranial development improved significantly after treatment with the sALP composition (Figs. 5A-5C). The patient developed coronal synostosis at one year of age requiring posterior cranial vault remodeling and distraction (Figs. 6A-6C). The patient exhibited significant improvement following treatment with the sALP composition in combination with a cranial vault remodeling procedure.

***Example 7. Summary of Case Series***

In the setting of craniosynostosis, CSF outflow can be reduced with impaired CSF absorption secondary to venous sinus hypertension. With the skull unable to expand in craniosynostosis, intracranial hypertension occurs. The goal of surgical treatment of HPP-related craniosynostosis is to substantially increase the intracranial volume, thereby decreasing ICP. As seen in our Patient 1 and 2, symptoms associated with suspected elevated ICP (e.g., papilledema, headaches, and emesis) can be reversed with cranial volume augmentation. Treatment with a sALP composition ENB-0040 (asfotase alfa; SEQ ID NO: 19) has improved patient outcomes for those children affected by HPP and has now made it possible to diagnose and surgically treat the secondary effects of the disease, such as craniosynostosis. In our case series, all 4 patients received a sALP composition along with a cranial vault remodeling procedure to treat craniosynostosis (Table 2).

The sALP composition was administered prior to a cranial vault remodeling procedure in one patient, and was administered after a cranial vault remodeling procedure in three patients. The outcomes from the preoperative and postoperative administration of sALP approaches were similar, and the patients did not have surgical complications. Calvarial reconstitution was excellent in the postoperative period for all four patients. These results suggest that surgical correction of HPP-associated craniosynostosis is safe and effective when used in combination with sALP administration.

**Table 2. Clinical summary of patients.**

| Case No. | Age (yrs), Sex | Neurological or skeletal Symptoms | Imaging Modality | Results | sALP | Surgery | Improved Neurological Symptoms |
|---|---|---|---|---|---|---|---|
| 1 | 3 yrs, 9 mos<br>M | Irritability; nausea; vomiting; head-aches; bilateral papilledema | Head CT | Left coronal craniosynostosis; sagittal craniosynostosis; widening of right coronal suture; persistent anterior fontanelle; vol loss of right frontal lobe; prominent scalp veins at vertex, skull base, anterior frontal lobe | After surgery | Yes | Yes |
| | | | Brain MRI | Vol loss of right frontal lobe; congenial absence of right sigmoid sinus; multiple venous anomalies | | | |
| 2 | 5 yrs, 6 mos<br>F | Headaches; nystagmus; decreased visual acuity; microcephaly | Head CT | Scalloping of inner table of skull; absence of extraaxial spaces; left coronal craniosynostosis; sagittal craniosynostosis | After surgery | Yes | Yes |
| | | | Brain MRI | Dysmorphic calvaria | | | |
| 3 | 8 mos<br>F | Brachy-cephaly; ventilator dependence hypotonia | Head CT | Bilateral coronal craniosynostosis | Prior to surgery | Yes | No papilledema |
| 4 | 22 mos<br>F | Bilateral papilledema, hypotonia | Head CT | Left coronal craniosynostosis; sagittal craniosynostosis; metopic synostosis | After surgery | Yes | Yes |
| 5 | M | Brachy-cephaly; ventilator dependence; hypotonia | Head CT | Bilateral coronal craniosynostosis, metopic craniosynostosis | Prior to surgery | Yes | No papilledema |

## 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. Indeed, various modifications of the described modes for carrying out the methods according to the disclosure that are obvious to those skilled in the art are

intended to be within the scope of the claimed invention. This application is intended to cover any variations, uses, or adaptations of the present disclosure following, in general, the principles of the disclosure and including such departures from the present disclosure come within known customary practice within the art to which the disclosure pertains and may be applied to the essential features herein before set forth.

## CLAIMS

1. A method of treating craniosynostosis in a patient having hypophosphatasia (HPP) and exhibiting or likely to have increased intracranial pressure (ICP), comprising administering a soluble alkaline phosphatase (sALP) to the patient in combination with at least one cranial vault remodeling procedure.

2. The method of claim 1, wherein the sALP is administered to the patient prior to the cranial vault remodeling procedure.

3. The method of claim 2, wherein the sALP is administered to the patient about two months to about 1 day, particularly six weeks, one month, three weeks, two weeks, one week, 6 days, 5 days, four days, or two days, prior to the cranial vault remodeling procedure.

4. The method of claim 3, wherein the sALP is administered about three weeks prior to the at least one cranial vault remodeling procedure.

5. The method of claim 1, wherein the sALP is administered to the patient after the at least one cranial vault remodeling procedure.

6. The method of claim 5, wherein the sALP is administered to the patient about two months to about 1 day, particularly six weeks, one month, three weeks, two weeks, one week, 6 days, 5 days, four days, or two days, after the cranial vault remodeling procedure.

7. The method of claim 6, wherein the sALP is administered about three weeks after the at least one cranial vault remodeling procedure.

8. The method of claim 1, wherein the patient is diagnosed with craniosynostosis prior to administration of the sALP.

9. The method of claim 1, wherein the patient is diagnosed with craniosynostosis prior to the cranial vault remodeling procedure.

10. The method of any one of claims 1-9, wherein the patient is a human.

11. The method of any one of claims 1-10, wherein the HPP is infantile HPP, childhood HPP, perinatal benign HPP, or perinatal lethal HPP.

12. The method of any one of claims 1-11, wherein the patient exhibits one or more additional symptoms of craniosynostosis.

13. The method of any one of claims 1-12, wherein the sALP is administered to the patient prior to premature fusion of cranial sutures.

14. The method of any one of claims 1-13, wherein the method further comprises monitoring ICP in the patient.

15. The method of any one of claims 1-14, wherein the method further comprises monitoring one or more additional symptoms of craniosynostosis in the patient.

16. The method of claim 15, wherein the monitoring comprises at least one of radiography, ultrasonography, clinical examination, and/or determination of sALP activity.

17. The method of claim 16, wherein the radiography comprises a computed tomography (CT) scan.

18. The method of claim 16, wherein the determination of sALP activity comprises measuring at least one of phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and/or pyridoxal 5'-phosphate (PLP) in a serum and/or blood sample from the patient.

19. The method of any one of claims 1-18, wherein the sALP is administered in an amount that is therapeutically effective to treat increased ICP.

20. The method of any one of claims 1-19, wherein the sALP is administered in an amount that is therapeutically effective to treat one or more additional symptoms of craniosynostosis.

21. The method of any one of claims 12, 15, or 20, wherein the one or more additional symptoms of craniosynostosis comprises headaches, irritability, nausea and emesis (vomiting), pulsatile tinnitus, hearing loss, abnormal skull and facial shapes, airway impairments, obstructive sleep apnea, Chiari Type I malformation, brain abnormalities, blindness, vision impairment, double vision, decreased visual acuity, deafness, seizures, impairments in mental development, herniation of cerebellar tonsils, syringomyelia, bilateral papilledema, nystagmus, microcephaly, brachycephaly, dolichocephaly, ventilator dependence, and/or chronic optic nerve edema.

22. The method of any one of claims 1-21, wherein the sALP is administered in an amount that is therapeutically effective to treat at least one symptom of HPP.

