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

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
27 May 2022 (27.05.2022)

WIPO | PCT

(10) International Publication Number
WO 2022/109344 A1

(51) International Patent Classification:
A61K 38/10 (2006.01) *A61P 9/00* (2006.01)
A61K 38/46 (2006.01) *A61P 13/12* (2006.01)
A61P 19/04 (2006.01)

(21) International Application Number:
PCT/US2021/060207

(22) International Filing Date:
19 November 2021 (19.11.2021)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:

63/116,106	19 November 2020 (19.11.2020)	US
63/116,086	19 November 2020 (19.11.2020)	US
63/116,093	19 November 2020 (19.11.2020)	US
63/219,229	07 July 2021 (07.07.2021)	US
63/237,351	26 August 2021 (26.08.2021)	US

(71) Applicant: **INOZYME PHARMA, INC.** [US/US]; 321 Summer Street, Suite 400, Boston, MA 02210 (US).

(72) Inventors: **HUERTAS, Pedro**; 321 Summer Street, Suite 400, Boston, MA 02210 (US). **WENKERT, Deborah**; 321 Summer Street, Suite 400, Boston, MA 02210 (US).

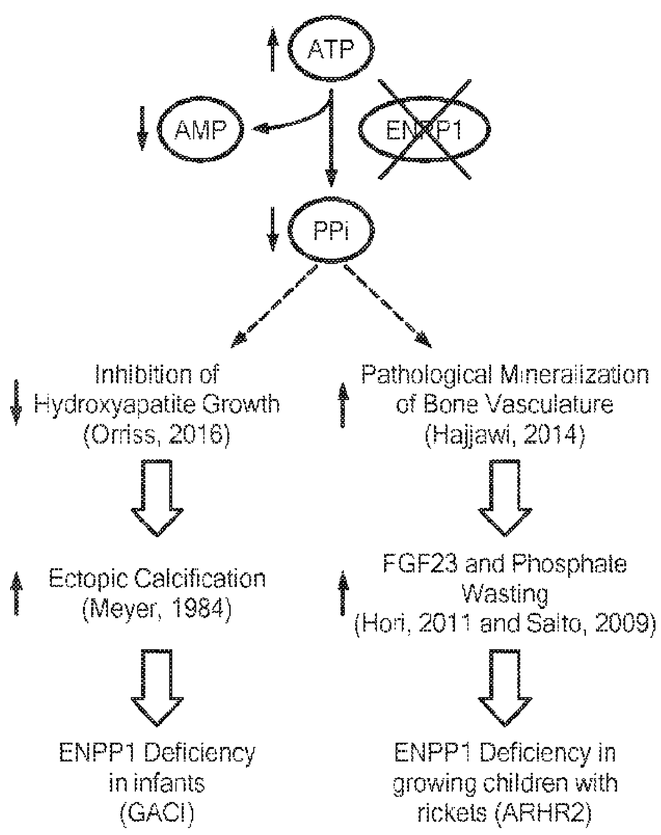
(74) Agent: **WILLIAMS, Kathleen, M.** et al.; Nutter Mcclennen & Fish LLP, Seaport West, 155 Seaport Boulevard, Boston, MA 02210-2604 (US).

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

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

(54) Title: TREATMENT OF ENPP1 DEFICIENCY AND ABCC6 DEFICIENCY

(57) Abstract: The present disclosure provides, among other things, specific doses of an ENPP1 agent for in vivo treatment of an ENPP1 deficiency, such as for treatment of Generalized Arterial Calcification of Infancy (GACI), Autosomal Recessive Hypophosphatemic Rickets 2 (ARHR2), and other diseases resulting from pathological calcification, ENPP1 deficiency, ABCC6 deficiency such as diseases or disorders involving ectopic calcification of soft tissue in a subject.



AMP/ATP = adenosine mono-/tri- phosphate;
ENPP1 = ectonucleotide pyrophosphatase/phosphodiesterase 1;
FGF23 = fibroblast growth factor 23;
PPi = inorganic pyrophosphate
References: Orriss 2016, Meyer 1984, Hajjawi 2014, Hori 2011, Saito 2009

FIGURE 1:
Overview of ENPP1 Deficiency

[Continued on next page]

WO 2022/109344 A1

Published:

— *with international search report (Art. 21(3))*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))*
— *with sequence listing part of description (Rule 5.2(a))*

Treatment of ENPP1 Deficiency and ABCC6 Deficiency

Cross-Reference to Related Applications

This application claims priority to U.S. Provisional Patent Application No. **63/116,086** entitled "*Treatment of ENPP1 Deficiency*" filed on November 19, 2020 (Attorney Docket No. 4427-10901), U.S. Provisional Patent Application No. **63/116,093** entitled "*Treatment of diseases of pathological calcification*" filed on November 19, 2020 (Attorney Docket No. 4427-11201), U.S. Provisional Patent Application No. **63/116,106** entitled "*Treatment of ABCC6 Deficiency*" filed on November 19, 2020 (Attorney Docket No. 4427-11001), U.S. Provisional Patent Application No. **63/219,229** entitled "*Treatment of ENPP1 Deficiency*" filed on July 7, 2021 (Attorney Docket No. 4427-10903), and U.S. Provisional Patent Application No. **63/237,351** entitled "*Treatment of ABCC6 Deficiency*" filed on August 26, 2021 (Attorney Docket No. 4427-11003), all of which applications are hereby incorporated by reference in their entirety.

Field

The field of the invention relates to treatment of ENPP1 deficiency and ABCC6 deficiencyby enzyme replacement.

Sequence Listing

This application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on November 19th, 2021, is named 4427-10902_sequence_ST25.txt and is 93.112 bytes in size.

Background

Calcification in biological systems is a complex process by which calcium salts are maintained at higher concentrations in noncirculating matrices than in regional circulating humoral or other mobile fluids. The principal result of normal calcification is the concentration of calcium and associated inorganic salts in crystalline patterns of similar arrangement and chemical composition in specialized intercellular matrices, all of which might vary among the different species. The net effect of pathological calcification is the concentration of calcium and associated inorganic salts with a greater-than-normal range in chemical composition or

diversity of pattern, not only in these specialized matrices but also in other intercellular, extracellular, and cellular materials leading to several disease states.

Some common examples of disease states of pathological calcification include but are not limited to kidney and bladder stones, dental pulp stones, gall stones, salivary gland stones, chronic calculous prostatitis, testicular microliths, calcification in hemodialysis patients, atherosclerosis, malacoplakia, scleroderma (systemic sclerosis), calcinosis cutis, calcific aortic stenosis, calcific tenditis, synovitis and arthritis, diffuse interstitial skeletal hyperostosis, juvenile dermatomyositis, Generalized Arterial Calcification of Infancy (GACI), Ossification of the Posterior Longitudinal Ligament (OPLL), hypophosphatemic rickets, osteoarthritis, calcification of atherosclerotic plaques, Chronic Kidney Disease (CKD), End Stage Renal Disease (ESRD), Pseudoxanthoma elasticum (PXE), ankylosing spondylitis, hardening of the arteries, calciphylaxis, and systemic lupus erythematosus.

ENPP1 Deficiency is a rare, genetic disorder caused by inactivating mutations in the *ENPP1* gene that encodes the ENPP1 enzyme. ENPP1 is an integral transmembrane protein whose extracellular domains carry pyrophosphatase and phosphodiestrerase activities. ENPP1 converts extracellular ATP to inorganic pyrophosphate (PPi) and AMP.

ENPP1 Deficiency causes hypopyrophosphatemia and hypoadenosinemia which, in turn, leads to ectopic (especially arterial) calcification (described in literature as Generalized Arterial Calcification of Infancy [GACI]), skeletal dysfunction secondary to rickets and osteomalacia (described in literature as Autosomal Recessive Hypophosphatemic Rickets 2 [ARHR2]) and occlusive neo-intimal proliferation. Beyond symptomatic and palliative interventions, no targeted therapy exists for this disease. Thus, ENPP1 Deficiency has a high unmet medical need. Infants with ENPP1 Deficiency have high mortality in the first 0 to 6 months of life and children and adults with ENPP1 Deficiency experience ongoing risk for organ calcification and dysfunction, debilitating rickets that progresses to osteomalacia in adulthood with severe bone and joint pain, fatigue, muscle weakness, and repeated bone fractures, all symptoms that lead to poor quality of life and function. Hypopyrophosphatemia causes a reactive increase in fibroblast growth factor 23 (FGF23) leading to hyperphosphaturia (the ENPP1 Deficiency "biochemical axis"), an essential feature in the pathophysiology of ENPP1 Deficiency.