23. The method of claim 22, wherein the symptom of HPP comprises premature loss of deciduous teeth, incomplete bone mineralization, elevated blood and/or urine levels of inorganic pyrophosphate ($PP_i$), elevated blood and/or urine levels of phosphoethanolamine (PEA), elevated blood and/or urine levels of pyridoxal 5'-phosphate (PLP), hypomineralization, rachitic ribs, hypercalciuria, short stature, skeletal deformity, waddling gait, bone pain, bone fracture, HPP-related seizure, inadequate weight gain, rickets, and/or calcium pyrophosphate dihydrate crystal deposition.

24. The method of any one of claims 1-23, wherein the sALP is formulated in a pharmaceutical composition, with a pharmaceutically acceptable carrier.

25. The method of claim 24, wherein the pharmaceutically acceptable carrier is saline.

26. The method of claim 24 or 25, wherein the pharmaceutical composition is formulated for intramuscular, subcutaneous, intravenous, oral, nasal, sublingual, intrathecal, or intradermal administration.

27. The method of any one of claims 24-26, wherein the pharmaceutical composition is formulated for daily or weekly administration.

28. The method of any one of claims 1-27, wherein the sALP is administered to the patient at a dosage of about 0.1 mg/kg to about 20 mg/kg, or at a weekly dosage of about 0.5 mg/kg to about 140 mg/kg.

29. The method of any one of claims 1-28, wherein the sALP is physiologically active toward phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and pyridoxal 5'-phosphate (PLP).

30. The method of any one of claims 1-29, wherein the sALP is catalytically competent to improve skeletal mineralization in bone.

31. The method of any one of claims 1-30, wherein the sALP is the soluble extracellular domain of an alkaline phosphatase.

32. The method of any one of claims 1-31, wherein the sALP is selected from the group consisting of tissue non-specific alkaline phosphatase (TNALP), placental alkaline phosphatase (PALP), germ cell alkaline phosphatase (GCALP), and intestinal alkaline phosphatase (IALP).

33. The method of claim 32, wherein the sALP is TNALP.

34. The method of claim 33, wherein:

a) the TNALP comprises an amino acid sequence as set forth in SEQ ID NOs: 1, 2, 3, 4, or 5;
b) the TNALP comprises an amino acid sequence as set forth in SEQ ID NO: 6;
c) the TNALP comprises an amino acid sequence as set forth in SEQ ID NO: 7;
d) the TNALP comprises an amino acid sequence as set forth in SEQ ID NO: 8;
e) the TNALP comprises an amino acid sequence as set forth in SEQ ID NO: 9;
f) the TNALP comprises an amino acid sequence as set forth in SEQ ID NO: 10;
g) the TNALP comprises an amino acid sequence as set forth in SEQ ID NO: 11, 12, or 13; or
h) the TNALP comprises an amino acid sequence as set forth in SEQ ID NO: 14.

35. The method of claim 32, wherein
a) the PALP comprises an amino acid sequence as set forth in SEQ ID NOs: 15 or 16;
b) the GCALP comprises an amino acid sequence as set forth in SEQ ID NO: 17; or
c) the IALP comprises an amino acid sequence as set forth in SEQ ID NO: 18.

36. The method of any one of claims 1-34, wherein the sALP comprises a polypeptide having the structure selected from the group consisting of Z-sALP-Y-spacer-X-$W_n$-V and Z-$W_n$-X-sALP-Y-spacer-V, wherein:
V is absent or is an amino acid sequence of at least one amino acid;
X is absent or is an amino acid sequence of at least one amino acid;
Y is absent or is an amino acid sequence of at least one amino acid;
Z is absent or is an amino acid sequence of at least one amino acid; and
$W_n$ is a bone-targeting moiety.

37. The method of claim 36, wherein the structure is Z-sALP-Y-spacer-X-$W_n$-V.

38. The method of claim 36, wherein the bone-targeting moiety is a polyaspartic or polyglutamic region.

39. The method of claim 38, wherein n = 1-50.

40. The method of claim 39, wherein n = 3-30.

41. The method of claim 40, wherein n = 5-15.

42. The method of claim 41, wherein n=10.

43. The method of any one of claims 36-42, wherein the spacer comprises a fragment crystallizable (Fc) region.

44. The method of claim 43, wherein the Fc region comprises a CH2 domain, a CH3 domain, and a hinge region.

45. The method of claim 44, wherein the Fc is a constant domain of an immunoglobulin selected from the group consisting of IgG-1, IgG-2, IgG-3, and IgG-4.

46. The method of claim 45, wherein the Fc is a constant domain of an immunoglobulin IgG-1.

47. The method of claim 36, wherein at least one of V, Z, and the spacer is absent.

48. The method of claim 36, wherein Y is two amino acid residues.

49. The method of claim 48, wherein Y is leucine-lysine.

50. The method of claim 36, wherein X is two amino acid residues.

51. The method of claim 50, wherein X is aspartate-isoleucine.

52. The method of any one of claims 1-34 or 36-51, wherein the sALP comprises an amino acid sequence as set forth in SEQ ID NO: 19.

53. The method of claim 52, wherein the sALP is the amino acid sequence of SEQ ID NO: 19.

54. The method of any one of claims 1-53, wherein the patient exhibits an improvement in one or more symptoms of craniosynostosis.

55. The method of claim 54, wherein the one or more symptoms comprises headaches, irritability, nausea and emesis (vomiting), pulsatile tinnitus, hearing loss, abnormal skull and facial shapes, airway impairments, obstructive sleep apnea, Chiari Type I malformation, brain abnormalities, blindness, vision impairment, double vision, decreased visual acuity, deafness, seizures, impairments in mental development, herniation of cerebellar tonsils, syringomyelia, bilateral papilledema, nystagmus, microcephaly, brachycephaly, dolichocephaly, ventilator dependence, and/or chronic optic nerve edema.

56. The method of any one of claims 1-55, wherein the patient exhibits an improvement in one or more neurological symptoms.

57. Use of the sALP of any one of claims 1-56 in the manufacture of a medicament for treating or preventing craniosynostosis.

58. A method of diagnosing HPP in a patient, the method comprising determining the level of TNALP activity in a patient having craniosynostosis.

59. The method of claim 58, wherein the determination of TNALP activity comprises measuring at least one of phosphoethanolamine (PEA), inorganic pyrophosphate (PPi), and/or pyridoxal 5'-phosphate (PLP) in a serum and/or blood sample from the patient.

60. A method of diagnosing HPP in a patient, the method comprising determining the presence of a mutation in TNALP in a patient having craniosynostosis.