ENPP1 Deficiency is characterized biochemically by low plasma PPi levels and clinically characterized by vascular calcification in infants (GACI Type phenotype) and rickets (ARHR2 phenotype) post-infancy and intimal proliferation. GACI (generalized arterial calcification of infants) is a severe disease occurring in infants and involving extensive arterial calcification (*Albright, et al., 2015, Nature Comm. 10006*).

ABCC6 deficiency is a rare, inherited, genetic inborn error of metabolism caused by mutations in the ABCC6 gene. ABCC6 deficiency is inherited as a recessive trait in which the genetic mutations result in decreased or absent activity of the ABCC6 protein (also known as MRP6 (multi-drug resistance protein 6), ATP-binding cassette sub-family C member 6 (ABCC6) and multi-specific organic anion transporter E (MOAT-E)). The deficiency leads to low plasma levels of PPi and is associated with pathological mineralization in blood vessels and soft tissues throughout the body, resulting in significant morbidity, including blindness, life-threatening cardiovascular complications, and skin calcification throughout the body, resulting in significant morbidity, including blindness, life-threatening cardiovascular complications, and skin calcification. The pathological mineralization associated with ABCC6 deficiency is the result of ectopic calcification in elastic fibers, a component of connective tissue, which provides strength and flexibility to structures throughout the body. Ectopic calcification can affect function in elastic fibers in the eyes, blood vessels, and skin, and less frequently in other areas such as the digestive tract.

Mutations in ABCC6 cause pseudoxanthoma elasticum (PXE).[9] The most common mutations, R1141X and 23-29del, account for about 25% of identified mutations. Le Saux O, et al. (2001). "*A spectrum of ABCC6 mutations is responsible for pseudoxanthoma elasticum*". Am. J. Hum. Genet. 69 (4): 749–64. Pfendner EG, et al. (2007). "*Mutation Detection in the ABCC6 Gene and Genotype-Phenotype Analysis in a Large International Case Series Affected by Pseudoxanthoma Elasticum*". Journal of Medical Genetics. 44 (10): 621–8.

Premature atherosclerosis is also associated with mutations in the ABCC6 gene, even in those without PXE. Trip MD, et al. (2002). "Frequent mutation in the ABCC6 gene (R1141X) is associated with a strong increase in the prevalence of coronary artery disease". Circulation. 106 (7): 773–5.

Deficiency of ABCC6 in mouse models of ischemia leads to larger infarcts, which can be rescued by ABCC6 overexpression. Mungrue IN, et al. (2011). "*ABCC6 deficiency causes increased infarct size and apoptosis in a mouse cardiac ischemia-reperfusion model*". Arterioscler Thromb Vasc Biol. 31 (12): 2806–12.

Some infants with ABCC6 deficiency are diagnosed with a vascular calcification condition resembling the acute infantile form of ENPP1 deficiency. In older patients, ABCC6 deficiency presents as pseudoxanthoma elasticum, or PXE, a rare disorder in which individuals develop calcification of soft connective tissues, including in the eyes, cardiovascular system, and skin. Individuals with PXE often have abnormalities in the eyes, such as a change in the pigmented cells of the retina or angioid streaks that occur when tiny cracks form in Bruch's membrane, the elastic membrane beneath the retina. Choroidal neovascularization — subsequent bleeding and scarring of the retina — may also occur, which, together with the damage to Bruch's membrane, can cause vision loss. A recent report stated that 37 percent of PXE patients over the age of 50 experienced visual impairment and 15 percent were legally blind. (Risseeuw S, Ossewaarde-van Norel J, Klayer CCW, Colijn JM, Imhof SM, van Leeuwen R. Visual acuity in pseudoxanthoma elasticum. Retina. 2019;39(8):1580-1587). Pathological mineralization of the vessels that carry blood from the heart to the rest of the body may cause other signs and symptoms of PXE. Ectopic calcification narrows blood vessels, particularly in the lower extremities, and leads to claudication, a condition characterized by cramping and pain during exercise due to decreased blood flow to the arms and legs. Individuals with PXE may also have yellowish bumps called papules on the neck, underarms, and other areas of the skin surrounding the joints. These papules are painful, can impair joint movement, and indicate a general, systemic, pathological process of soft tissue calcification.

PPi is modulator of bone mineralization and a potent inhibitor of calcium hydroxyapatite crystal deposition and is essential for prevention of harmful soft tissue calcification.

PPi functions as a potent inhibitor of ectopic tissue mineralization by binding to nascent hydroxyapatite (HA) crystals, thereby preventing the future growth of these crystals. ENPP1 generates PPi via hydrolysis of nucleotide triphosphates (NTPs), Progressive Ankylosis Protein (ANK) transports intracellular PPi into the extracellular space, and Tissue Non-specific Alkaline Phosphatase (TNAP) removes PPi via direct hydrolysis of PPi into Pi.

Ectopic tissue mineralization is associated with numerous human diseases, including chronic joint disease and acutely fatal neonatal syndromes. To prevent unwanted tissue calcification, factors that promote and inhibit tissue mineralization must be kept in tight balance. The balance of extracellular inorganic pyrophosphate (PPi) and phosphate (Pi) is an important regulator of ectopic tissue mineralization. The activity of the three extracellular enzymes -TNAP, ANK, and ENPP1 - tightly control the concentration of Pi and PPi in mammals at μ mM and 2-3 μM respectively. PPi is a regulator of biomineralization, inhibiting the formation of basic calcium phosphate from amorphous calcium phosphate.

ENPP1 polypeptides have been shown to be effective in treating certain diseases of ectopic tissue calcification. ENPP1-Fc has been shown to reduce generalized arterial calcifications in a mouse model for GACI (generalized arterial calcification of infants), which is a severe disease occurring in infants and involving extensive arterial calcification (Albright, et al., 2015, Nature Comm. 10006). Fusion proteins of ENPP1 also have been described to treat diseases of severe tissue calcification (*see*, *e.g.*, PCT Application Publication Nos. WO 2014/126965 and WO 2016/187408), and a fusion protein of ENPP1 comprising a negatively-charged bone-targeting domain has been described to treat GACI (PCT Application Publication Nos. WO 2011/113027 and WO 2012/125182).

Currently no clinically approved treatments exist for these ultra-rare genetic, chronic, progressive, and life-threatening diseases in which patients experience devastating effects on multiple systems of the body, leading to life-threatening or debilitating complications.

Summary

The disclosure relates to administration of an ENPP1 agent to a subject at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject.

In an aspect, the disclosure relates to administering to a subject having an ENPP1 deficiency or to a subject having an ABCC6 deficiency, an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject in order to restore a physiological level of ENPP1 protein and/or activity in the plasma or tissues of the subject. A physiological level of ENPP1 activity in the plasma or tissues, as used herein, is an amount or concentration of ENPP1 activity sufficient to achieve and maintain a physiological level of PPi in human serum.

In an aspect, the disclosure relates to administering to a subject having an ENPP1 deficiency or to a subject having an ABCC6 deficiency, an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject in order to restore a physiological level of Pi and/or PPi in the plasma of the subject. The physiological level of Pi and PPi in human serum (and in mammals generally) is 1-3 mM and 2-3 μM respectively.

In an aspect, the disclosure relates to a method for preventing progression of or reducing vascular calcification in a subject with ENPP1 Deficiency or in a subject having an ABCC6 deficiency, the method comprising: to thereby prevent the progression of or reduce vascular calcification in the subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce vascular calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing pathological calcification in a subject with ENPP1 Deficiency or in a subject having an ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce pathological calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing tissue calcification in a subject with ENPP1 Deficiency or a subject having an ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing pathological ossification in a subject with ENPP1 Deficiency or in a subject having an ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject.

In an aspect, the disclosure relates to a method for increasing circulating pyrophosphate (PPi) in a subject with ENPP1 Deficiency or in a subject having an ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

In an aspect, the disclosure relates to a method for increasing pyrophosphatase activity in a subject with ENPP1 Deficiency or in a subject having an ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

In an aspect, the disclosure relates to a method for ameliorating one or more symptoms of ENPP1 Deficiency in a subject or one or more symptoms of ABCC6 deficiency in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby ameliorate one or more symptoms of ENPP1 Deficiency or one or more symptoms of ABCC6 Deficiency in the subject.

In an aspect, the disclosure relates to a method for treating a subject with ENPP1 Deficiency or a subject having an ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby treat the subject.