61. The method of claim 60, wherein the mutation in TNALP is associated with HPP.

FIG. 1A

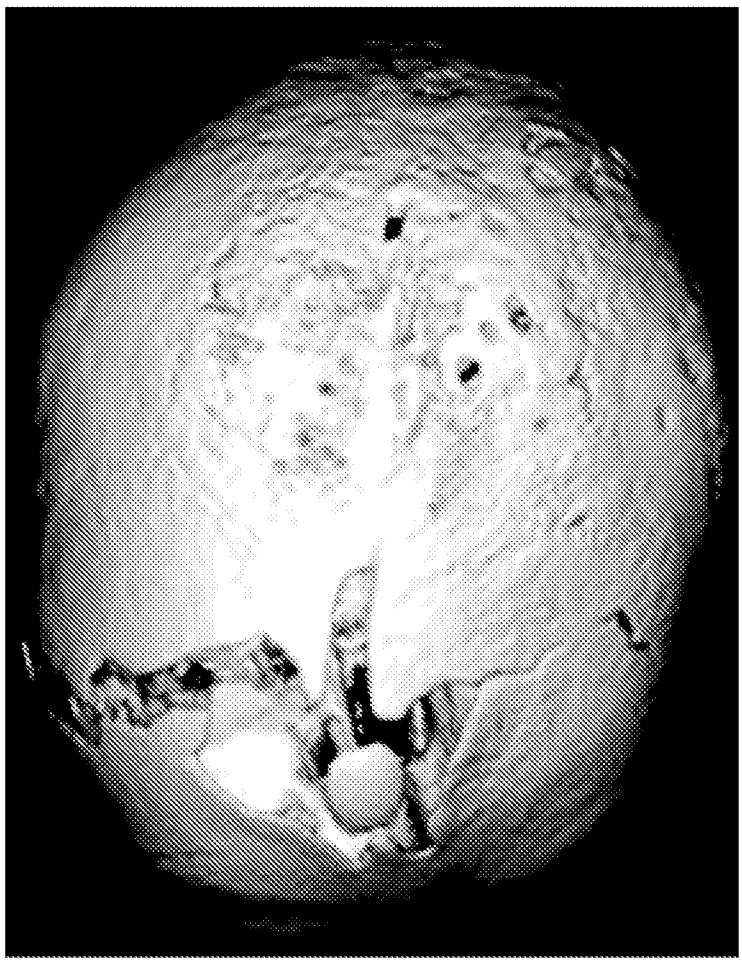


FIG. 1B

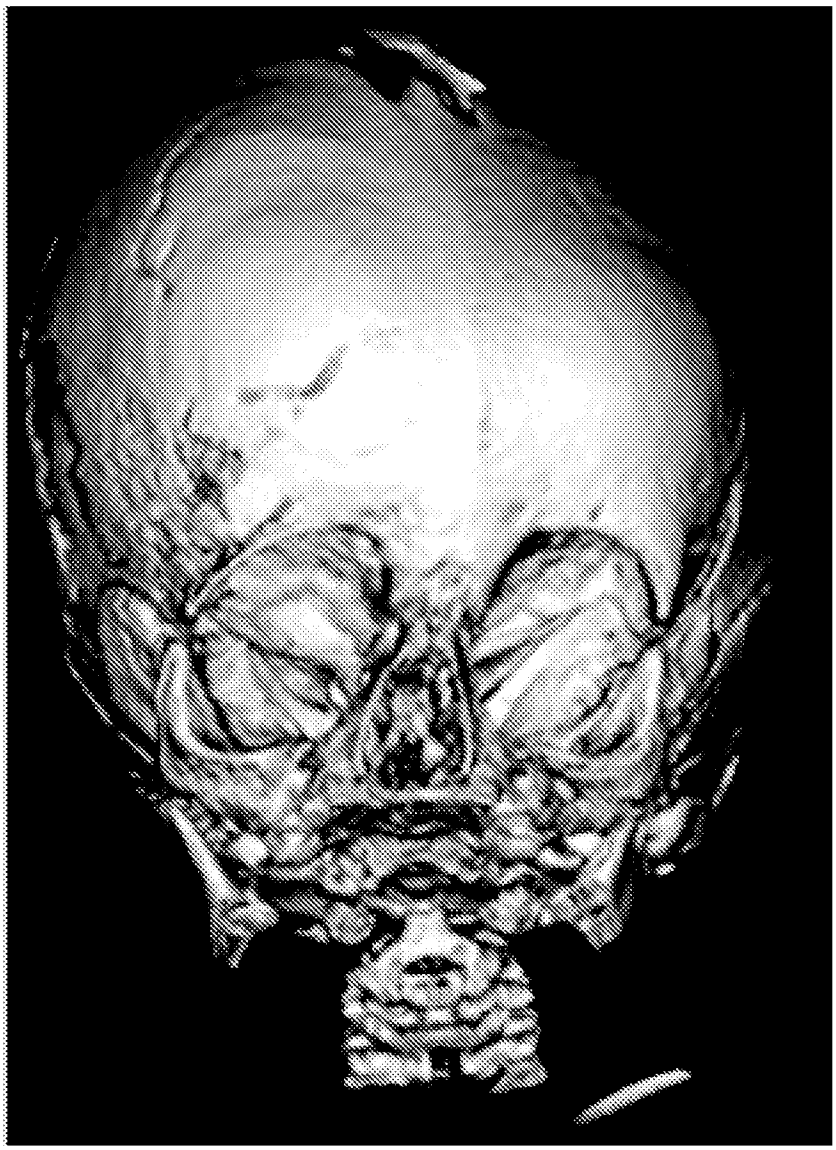


FIG. 2A

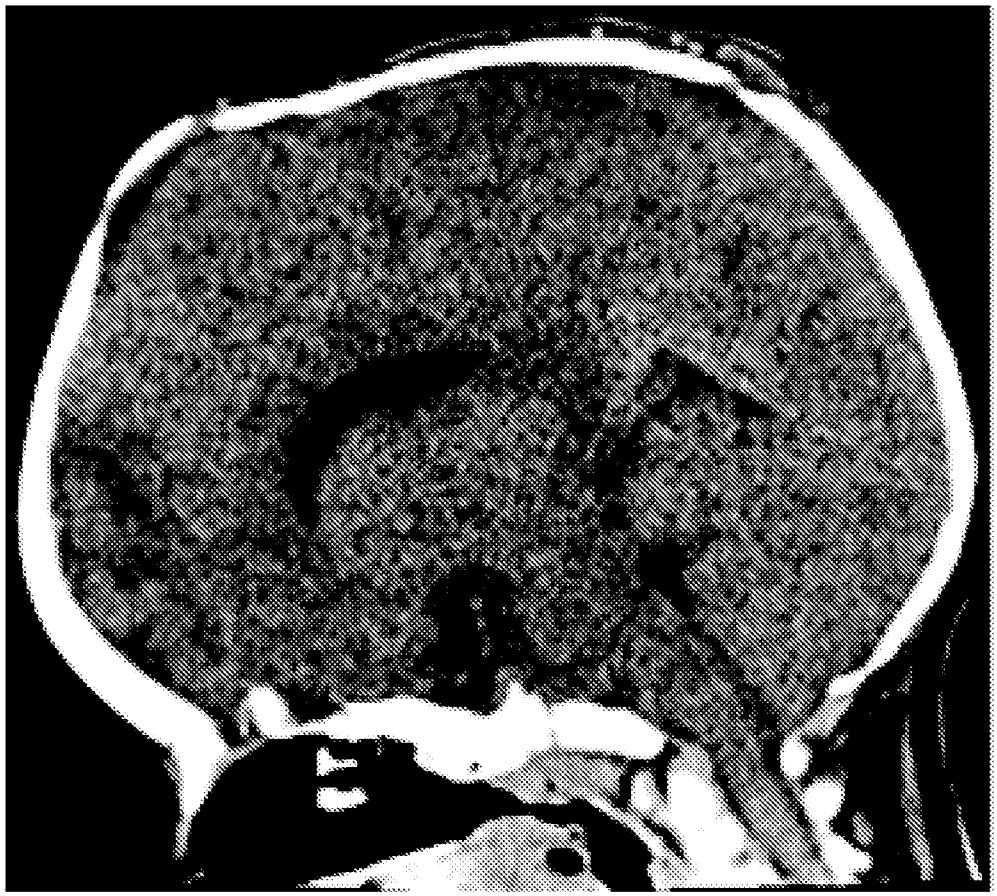


FIG. 2B

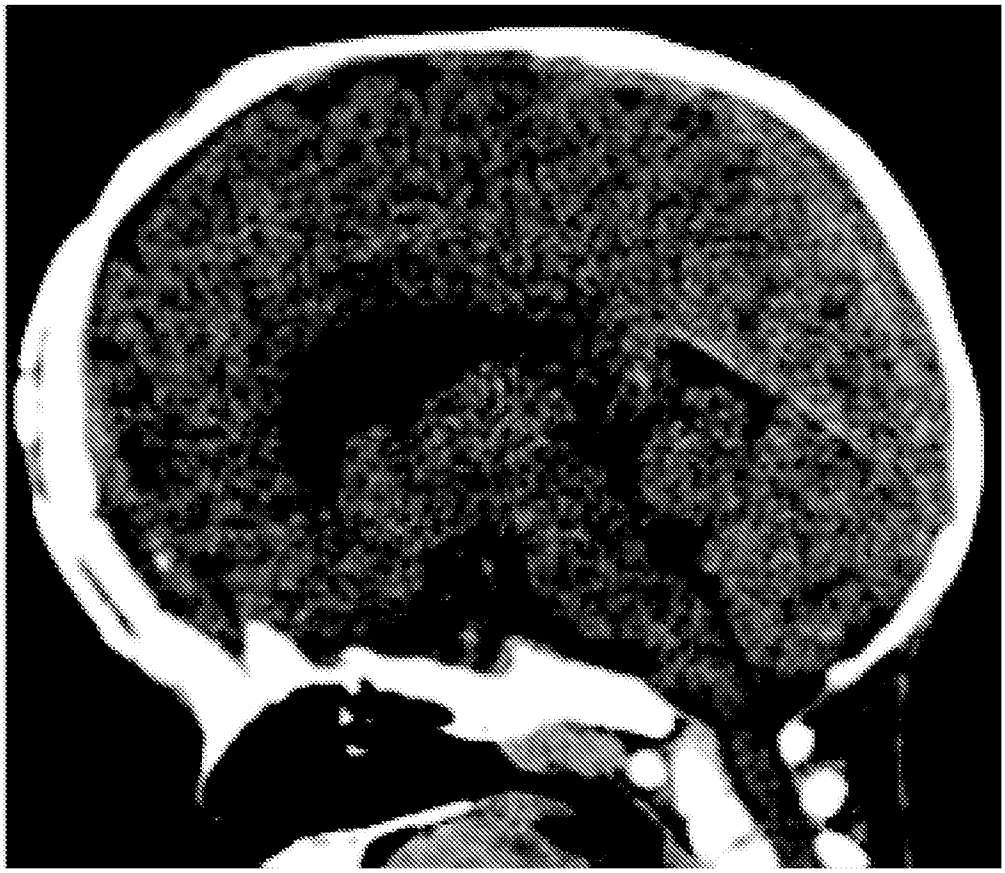


FIG. 3A

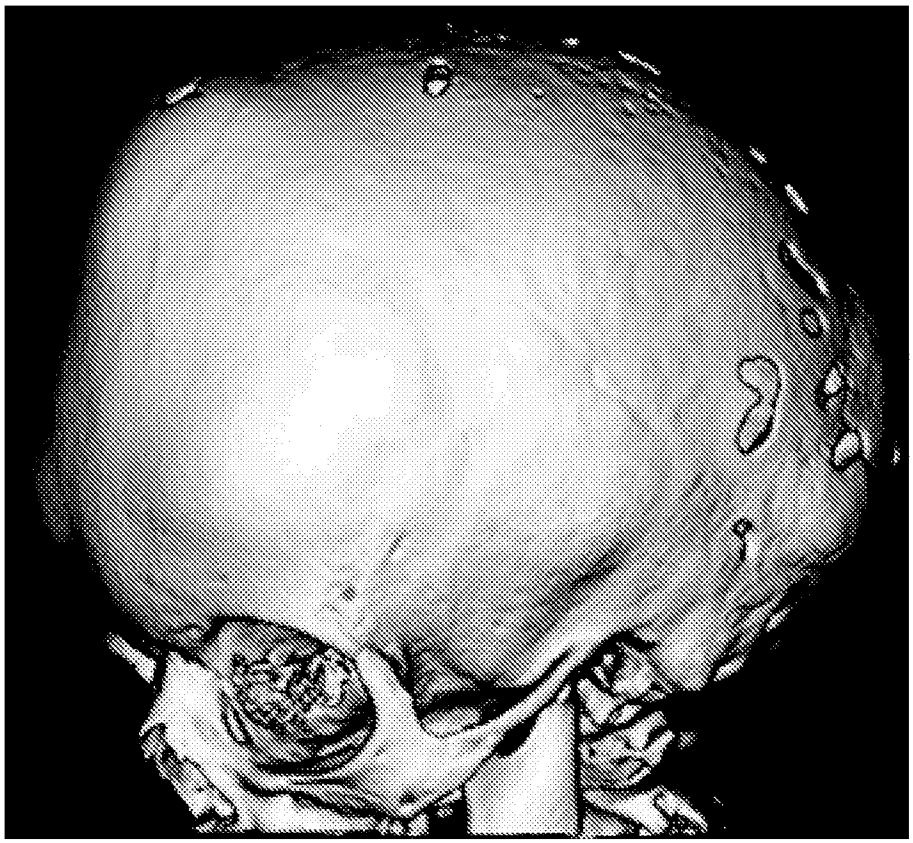


FIG. 3B

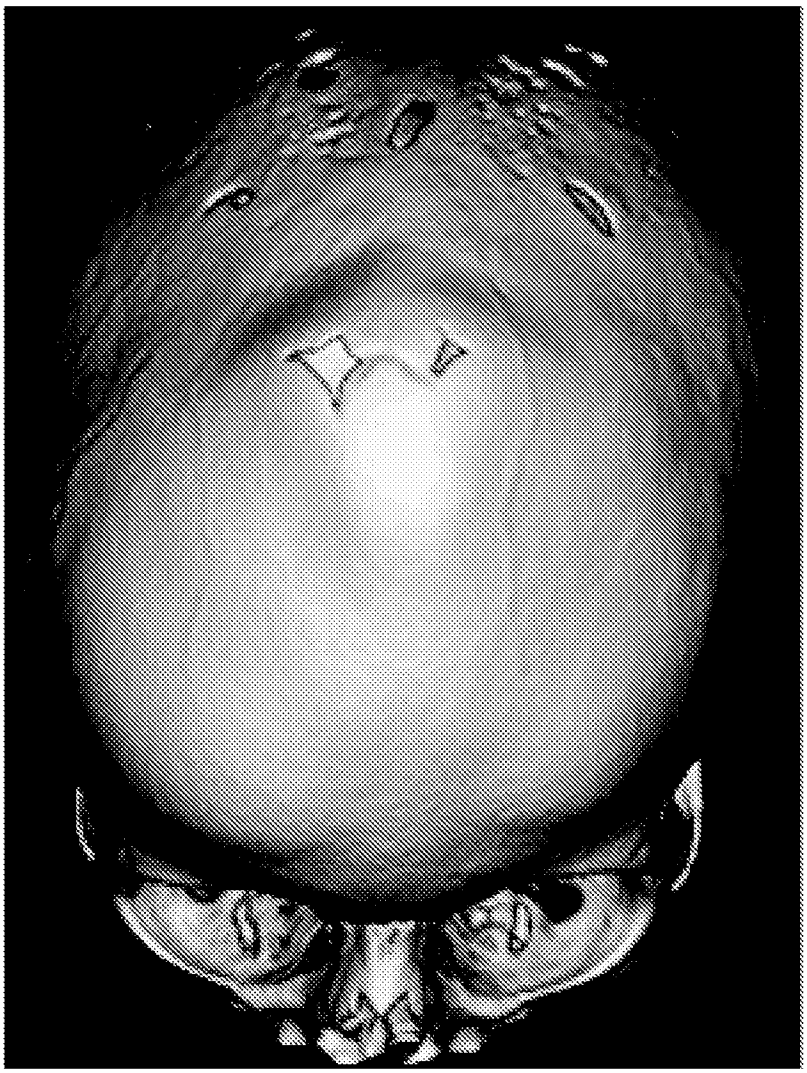


FIG. 3C

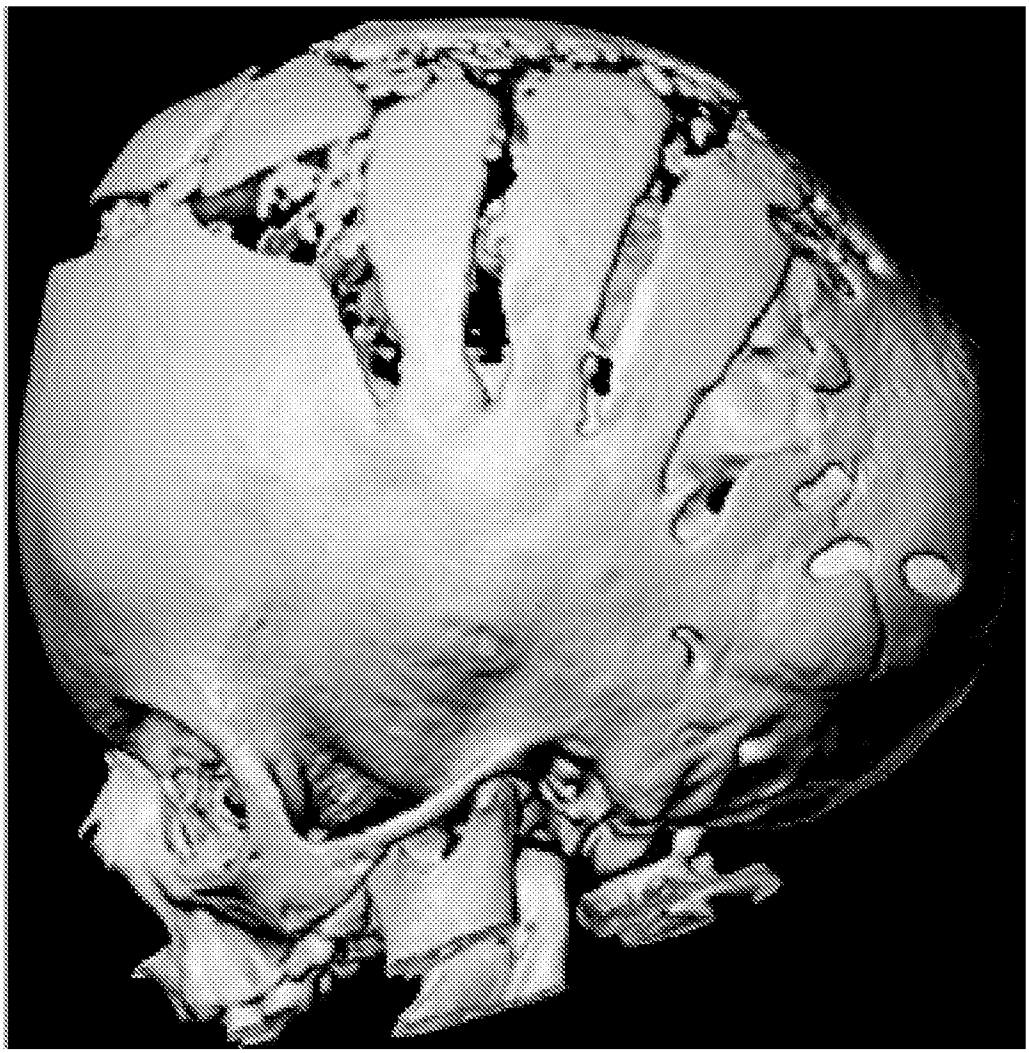


FIG. 4A

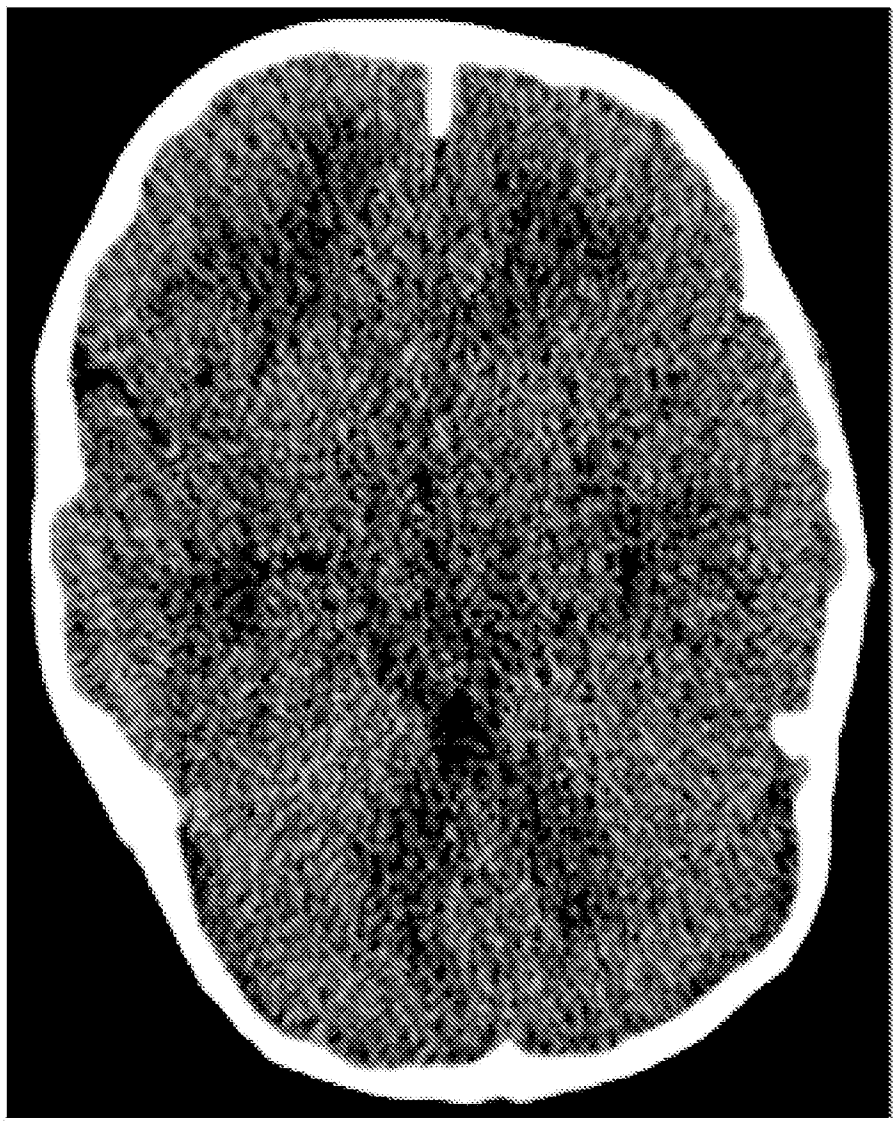


FIG. 4B

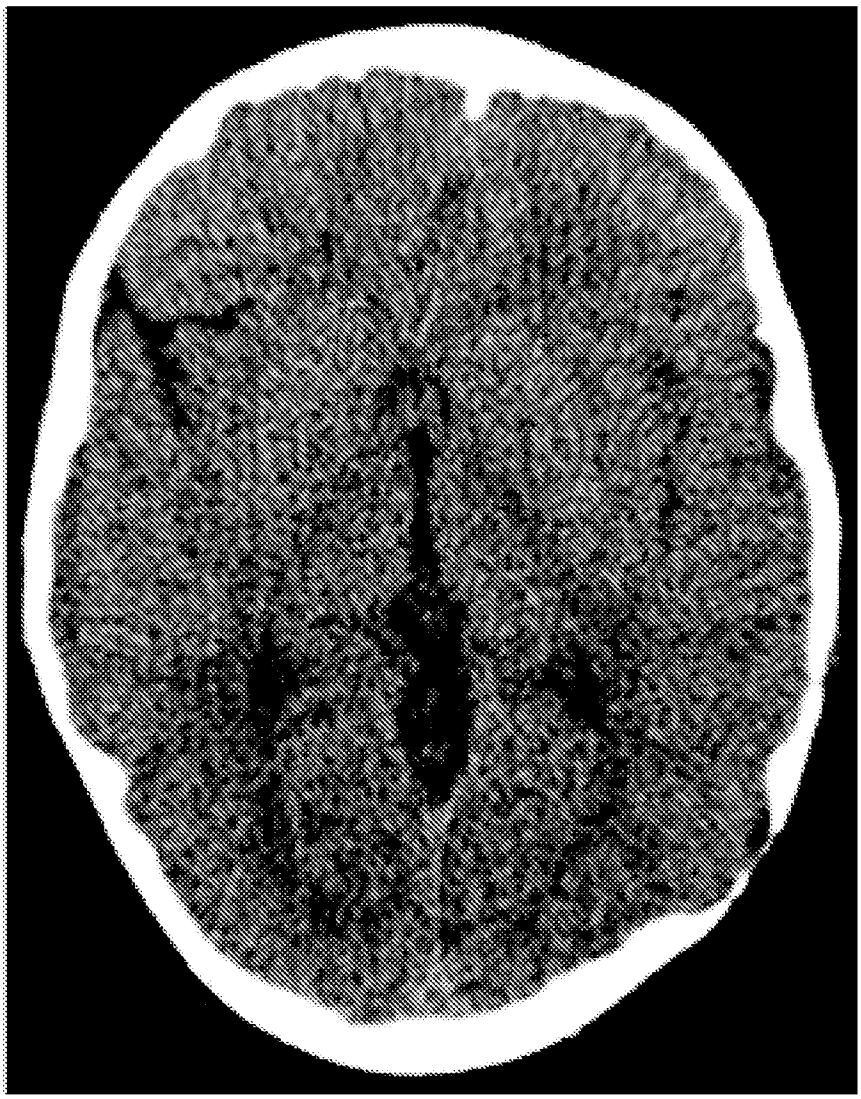


FIG. 5A