In an aspect, the disclosure relates to methods in which the administered dose is about 0.2 mg per kilogram of the subject.

In some embodiments, the disclosure relates to methods in which the administered dose is 0.2 (± 1-10 %) mg per kilogram of the subject. For example, the dose can be 0.22 mg/kg (0.2 +10% mg/kg), or 0.18 mg/kg (0.2-10% mg/kg), or 0.21 mg/kg (0.2 + 5% mg/kg), or 0.19 mg/kg (0.2-5% mg/kg) or 0.202 mg/kg (0.2 + 1% mg/kg) or 0.198 mg/kg (0.2 -1% mg/kg) of the subject.

In an aspect, the disclosure relates to methods in which the administered is about 0.6 mg per kilogram of the subject.

In some embodiments, the disclosure relates to methods in which the administered dose is 0.6 (± 1-5 %) mg per kilogram of the subject. For example, the dose can be 0.63 mg/kg (0.6 +5% mg/kg), or 0.57 mg/kg (0.6-5% mg/kg), or 0.624 mg/kg (0.6 + 4% mg/kg), or 0.576 mg/kg (0.6-4% mg/kg) or 0.618 mg/kg (0.6 + 3% mg/kg) or 0.582 mg/kg (0.6 -3% mg/kg) or 0.612 mg/kg (0.6+2% mg/kg) or 0.588 mg/kg (0.6-2% mg/kg) or 0.606 mg/kg (0.6 +1% mg/kg) or 0.594 mg/kg (0.6-1% mg/kg) of the subject.

In an aspect, the disclosure relates to methods in which the administered is about 1.8 mg per kilogram of the subject.

In some embodiments, the disclosure relates to methods in which the administered dose is 1.8 (± 1-5 %) mg per kilogram of the subject. For example, the dose can be 1.89 mg/kg (1.8 +5% mg/kg), or 1.71 mg/kg (1.8-5% mg/kg), or 1.872 mg/kg (1.8 + 4% mg/kg), or 1.728 mg/kg (1.8-4% mg/kg) or 1.854 mg/kg (1.8 + 3% mg/kg) or 1.746 mg/kg (1.8 -3% mg/kg) or 1.836 mg/kg (1.8+2% mg/kg) or 1.764 mg/kg (1.8-2% mg/kg) or 1.818 mg/kg (1.8 +1% mg/kg) or 1.782 mg/kg (1.8-1% mg/kg) of the subject.

In yet another aspect, the disclosure relates to a method for treating a subject with ENPP1 Deficiency or a subject with ABCC6 deficiency, the method comprising administering to the subject an ENPP1 agent in an amount effective to treat, or otherwise ameliorate one or more symptoms associated with, the subject's ENPP1 deficiency or the subject's ABCC6 deficiency.

In some embodiments of any of the methods described herein, the subject can be, e.g., one who has discontinued (e.g., at least 14 days prior to treatment with the ENPP1 agent) treatment with (or is otherwise not receiving at the time of treatment with the ENPP1 agent) one or more (or all) of the following: a bisphosphonate, an anti-FGF23 antibody or FGF23 antagonist, a calcimimetic, an antacid, a corticosteroid, or a PTH suppressor.

In some embodiments of any of the methods described herein, the subject is one who has been pre-treated with one or more statins and/or one or more proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. Preferably, such pre-treatment include consistent dosage and frequency of the one or more statins and/or one or more PCSK9 inhibitors for 3 or more years prior to treatment according to methods described herein.

In some embodiments of any of the methods described herein, the subject has not been diagnosed with a malignancy within 5 years prior to treatment according to methods described herein, with the exception of non-melanoma skin cancers and/or cervical carcinoma in situ.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is administered at least one time per week.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is administered at least two times per week.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is administered to the subject at least two times per week following an initial dose.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is administered subcutaneously.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is self-administered.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is administered under a dosing regimen comprising: (a) an initial dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject and (b) about seven days after the initial dose, twice weekly administration of maintenance doses of the ENPP1 agent of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject.

In an aspect, the disclosure relates to methods in which the initial dose of the ENPP1 agent and the maintenance dose of the ENPP1 agent are the same amount.

In an aspect, the disclosure relates to methods in which the administered ENPP1 agent comprises the catalytic domain of ENPP1.

In an aspect, the disclosure relates to methods in which the administered ENPP1 agent comprises the nuclease domain of ENPP1.

In an aspect, the disclosure relates to methods in which the administered ENPP1 agent comprises the extracellular domain of ENPP1.

In an aspect, the disclosure relates to methods in which the administered ENPP1 agent comprises the catalytic and nuclease domains of ENPP1.

In an aspect, the disclosure relates to methods in which the administered ENPP1 agent comprises a heterologous moiety; the heterologous moiety may be a polypeptide.

In an aspect, the disclosure relates to methods in which the heterologous moiety increases the circulating half-life of the ENPP1 agent relative to the circulating half-life of the ENPP1 agent lacking the heterologous moiety.

In an aspect, the disclosure relates to methods in which the heterologous moiety comprises the Fc region of an immunoglobulin molecule; the immunoglobulin molecules may be a human immunoglobulin molecule; the immunoglobulin molecule may be an IgG1.

In an aspect, the disclosure relates to methods in which the heterologous moiety comprises albumin; the heterologous moiety comprises serum albumin; the heterologous moiety comprises human serum albumin.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising amino acid residues 99 (**P**SCAKE) to 925(QE**D**) of SEQ ID NO:1.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising amino acid residues 1 (**F**TAGLKPSCAKE) to 833 (QE**D**) of SEQ ID NO:3.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising the amino acid sequence depicted in SEQ ID NO:2.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising the amino acid sequence depicted in SEQ ID NO:3.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising the amino acid sequence depicted in SEQ ID NO:4.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising the amino acid sequence depicted in SEQ ID NO:5.

In an aspect, the disclosure relates to methods in which the subject has or is suspected of having generalized arterial calcification of infancy (GACI).

In an aspect, the disclosure relates to methods in which the subject has or is suspected of having autosomal recessive hypophosphatemic rickets type 2 (ARHR2).

In an aspect, the disclosure relates to methods in which the subject has or is suspected of having Pseudoxanthoma elasticum (PXE).

In an aspect, the disclosure relates to methods in which the subject having ABCC6 deficiency and exhibits symptoms similar to a subject having autosomal recessive hypophosphatemic rickets type 2 (ARHR2) or generalized arterial calcification of infancy (GACI).

In an aspect, the disclosure relates to administering to a subject having pathological calcification an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject in order to restore a physiological level of Pi and/or PPi in the plasma of the subject. The physiological level of Pi and PPi in human serum (and in mammals generally) is 1-3 mM and 2-3 μM respectively.

In an aspect, the disclosure relates to a method for preventing progression of or reducing vascular calcification in a subject having pathological calcification, the method comprising: to thereby prevent the progression of or reduce vascular calcification in the subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce vascular calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing pathological calcification in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce pathological calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing tissue calcification in a subject, the method comprising: administering to the subject

an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject

In an aspect, the disclosure relates to a method for preventing the progression of or reducing pathological ossification in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject.

In an aspect, the disclosure relates to a method for increasing circulating pyrophosphate (PPi) in a subject with pathological calcification, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

In an aspect, the disclosure relates to a method for increasing pyrophosphatase activity in a subject with pathological calcification, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

In an aspect, the disclosure relates to a method for ameliorating one or more symptoms of pathological calcification in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby ameliorate one or more symptoms of pathological calcification in the subject.

In an aspect, the disclosure relates to a method for treating a subject with pathological calcification, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby treat the subject.

In an aspect, the disclosure relates to methods in which the administered dose is about 0.2 mg per kilogram of the subject.

In some embodiments, the disclosure relates to methods in which the administered dose is 0.2 (± 1-10 %) mg per kilogram of the subject. For example, the dose can be 0.22 mg/kg (0.2 +10% mg/kg), or 0.18 mg/kg (0.2-10% mg/kg), or 0.21 mg/kg (0.2 + 5% mg/kg), or 0.19 mg/kg (0.2-5% mg/kg) or 0.202 mg/kg (0.2 + 1% mg/kg) or 0.198 mg/kg (0.2 -1% mg/kg) of the subject.

In an aspect, the disclosure relates to methods in which the administered is about 0.6 mg per kilogram of the subject.

In some embodiments, the disclosure relates to methods in which the administered dose is 0.6 (± 1-5 %) mg per kilogram of the subject. For example, the dose can be 0.63 mg/kg (0.6 +5% mg/kg), or 0.57 mg/kg (0.6-5% mg/kg), or 0.624 mg/kg (0.6 + 4% mg/kg), or 0.576 mg/kg (0.6-4% mg/kg) or 0.618 mg/kg (0.6 + 3% mg/kg) or 0.582 mg/kg (0.6 -3% mg/kg) or 0.612 mg/kg (0.6+2% mg/kg) or 0.588 mg/kg (0.6-2% mg/kg) or 0.606 mg/kg (0.6 +1% mg/kg) or 0.594 mg/kg (0.6-1% mg/kg) of the subject.

In an aspect, the disclosure relates to methods in which the administered is about 1.8 mg per kilogram of the subject.

In some embodiments, the disclosure relates to methods in which the administered dose is 1.8 (± 1-5 %) mg per kilogram of the subject. For example, the dose can be 1.89 mg/kg (1.8 +5% mg/kg), or 1.71 mg/kg (1.8-5% mg/kg), or 1.872 mg/kg (1.8 + 4% mg/kg), or 1.728 mg/kg (1.8-4% mg/kg) or 1.854 mg/kg (1.8 + 3% mg/kg) or 1.746 mg/kg (1.8 -3% mg/kg) or 1.836 mg/kg (1.8+2% mg/kg) or 1.764 mg/kg (1.8-2% mg/kg) or 1.818 mg/kg (1.8 +1% mg/kg) or 1.782 mg/kg (1.8-1% mg/kg) of the subject.

In yet another aspect, the disclosure relates to a method for treating a subject with pathological calcification, the method comprising administering to the subject an ENPP1 agent in an amount effective to treat, or otherwise ameliorate one or more symptoms associated with, the subject's pathological calcification.

In some embodiments of any of the methods described herein, the subject can be, e.g., one who has discontinued (e.g., at least 14 days prior to treatment with the ENPP1 agent) treatment with (or is otherwise not receiving at the time of treatment with the ENPP1 agent) one or more (or all) of the following: a bisphosphonate, an anti-FGF23 antibody or FGF23 antagonist, a calcimimetic, an antacid, a corticosteroid, or a PTH suppressor.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is administered at least one time per week.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is administered at least two times per week.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is administered to the subject at least two times per week following an initial dose.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is administered subcutaneously.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is self-administered.

In an aspect, the disclosure relates to methods in which the ENPP1 agent is administered under a dosing regimen comprising: (a) an initial dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject and (b) about seven days after the initial dose, twice weekly administration of maintenance doses of the ENPP1 agent of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject.

In an aspect, the disclosure relates to methods in which the initial dose of the ENPP1 agent and the maintenance dose of the ENPP1 agent are the same amount.

In an aspect, the disclosure relates to methods in which the administered ENPP1 agent comprises the catalytic domain of ENPP1.

In an aspect, the disclosure relates to methods in which the administered ENPP1 agent comprises the nuclease domain of ENPP1.

In an aspect, the disclosure relates to methods in which the administered ENPP1 agent comprises the extracellular domain of ENPP1.

In an aspect, the disclosure relates to methods in which the administered ENPP1 agent comprises the catalytic and nuclease domains of ENPP1.

In an aspect, the disclosure relates to methods in which the administered ENPP1 agent comprises a heterologous moiety; the heterologous moiety may be a polypeptide.

In an aspect, the disclosure relates to methods in which the heterologous moiety increases the circulating half-life of the ENPP1 agent relative to the circulating half-life of the ENPP1 agent lacking the heterologous moiety.

In an aspect, the disclosure relates to methods in which the heterologous moiety comprises the Fc region of an immunoglobulin molecule; the immunoglobulin molecules may be a human immunoglobulin molecule; the immunoglobulin molecule may be an IgG1.

In an aspect, the disclosure relates to methods in which the heterologous moiety comprises albumin; the heterologous moiety comprises serum albumin; the heterologous moiety comprises human serum albumin.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising amino acid residues 99 (**P**SCAKE) to 925 (QE**D**)of SEQ ID NO:1.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising amino acid residues 1 (**F**TAGLKPSCAKE) to 833 (QE**D**) of SEQ ID NO:3.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising the amino acid sequence depicted in SEQ ID NO:2.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising the amino acid sequence depicted in SEQ ID NO:3.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising the amino acid sequence depicted in SEQ ID NO:4.

In an aspect, the disclosed ENPP1 agent and methods of its use relates to an ENPP1 agent comprising the amino acid sequence depicted in SEQ ID NO:5.

In an aspect, the disclosure relates to methods in which the subject has or is suspected of having diseases of pathological calcification other than generalized arterial calcification of infancy (GACI).

In an aspect, the disclosure relates to methods in which the subject has or is suspected of having diseases of pathological calcification other than autosomal recessive hypophosphatemic rickets type 2 (ARHR2).

In an aspect, the disclosure relates to methods in which the subject has or is suspected of having diseases of pathological calcification other than Pseudoxanthoma elasticum (PXE).

In an aspect, the disclosure relates to methods in which the subject has or is suspected of having one or more of kidney and bladder stones, dental pulp stones, gall stones, salivary gland stones, chronic calculous prostatitis, testicular microliths, calcification in hemodialysis patients, atherosclerosis, malacoplakia, scleroderma (systemic sclerosis), ARHR2, calcinosis cutis, calcific aortic stenosis, calcific tenditis, synovitis and arthritis, diffuse interstitial skeletal hyperostosis, juvenile dermatomyositis, Generalized Arterial Calcification of Infancy (GACI), Ossification of the Posterior Longitudinal Ligament (OPLL), hypophosphatemic rickets, osteoarthritis, calcification of atherosclerotic plaques, Chronic Kidney Disease (CKD), End Stage Renal Disease (ESRD), Pseudoxanthoma elasticum (PXE), ankylosing spondylitis, hardening of the arteries, calciphylaxis, and systemic lupus erythematosus.

In some embodiments of any of the methods described herein, the subject is characterized by one or more of the inclusion criteria described herein. For example, a subject described herein can have a plasma PPi concentration of below approximately 1300nM, e.g., prior to treatment with an ENPP1 agent. In some embodiments, the subject is not characterized by any of the exclusion criteria described herein. For example, the subject can be one who does not have advanced eye disease prior to, or during, treatment with an ENPP1 agent. In some embodiments, the subject does not have advanced eye disease requiring anti-VEGF treatment (for example) prior to, or during, treatment with an ENPP1 agent.

DESCRIPTION

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 presents a schematic overview of ENPP1 Deficiency.

Figure 2 shows the full, unprocessed amino acid sequence of wild-type ENPP1 precursor protein (SEQ ID NO: 1). The cytosolic and transmembrane regions are underlined. Potential N-glycosylation sites are in bold. PSCAKE (residues 99-104; boxed) is the start of

soluble ENPP1 protein portion which includes SMB1 (residues 104-144) and SMB2 (residues 145-189).

Figure 3 illustrates certain domains of human ENPP1.

Figure 4 shows the amino acid sequence of a soluble wild-type ENPP1 polypeptide (SEQ ID NO: 2).

Figure 5A and **Figure 5B** show a multiple sequence alignment of various vertebrate soluble ENPP1 polypeptides and human soluble ENPP1 polypeptide (SEQ ID NOs: 1 and 7-10). The various soluble ENPP1 polypeptides correspond to the following species and represent regions of the specific NCBI accession number: Mouse (NCBI accession NP_001295256.1; SEQ ID NO:7), Cow (NCBI accession NP_001193141; SEQ ID NO: 8), Rabbit (NCBI accession NP_001162404.1; SEQ ID NO:9), Human (NCBI accession NP_006199.2; SEQ ID NO: 1), and Baboon (NCBI accession NP_001076211.2; SEQ ID NO: 10).

Figure 6 presents a schematic overview of ABCC6 Deficiency.

DEFINITIONS

The terms used in this specification generally have their ordinary meanings in the art, within the context of this disclosure and in the specific context where each term is used. Certain terms are discussed below or elsewhere in the specification, to provide additional guidance to the practitioner in describing the compositions and methods of the disclosure and how to make and use them. The scope or meaning of any use of a term will be apparent from the specific context in which the term is used.

An "*ENPP1 Deficiency*" is characterized by a reduced level of ENPP1 enzymatic activity in serum or plasma of a subject. ENPP1 Deficiency is a rare, genetic disorder caused by inactivating mutations in the *ENPP1* gene that encodes the ENPP1 enzyme. ENPP1 is an integral transmembrane protein whose extracellular domains carry pyrophosphatase and phosphodiestrerase activities. ENPP1 converts extracellular ATP to inorganic pyrophosphate (PPi) and AMP.