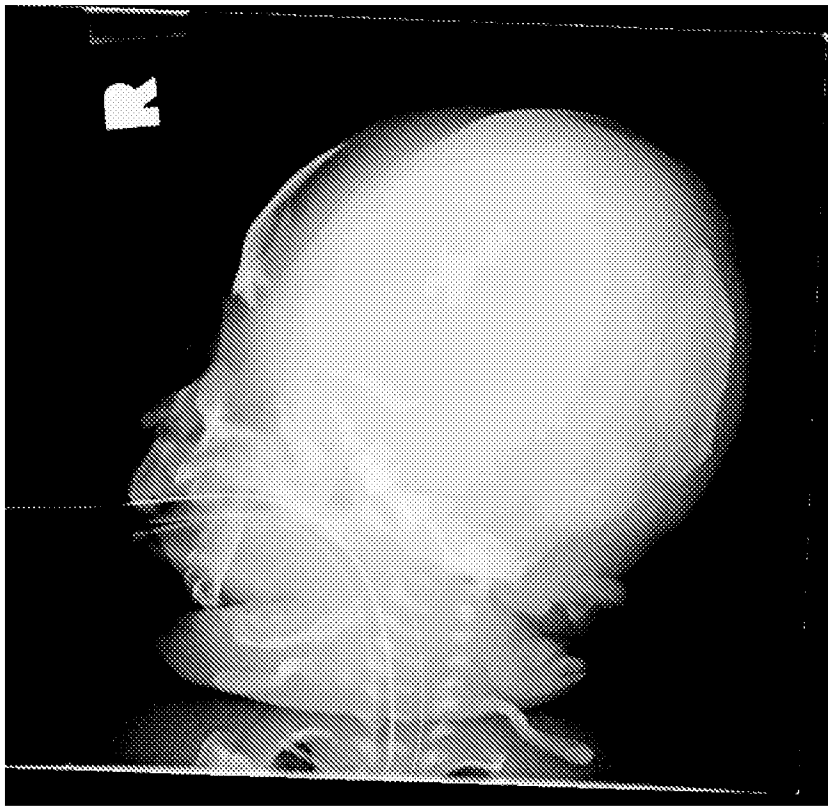


FIG. 5B

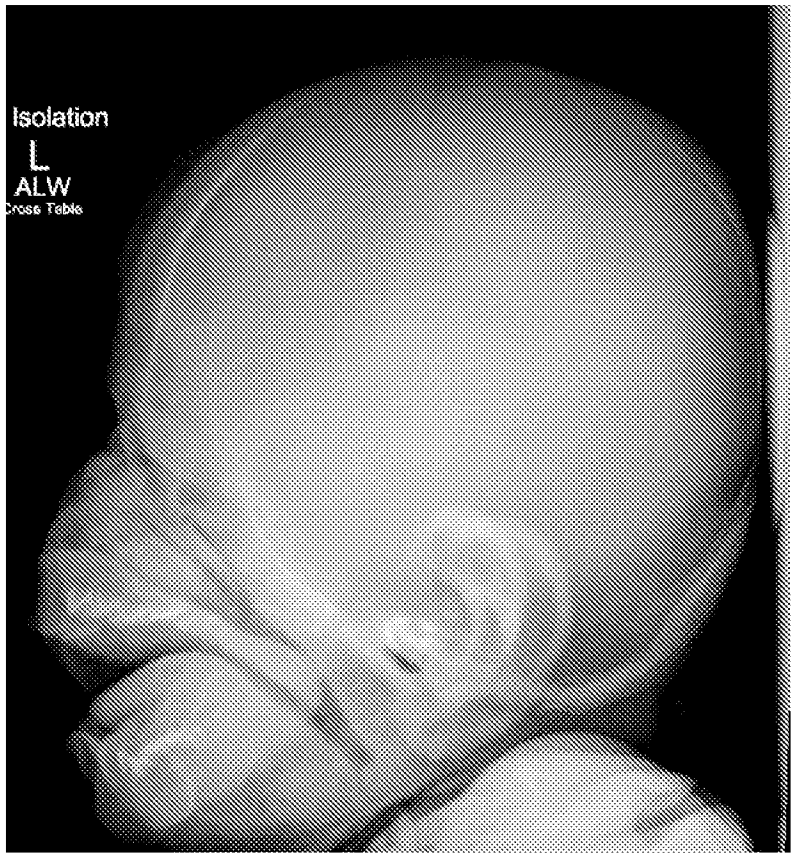


FIG. 5C

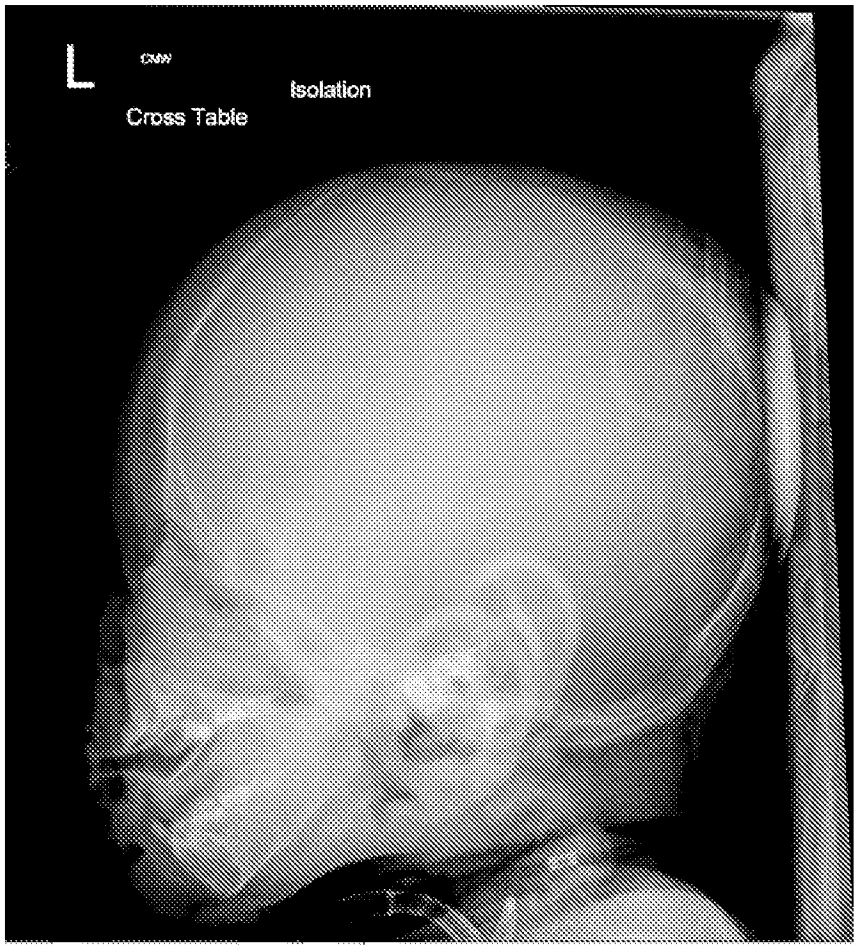


FIG. 6A

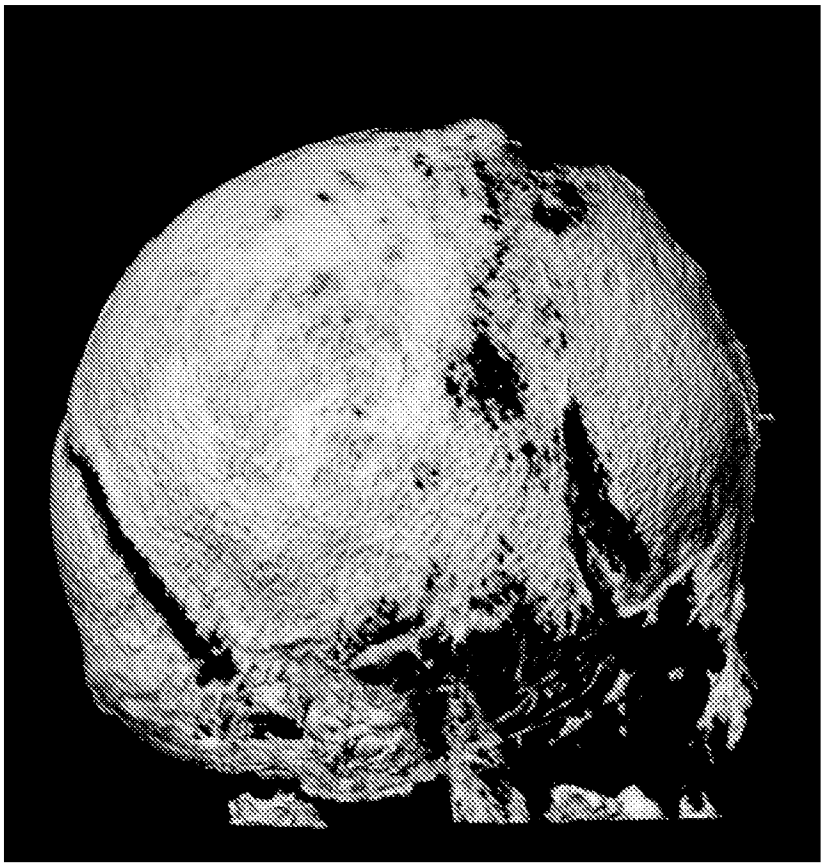


FIG. 6B

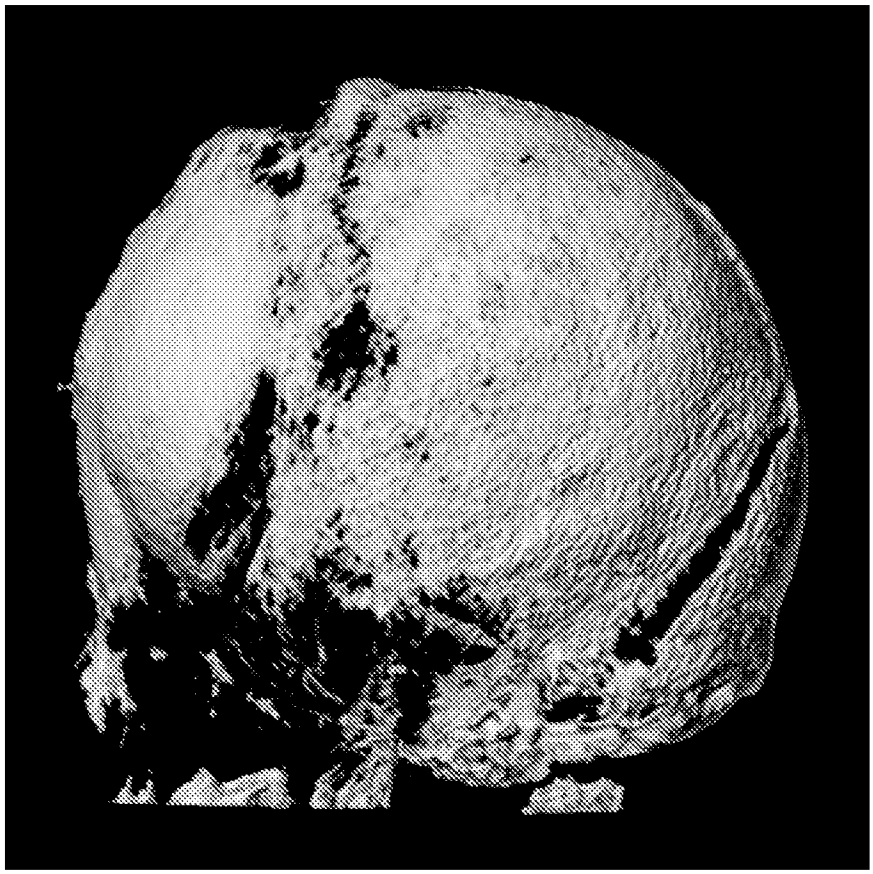
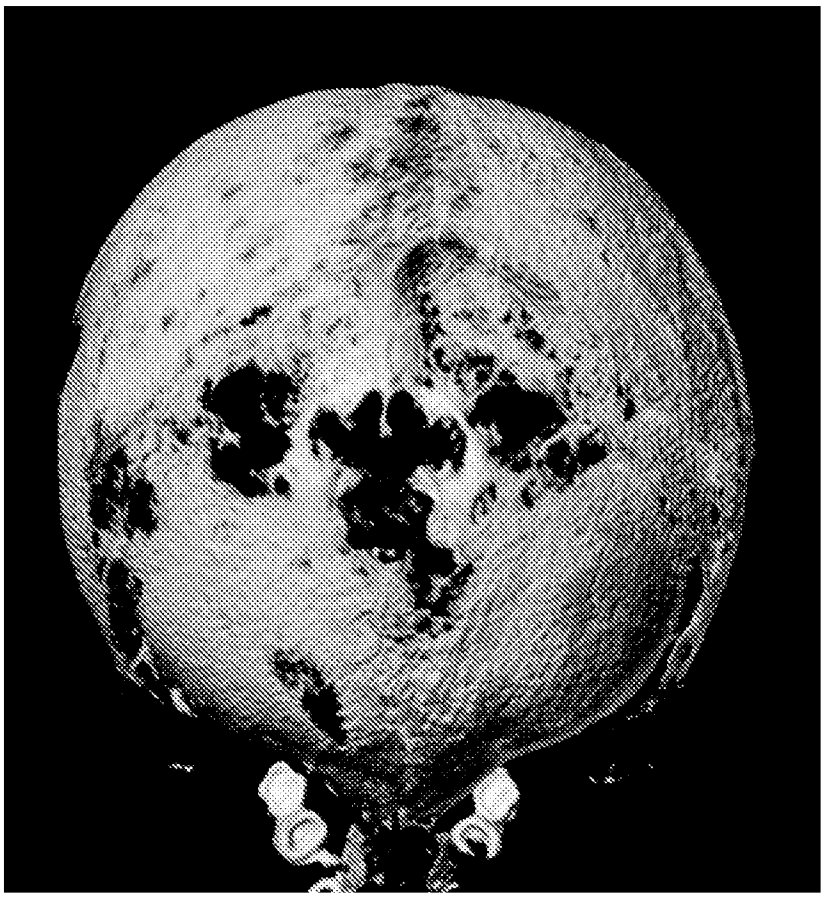


FIG. 6C



# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/58498

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 38/46, A61K 48/00 (2015.01)
CPC - A61K 38/00, A61K 48/00
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)
IPC(8): A61K 38/46, A61K 48/00 (2015.01)