ENPP1 Deficiency causes hypopyrophosphatemia and hypoadenosinemia which, in turn, leads to ectopic (especially arterial) calcification (described in literature as Generalized

Arterial Calcification of Infancy [GACI]), skeletal dysfunction secondary to rickets and osteomalacia (described in literature as Autosomal Recessive Hypophosphatemic Rickets 2 [ARHR2]) and occlusive neo-intimal proliferation. Beyond symptomatic and palliative interventions, no targeted therapy exists for this disease. Thus, ENPP1 Deficiency has a high unmet medical need. Infants with ENPP1 Deficiency have high mortality in the first 0 to 6 months of life and children and adults with ENPP1 Deficiency experience ongoing risk for organ calcification and dysfunction, debilitating rickets that progresses to osteomalacia in adulthood with severe bone and joint pain, fatigue, muscle weakness, and repeated bone fractures, all symptoms that lead to poor quality of life and function. Hypopyrophosphatemia causes a reactive increase in fibroblast growth factor 23 (FGF23) leading to hyperphosphaturia (the ENPP1 Deficiency "biochemical axis"), an essential feature in the pathophysiology of ENPP1 Deficiency.

ENPP1 Deficiency is characterized biochemically by low plasma PPi levels and clinically characterized by vascular calcification in infants (GACI Type phenotype) and rickets (ARHR2 phenotype) post-infancy and intimal proliferation. GACI (generalized arterial calcification of infants) is a severe disease occurring in infants and involving extensive arterial calcification (Albright, et al., 2015, Nature Comm. 10006).

Reduced ENPP1 enzymatic activity may occur via a reduction in the amount of ENPP1 enzyme present in serum or plasma of a subject relative to the amount of ENPP1 enzyme present in serum or plasma of a normal subject, and/or a reduction in the level of enzymatic activity of ENPP1 detected in serum or plasma of a subject relative to the level of enzymatic activity of ENPP1 detected in a normal subject, and/or via a defect in expression of ENPP1 gene at the transcriptional (RNA and/or mRNA) or translational levels, for example via a defect in the ENPP1 gene or a control element affecting ENPP1 gene expression, relative to normal expression the ENPP1 gene in a subject. ENPP1 enzymatic activity is defined below.

"*Enzymatically active*" with respect to an ENPP1 polypeptide, or, as used herein, "enzymatic activity" with respect to an ENPP1 polypeptide, is defined as possessing ATP hydrolytic activity into AMP and PPi and/or AP3a hydrolysis to ATP. NPP1 readily hydrolyze ATP into AMP and PPi. The steady-state Michaelis-Menten enzymatic constants of NPP1 are determined using ATP as a substrate. NPP1 can be demonstrated to cleave ATP by HPLC analysis of the enzymatic reaction, and the identity of the substrates and products of

the reaction are confirmed by using ATP, AMP, and ADP standards. The ATP substrate degrades over time in the presence of NPP1, with the accumulation of the enzymatic product AMP. Using varying concentrations of ATP substrate, the initial rate velocities for NPP1 are derived in the presence of ATP, and the data is fit to a curve to derive the enzymatic rate constants. At physiologic pH, the kinetic rate constants of NPP1 are Km=144 μM and kcat=7.8 s-1.

As used herein the term "*plasma pyrophosphate (PPi) levels*" refers to the amount of pyrophosphate present in plasma of animals. In certain embodiments, animals include rat, mouse, cat, dog, human, cow and horse. It is necessary to measure PPi in the plasma rather than serum because of release from platelets. There are several ways to measure PPi, one of which is by enzymatic assay using uridine-diphosphoglucose (UDPG) pyrophosphorylase (*Lust & Seegmiller, 1976, Clin. Chim. Acta 66:241-249; Cheung & Suhadolnik, 1977, Anal. Biochem. 83:61-63*) with modifications.

Typically, plasma PPi levels in healthy human subjects range from about 1μm to about 3 μM, in some cases between 1-2 μm. A normal level of ENPP1 in plasma refers to the amount of ENPP1 protein required to maintain a normal level of plasma pyrophosphate (PPi) in a healthy subject. A normal level of PPi in healthy humans corresponds to 2-3 μM. Subjects who have a deficiency of ENPP1 exhibit low PPi levels which range from at least 10% below normal levels, at least 20% below normal levels, at least 30% below normal levels, at least 40% below normal levels, at least 50% below normal levels, at least 60% below normal levels, at least 70% below normal levels, at least 80% below normal levels and combinations thereof. In patients afflicted with GACI, the PPi levels are found to be less than 1 μm and in some cases are below a detectable level. In patients afflicted with PXE, the PPi levels are below 0.5 μm. (*Arterioscler Thromb Vasc Biol. 2014 Sep;34(9):1985-9; Braddock et al., Nat Commun. 2015; 6: 10006.)*

An "*ABCC6 Deficiency*" may be indicated in a number of ways, for example, by a reduced expression levels of ABCC6 enzyme in a subject. Subjects having ABCC6 deficiency can be identified by the presence of one or more physiological symptoms such as ocular calcification, skin calcification yellow papules, angioid streaks, visual impairment, calcification of soft connective tissues including the cardiovascular system, and/or, the presence of ABCC6 mutations that affect the activity and expression of ABCC6 protein. Details on diagnosis and

classification of ABCC6 mutations are known in art. (*Expert Opin Orphan Drugs. 2014 Jun 1; 2(6): 567–577*).

ABCC6 deficiency may result in PXE. Reduced ABCC6 enzymatic activity may occur via a reduction in the amount of ABCC6 enzyme present in a subject relative to the amount of ABCC6 enzyme present in a normal subject, and/or a reduction in the level of enzymatic activity of ABCC6 in a subject relative to the level of enzymatic activity of ABCC6 detected in a normal subject, and/or via a defect in expression of ABCC6 gene at the transcriptional (RNA and/or mRNA) or translational levels, for example via a defect in the ABCC6 gene or a control element affecting ABCC6 gene expression, relative to normal expression the ABCC6 gene in a subject.

A subject with ABCC6 deficiency can be identified by screening for ABCC6 gene mutations. Several methods for detecting gene mutations are known in art. For instance, detection of mutation in ABCC6 genes can performed by following the protocols described in J Med Genet. 2007 Oct; 44(10): 621–628. (*Mutation detection in the ABCC6 gene and genotype–phenotype analysis in a large international case series affected by pseudoxanthoma elasticum, Ellen G Pfendner, et al*)

"*ABCC6 deficient patient*" or "*ABCC6 deficient subject*" as used herein, refers to a patient having at least one pathogenic mutation in the ABCC6 gene that affects activity and/or expression of ABC66 protein.

"P*athological calcification*": As used herein, the term refers to the abnormal deposition of calcium salts in soft tissues, secretory and excretory passages of the body causing it to harden. There are two types, dystrophic calcification which occurs in dying and dead tissue and metastatic calcification which elevated extracellular levels of calcium (hypercalcemia), exceeding the homeostatic capacity of cells and tissues. Calcification can involve cells as well as extracellular matrix components such as collagen in basement membranes and elastic fibers in arterial walls. Some examples of tissues prone to calcification include: Gastric mucosa – the inner epithelial lining of the stomach, Kidneys and lungs, Cornea, Systemic arteries and Pulmonary veins.

"*Pathological ossification*": As used herein, the term refers to a pathological condition in which bone arises in tissues not in the osseous system and in connective tissues usually not manifesting osteogenic properties. Ossification is classified into three types

depending on the nature of the tissue or organ being affected, endochondral ossification is ossification that occurs in and replaces cartilage. Intramembranous ossification is ossification of bone that occurs in and replaces connective tissue. Metaplastic ossification the development of bony substance in normally soft body structures; called also heterotrophic ossification.

A "*deficiency*" of NPP1 refers to a condition in which the subject has less than or equal to 5%-10% of normal levels of NPP1 in blood plasma. Normal levels of NPP1in healthy human subjects is approximately between 10 to 30 ng/ml. (Am J Pathol. 2001 Feb; 158(2): 543–554.)

A "*deficiency*" of ABCC6 refers to a condition in which the subject has less than or equal to 5%-10% of normal levels of ABCC6 expression. ABCC6 protein is expressed in liver and levels of ABCC6 protein can be measured by means of liver biopsy.

A "*low*" level of PPi refers to a condition in which the subject has less than or equal to 2%-5% of normal levels of plasma pyrophosphate (PPi). Normal levels of Plasma PPi in healthy human subjects is approximately 1.8 to 2.6 μM. (Arthritis and Rheumatism, Vol. 22, No. 8 (August 1979))

"*Ectopic calcification*" refers to a condition characterized by a pathologic deposition of calcium salts in tissues or bone growth in soft tissues.

"*Ectopic calcification of soft tissue*" refers to inappropriate biomineralization, typically composed of calcium phosphate, hydroxyapatite, calcium oxalates and ocatacalcium phosphates occurring in soft tissues leading to loss of hardening of soft tissues. "Arterial calcification" refers to ectopic calcification that occurs in arteries and heart valves leading to hardening and or narrowing of arteries. Calcification in arteries is correlated with atherosclerotic plaque burden and increased risk of myocardial infarction, increased ischemic episodes in peripheral vascular disease, and increased risk of dissection following angioplasty.

"*Venous calcification*" refers to ectopic calcification that occurs in veins that reduces the elasticity of the veins and restricts blood flow which can then lead to increase in blood pressure and coronary defects

"*Vascular calcification*" refers to the pathological deposition of mineral in the vascular system. It has a variety of forms, including intimal calcification and medial calcification, but can also be found in the valves of the heart. Vascular calcification is associated with atherosclerosis, diabetes, certain heredity conditions, and kidney disease, especially CKD. Patients with vascular calcification are at higher risk for adverse cardiovascular events. Vascular calcification affects a wide variety of patients. Idiopathic infantile arterial calcification is a rare form of vascular calcification where the arteries of neonates calcify.

"*Brain calcification*" (BC) refers to a nonspecific neuropathology wherein deposition of calcium and other mineral in blood vessel walls and tissue parenchyma occurs leading to neuronal death and gliosis. Brain calcification is" often associated with various chronic and acute brain disorders including Down's syndrome, Lewy body disease, Alzheimer's disease, Parkinson's disease, vascular dementia, brain tumors, and various endocrinologic conditions

Calcification of heart tissue refers to accumulation of deposits of calcium (possibly including other minerals) in tissues of the heart, such as aorta tissue and coronary tissue.

"Chronic kidney disease (CKD)" As used herein, the term refers to abnormalities of kidney structure or function that persist for more than three months with implications for health. Generally excretory, endocrine and metabolic functions decline together in most chronic kidney diseases. Cardiovascular disease is the most common cause of death in patients with chronic kidney disease (CKD) and vascular calcification is one of the strongest predictors of cardiovascular risk. With decreasing kidney function, the prevalence of vascular calcification increases and calcification occurs years earlier in CKD patients than in the general population. Preventing, reducing and/or reversing vascular calcification may result in increased survival in patients with CKD.

Clinical symptoms of chronic kidney diseases include itching, muscle cramps, nausea, lack of appetite, swelling of feet and ankles, sleeplessness and labored breathing. Chronic kidney disease if left untreated tends to progress into End stage renal disease (ESRD). Common symptoms of ESRD include an inability to urinate, fatigue, malaise, weight loss, bone pain, changes in skin color, a frequent formation of bruises, and edema of outer extremities like fingers, toes, hands and legs. Calciphylaxis or calcific uremic arteriolopathy (CUA) is a condition that causes calcium to build up inside the blood vessels

of the fat and skin. A subpopulation of patients suffering from ESRD can also develop Calciphylaxis. Common symptoms of Calciphylaxis include large purple net-like patterns on skin, deep and painful lumps that ulcerate creating open sores with black-brown crust that fails to heal, skin lesions on the lower limbs or areas with higher fat content, such as thighs, breasts, buttocks, and abdomen. A person with calciphylaxis may have higher than normal levels of calcium (hypercalcemia) and phosphate (hyperphosphatemia) in the blood. They may also have symptoms of hyperparathyroidism. Hyperparathyroidism occurs when the parathyroid glands make excess parathyroid hormone (PTH). Reduced plasma pyrophosphate (PPi) levels are also present in vascular calcification associated with end stage renal disease (ESRD).

Vascular calcifications associated with ESRD contributes to poor outcomes by increasing pulse pressure, causing or exacerbating hypertension, and inducing or intensifying myocardial infarctions and strokes. Most patients with ESRD do not die of renal failure, but from the cardiovascular complications of ESRD, and it is important to note that many very young patients with ESRD on dialysis possess coronary artery calcifications. The histologic subtype of vascular calcification associated with CKD is known as Mönckeburg's sclerosis, which is a form of vessel hardening in which calcium deposits are found in the muscular layers of the medial vascular wall. This form of calcification is histologically distinct from intimal or neo-intimal vascular wall calcification commonly observed in atherosclerosis but identical to the vascular calcifications observed in human CKD patients, and in the rodent models of the disease described herein.

"Generalized arterial calcification of infants (GACI)" (also known as IACI)", as used herein, refers to a disorder affecting the circulatory system that becomes apparent before birth or within the first few months of life. It is characterized by abnormal accumulation of the mineral calcium (calcification) in the walls of the blood vessels that carry blood from the heart to the rest of the body (the arteries). Calcification often occurs along with thickening of the lining of the arterial walls (the intima). These changes lead to narrowing (stenosis) and stiffness of the arteries, which forces the heart to work harder to pump blood. As a result, heart failure may develop in affected individuals, with signs and symptoms including difficulty breathing, accumulation of fluid (edema) in the extremities, a bluish appearance of the skin or lips (cyanosis), severe high blood pressure (hypertension), and an enlarged heart (cardiomegaly). People with GACI may also have calcification in other organs and tissues,

particularly around the joints. In addition, they may have hearing loss or softening and weakening of the bones referred to as rickets.

General arterial calcification (GACI) or Idiopathic Infantile Arterial Calcification (IIAC) characterized by abnormal accumulation of the mineral calcium (calcification) in the walls of the blood vessels that carry blood from the heart to the rest of the body (the arteries). The calcification often occurs along with thickening of the lining of the arterial walls (the intima). These changes lead to narrowing (stenosis) and stiffness of the arteries, which forces the heart to work harder to pump blood. As a result, heart failure may develop in affected individuals, with signs and symptoms including difficulty breathing, accumulation of fluid (edema) in the extremities, a bluish appearance of the skin or lips (cyanosis), severe high blood pressure (hypertension), and an enlarged heart (cardiomegaly).

The term "*Infant*" as used herein refers to a human child in his or her first year of life. Typically, infantile stage refers to the age during which the child is unable to walk.

"Arterial calcification" or "Vascular calcification" or "hardening of arteries", As used herein, the term refers to a process characterized by thickening and loss of elasticity of muscular arteries walls. The thickening and loss of elasticity occurs in two distinct sites, the intimal and medial layers of the vasculatures (Medial vascular calcification). Intimal calcification is associated with atherosclerotic plaques and medial calcification is characterized by vascular stiffening and arteriosclerosis. This results in a reduction of arterial elasticity and an increased propensity for morbidity and mortality due to the impairment of the cardiovascular system's hemodynamics.

"Mineral bone disorders (MBD)", as used herein, the term refers to a disorder characterized by abnormal hormone levels cause calcium and phosphorus levels in a person's blood to be out of balance. Mineral and bone disorder commonly occurs in people with CKD and affects most people with kidney failure receiving dialysis.

Osteopenia is a bone condition characterized by decreased bone density, which leads to bone weakening and an increased risk of bone fracture. Osteomalacia is a bone disorder characterized by decreased mineralization of newly formed bone. Osteomalacia is caused by severe vitamin D deficiency (which can be nutritional or caused by a hereditary syndrome) and by conditions that cause very low blood phosphate levels. Both osteomalacia and

osteopenia increase the risk of breaking a bone. Symptoms of osteomalacia include bone pain and muscle weakness, bone tenderness, difficulty walking, and muscle spasms.

"Age related osteopenia", as used herein refers to a condition in which bone mineral density is lower than normal. Generally, patients with osteopenia have a bone mineral density T-score of between -1.0 and -2.5. Osteopenia if left untreated progresses into Osetoporosis where bones become brittle and are extremely prone to fracture.

"Ossification of posterior longitudinal ligament (OPLL)", as used herein, the term refers to a hyperostotic (excessive bone growth) condition that results in ectopic calcification of the posterior longitudinal ligament. The posterior longitudinal ligament connects and stabilizes the bones of the spinal column. The thickened or calcified ligament may compress the spinal cord, producing myelopathy. Symptoms of myelopathy include difficulty walking and difficulty with bowel and bladder control. OPLL may also cause radiculopathy, or compression of a nerve root. Symptoms of cervical radiculopathy include pain, tingling, or numbness in the neck, shoulder, arm, or hand.