CPC: A61K 38/00, A61K 48/00

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC: 424/94.6, 514/44R (text search, terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PubWest, PatBase, Google Scholar, Google Patents: Cranisynostosis, hypophosphatasia, surgery, intracranial pressure, ICP, cranial vault remodel*, alkaline phosphatase, soluble, sALP

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category* | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| X<br>---<br>Y | KOSNIK-INFINGER et al., Enzyme replacement therapy for congenital hypophosphatasia allows for surgical treatment of related complex craniosynostosis: a case series, Neurosurg Focus, May 2015, Vol. 38, No. 5; pages 1-6; Abstract; page 2, col 1, para 2; page 5, col 1, para 3-4 | 1-10<br>---<br>58-61 |
| Y | MORNET et al., Identification of fifteen novel mutations in the tissue-nonspecific alkaline phosphatase (TNSALP) gene in European patients with severe hypophosphatasia, 1998, Vol. 6, pages 308-314; Abstract | 58-61 |
| Y | MILLAN et al., Enzyme Replacement Therapy for Murine Hypophosphatasia, Joural of Bone and Mineral Research, December 17, 2007; Vol. 23, No. 6, pages 777-787; Abstract | 59 |
| A | STRENSIQ Product Monograph (Alexion Pharma International) 14 August 2015 (14.08.2015) [retrieved on 05 January 2015 from http://alexionpharma.ca/documents/Strensiq-PM-asfotase-alfa-14Aug2015.aspx] page 4, para 1 | 1 |

☐ Further documents are listed in the continuation of Box C. ☐

* 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 |
|---|---|
| 05 January 2016 (05.01.2016) | 22 JAN 2016 |
| Name and mailing address of the ISA/US<br>Mail Stop PCT, Attn: ISA/US, Commissioner for Patents<br>P.O. Box 1450, Alexandria, Virginia 22313-1450<br>Facsimile No. 571-273-8300 | Authorized officer:<br>Lee W. Young<br>PCT Helpdesk: 571-272-4300<br>PCT OSP: 571-272-7774 |

Form PCT/ISA/210 (second sheet) (January 2015)

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/58498

**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:

# INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 15/58498

**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.: 11-57
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.