Clinical symptoms and signs caused by OPLL are categorized as: (1) myelopathy, or a spinal cord lesion with motor and sensory disturbance of the upper and lower limbs, spasticity, and bladder dysfunction; (2) cervical radiculopathy, with pain and sensory disturbance of the upper limbs; and (3) axial discomfort, with pain and stiffness around the neck. The most common symptoms in the early stages of OPLL include dysesthesia and tingling sensation in hands, and clumsiness. With the progression of neurologic deficits, lower extremity symptoms, such as gait disturbance may appear. OPLL is detected on lateral plain radiographs, and the diagnosis and morphological details of cervical OPLL have been clearly demonstrated by magnetic resonance imaging (MRI) and computed tomography (CT).

"Pseudoxanthoma elasticum (PXE)", as used herein, the term refers a progressive disorder that is characterized by the accumulation of deposits of calcium and other minerals (mineralization) in elastic fibers. Elastic fibers are a component of connective tissue, which provides strength and flexibility to structures throughout the body. In PXE, mineralization can affect elastic fibers in the skin, eyes, and blood vessels, and less frequently in other areas such as the digestive tract. People with PXE may have yellowish bumps called papules on their necks, underarms, and other areas of skin that touch when a joint bends. Mineralization of the blood vessels that carry blood from the heart to the rest of the body (arteries) may

cause other signs and symptoms of PXE. For example, people with this condition can develop narrowing of the arteries (arteriosclerosis) or a condition called claudication that is characterized by cramping and pain during exercise due to decreased blood flow to the arms and legs.

Pseudoxanthoma elasticum (PXE), also known as Grönblad–Strandberg syndrome, is a genetic disease that causes fragmentation and mineralization of elastic fibers in some tissues. The most common problems arise in the skin and eyes, and later in blood vessels in the form of premature atherosclerosis. PXE is caused by autosomal recessive mutations in the ABCC6 gene on the short arm of chromosome 16 (16p13.1). In some cases, a portion of infants survive GACI and end up developing Pseudoxanthoma elasticum (PXE) when they grow into adults. PXE is characterized by the accumulation of calcium and other minerals (mineralization) in elastic fibers, which are a component of connective tissue. Connective tissue provides strength and flexibility to structures throughout the body. Features characteristic of PXE that also occur in GACI include yellowish bumps called papules on the underarms and other areas of skin that touch when a joint bends (flexor areas); arterial stenosis, and abnormalities called angioid streaks affecting tissue at the back of the eye (retinal hemorrhage), which is detected during an eye examination.

"End stage renal disease (ESRD), as used herein, the term refers to an advanced stage of chronic kidney disease where kidneys of the patient are no longer functional. Common symptoms include fatigue associated with anemia (low blood iron), decreased appetite, nausea, vomiting, abnormal lab values including elevated potassium, abnormalities in hormones related to bone health, elevated phosphorus and/or decreased calcium, high blood pressure (hypertension), swelling in hands/legs/eyes/lower back (sacrum) and shortness of breath.

"Calcific uremic arteriolopathy (CUA)" or "Calciphylaxis", as used herein refers to a condition with high morbidity and mortality seen in patients with kidney disease, especially in those with end stage renal disease (ESRD). It is characterized by calcification of the small blood vessels located within the fatty tissue and deeper layers of the skin leading to blood clots, and the death of skin cells due to reduced blood flow caused by excessive calcification.

"Hypophosphatemic rickets", as used herein refers to a disorder in which the bones become soft and bend easily, due to low levels of phosphate in the blood. Symptoms usually

begin in early childhood and can range in severity from bowing of the legs, bone deformities; bone pain; joint pain; poor bone growth; and short stature.

"*Hereditary Hypophosphatemic Rickets*" as used herein refers to a disorder related to low levels of phosphate in the blood (hypophosphatemia). Phosphate is a mineral that is essential for the normal formation of bones and teeth. Most commonly, it is caused by a mutation in the PHEX gene. Other genes that can be responsible for the condition include the CLCN5, DMP1, ENPP1, FGF23, and SLC34A3 genes. Other signs and symptoms of *hereditary hypophosphatemic rickets* can include premature fusion of the skull bones (craniosynostosis) and dental abnormalities. The disorder may also cause abnormal bone growth where ligaments and tendons attach to joints (enthesopathy). In adults, hypophosphatemia is characterized by a softening of the bones known as osteomalacia. Another rare type of the disorder is known as *hereditary hypophosphatemic rickets with hypercalciuria (HHRH)* wherein in addition to hypophosphatemia, this condition is characterized by the excretion of high levels of calcium in the urine (hypercalciuria).

"X-linked hypophosphatemia (XLH)", as used herein, the term X-linked hypophosphatemia (XLH), also called X-linked dominant hypophosphatemic rickets, or X-linked Vitamin D-resistant rickets, is an X-linked dominant form of rickets (or osteomalacia) that differs from most cases of rickets in that vitamin D supplementation does not cure it. It can cause bone deformity including short stature and genu varum (bow leggedness). It is associated with a mutation in the PHEX gene sequence (Xp.22) and subsequent inactivity of the PHEX protein.

"Autosomal Recessive Hypophosphatemia Rickets type 2 (ARHR2)", as used herein, the term refers to a hereditary renal phosphate-wasting disorder characterized by hypophosphatemia, rickets and/or osteomalacia and slow growth. Autosomal recessive hypophosphatemic rickets type 2 (ARHR2) is caused by homozygous loss-of-function mutation in the ENPP1 gene.

"*Autosomal Dominant Hypophosphatemic Rickets (ADHR)"*, as used herein refers to a rare hereditary disease in which excessive loss of phosphate in the urine leads to poorly formed bones (rickets), bone pain, and tooth abscesses. ADHR is caused by a mutation in the fibroblast growth factor 23 (FGF23). ADHR is characterized by impaired mineralization of bone, rickets and/or osteomalacia, suppressed levels of calcitriol (1, 25-dihydroxyvitamin

D3), renal phosphate wasting, and low serum phosphate. Mutations in FGF23 render the protein more stable and uncleavable by proteases resulting in enhanced bioactivity of FGF23. The enhanced activity of FGF23 mutants reduce expression of sodium-phosphate co-transporters, NPT2a and NPT2c, on the apical surface of proximal renal tubule cells, resulting in renal phosphate wasting.

Hypophosphatemic rickets (previously called vitamin D-resistant rickets) is a disorder in which the bones become painfully soft and bend easily, due to low levels of phosphate in the blood. Symptoms may include bowing of the legs and other bone deformities; bone pain; joint pain; poor bone growth; and short stature. In some affected babies, the space between the skull bones closes too soon leading to craniosynostosis. Most patients display Abnormality of calcium-phosphate metabolism, Abnormality of dental enamel, Delayed eruption of teeth and long, narrow head (Dolichocephaly).

"*Pre-treatment*", as used herein, means treatment prior to commencement of a treatment method described herein.

"*PCSK9 inhibitor*" as used herein, the term refers to inhibitor that blocks the PCSK9 enzyme. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is an enzyme that binds to low-density lipoprotein receptors (LDL receptors), which stops LDL being removed from the blood, leading to an increase in blood levels of LDL. The PCSK9 inhibitor blocks the PCSK9 enzyme, resulting in more LDL receptors available to remove LDL from the blood, which produces in a decrease in LDL blood levels. Commonly known PCSK9 inhibitors include but not limited to Repatha (evolocumab), Praulent (alirocumab). See "PCSK9 inhibitors: A new era of lipid lowering therapy", Chaudhary et al., World J Cardiol. 2017 Feb 26; 9(2): 76–91.

"*Statins*", as used herein, the term refers to a class of lipid-lowering medications that reduce illness and mortality in those who are at high risk of cardiovascular disease. They lower the level of cholesterol in the blood by reducing the production of cholesterol by the liver Example of statins commonly known include but not limited to Atorvastatin (Lipitor), Lovastatin (Altoprev), Pitavastatin (Livalo, Zypitamag), Pravastatin (Pravachol), Rosuvastatin (Crestor, Ezallor), and Simvastatin (Zocor).

"*Malignancy*", as used herein, refers to the presence of cancerous cells that have the ability to spread to other sites in the body (metastasize) or to invade nearby (locally) and destroy tissues. Malignant cells tend to have fast, uncontrolled growth and do not die

normally due to changes in their genetic makeup. There are several main types of malignancy. Carcinoma is a malignancy that begins in the skin or in tissues that line or cover internal organs. Sarcoma is a malignancy that begins in bone, cartilage, fat, muscle, blood vessels, or other connective or supportive tissue. Leukemia is a malignancy that begins in blood-forming tissue, such as the bone marrow, and causes too many abnormal blood cells to be made. Lymphoma and multiple myeloma are malignancies that begin in the cells of the immune system. Central nervous system cancers are malignancies that begin in the tissues of the brain and spinal cord.

"*Nonmelanoma Skin Cancer*", as used herein, refer to any cancer that forms in the basal, squamous or Merkel cells of the skin. Melanoma is a cancer that develops in the skin's melanocytes.

"*Sponsor of study*" as used herein, refers to an individual, institution, company or organization (for example, a contract research organization) that takes the responsibility to initiate, manage or finance the clinical trial but does not actually conduct the investigation.

The term "*subject*", as used herein, refers to an individual, such as a mammal, such as a human, a non-human primate (e.g. chimpanzees and other apes and monkey species), a farm animal (e.g. birds, fish, cattle, sheep, pigs, goats, and horses), a domestic mammal (e.g. dogs and cats), or a laboratory animal (e.g. rodents, such as mice, rats and guinea pigs). The term includes a subject of any age or sex. In another embodiment the subject is a mammal, preferably a human.

A disease or disorder is "*alleviated*" if the severity of a symptom of the disease or disorder, the frequency with which such a symptom is experienced by a patient, or both, is reduced.

As used herein the terms "*alteration*," "*defect*," "*variation*" or "*mutation*" refer to a mutation in a gene in a cell that affects the function, activity, expression (transcription or translation) or conformation of the polypeptide it encodes, including missense and nonsense mutations, insertions, deletions, frameshifts and premature terminations.

A "*disease*" is a state of health of an animal wherein the animal cannot maintain homeostasis, and wherein if the disease is not ameliorated then the animal's health continues to deteriorate.

A "*disorder*" in an animal is a state of health in which the animal is able to maintain homeostasis, but in which the animal's state of health is less favorable than it would be in the absence of the disorder. Left untreated, a disorder does not necessarily cause a further decrease in the animal's state of health.

As used herein, the term "*immune response" or "immune reaction*" refers to the host's immune system to antigen in an invading (infecting) pathogenic organism, or to introduction or expression of foreign protein. The immune response is generally humoral and local; antibodies produced by B cells combine with antigen in an antigen-antibody complex to inactivate or neutralize antigen. Immune response is often observed when human proteins are injected into mouse model systems. Generally, the mouse model system is made immune tolerant by injecting immune suppressors prior to the introduction of a foreign antigen to ensure better viability.

As used herein, the term "*immune suppression*" is a deliberate reduction of the activation or efficacy of the host immune system using immune suppresant drugs to facilitate immune tolerance towards foreign antigens such as foreign proteins, organ transplants, bone marrow and tissue transplantation. Non-limiting examples of immunosuppressant drugs include anti-CD4(GK1.5) antibody, Cyclophosphamide, Azathioprine (Imuran), Mycophenolate mofetil (Cellcept), Cyclosporine (Neoral, Sandimmune, Gengraf), Methotrexate (Rheumatrex), Leflunomide (Arava), Cyclophosphamide (Cytoxan) and Chlorambucil (Leukeran).

As used herein, the term "*ENPP*" or "*NPP*" refers to ectonucleotide pyrophosphatase/ phosphodiesterase.

As used herein, the term "*ENPP1 protein*" or "*ENPP1 polypeptide*" refers to ectonucleotide pyrophosphatase/phosphodiesterase-1 protein encoded by the *ENPP1* gene. The encoded protein is a type II transmembrane glycoprotein and cleaves a variety of substrates, including phosphodiester bonds of nucleotides and nucleotide sugars and pyrophosphate bonds of nucleotides and nucleotide sugars. ENPP1 protein has a transmembrane domain and soluble extracellular domain. The extracellular domain is further subdivided into somatomedin B domain, catalytic domain (residues 186 to 586 of SEQ ID NO: 1) and the nuclease domain (residues 524 to 885 of SEQ ID NO: 1). The sequence and

structure of wild-type ENPP1 is described in detail in PCT Application Publication No. *WO 2014/126965* to Braddock, *et al.*, which is incorporated herein in its entirety by reference.

Mammal ENPP1 polypeptides, mutants, or mutant fragments thereof, have been previously disclosed in International PCT Application Publications No. *WO/2014/126965-Braddock et al., WO/2016/187408-Braddock et al., WO/2017/087936-Braddock et al., and WO2018/027024-Braddock et al.*, all of which are incorporated by reference in their entireties herein.

As used herein, the term "*ENPP1 precursor protein*" refers to ENPP1 with its signal peptide sequence at the ENPP1 N-terminus. Upon proteolysis, the signal sequence is cleaved from ENPP1 to provide the ENPP1 protein. Signal peptide sequences useful within the invention include, but are not limited to, Albumin signal sequence, Azurocidin signal sequence, ENPP1 signal peptide sequence, ENPP2 signal peptide sequence, ENPP7 signal peptide sequence, and/or ENPP5 signal peptide sequence.

As used herein, the term "*ENPP1-Fc construct*" refers to ENPP1 recombinantly fused and/or chemically conjugated (including both covalent and non-covalent conjugations) to an FcR binding domain of an IgG molecule (preferably, a human IgG). In certain embodiments, the C-terminus of ENPP1 is fused or conjugated to the N-terminus of the FcR binding domain.

As used herein, the term "*Fc*" refers to a human IgG (immunoglobulin) Fc domain. Subtypes of IgG such as IgG1, IgG2, IgG3, and IgG4 are contemplated for use as Fc domains.

As used herein, the "*Fc region or Fc polypeptide*" is the portion of an IgG molecule that correlates to a crystallizable fragment obtained by papain digestion of an IgG molecule. The Fc region comprises the C-terminal half of the two heavy chains of an IgG molecule that are linked by disulfide bonds. It has no antigen binding activity but contains the carbohydrate moiety and the binding sites for complement and Fc receptors, including the FcRn receptor. The Fc fragment contains the entire second constant domain CH2 (residues 231-340 of human IgG1, according to the Kabat numbering system) and the third constant domain CH3 (residues 341-447). The term "IgG hinge-Fc region" or "hinge-Fc fragment" refers to a region of an IgG molecule consisting of the Fc region (residues 231 -447) and a hinge region (residues 216-230) extending from the N-terminus of the Fc region. The term "constant

domain" refers to the portion of an immunoglobulin molecule having a more conserved amino acid sequence relative to the other portion of the immunoglobulin, the variable domain, which contains the antigen binding site. The constant domain contains the CH1, CH2 and CH3 domains of the heavy chain and the CHL domain of the light chain.

As used herein, the term "*fragment*," as applied to a nucleic acid, refers to a subsequence of a larger nucleic acid. A "fragment" of a nucleic acid can be at least about 15, 50-100, 100-500, 500-1000, 1000-1500 nucleotides, 1500-2500, or 2500 nucleotides (and any integer value in between). As used herein, the term "fragment," as applied to a protein or peptide, refers to a subsequence of a larger protein or peptide, and can be at least about 20, 50, 100, 200, 300 or 400 amino acids in length (and any integer value in between).

"*Isolated*" means altered or removed from the natural state. For example, a nucleic acid or a polypeptide naturally present in a living animal is not "isolated," but the same nucleic acid or polypeptide partially or completely separated from the coexisting materials of its natural state is "isolated." An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell.

As used herein, the term "*patient*," "*individu*al" or "*subject*" refers to a human.

As used herein, the term "*pharmaceutical composition*" or "*composition*" refers to a mixture of at least one compound useful within the invention with a pharmaceutically acceptable carrier. The pharmaceutical composition facilitates administration of the compound to a patient. Multiple techniques of administering a compound exist in the art including, but not limited to, subcutaneous, intravenous, oral, aerosol, inhalational, rectal, vaginal, transdermal, intranasal, buccal, sublingual, parenteral, intrathecal, intragastrical, ophthalmic, pulmonary, and topical administration.

As used herein, the term "*pharmaceutically acceptable*" refers to a material, such as a carrier or diluent, which does not abrogate the biological activity or properties of the compound, and is relatively non-toxic, *i.e.*, the material may be administered to an individual without causing undesirable biological effects or interacting in a deleterious manner with any of the components of the composition in which it is contained; for example, phosphate-buffered saline (PBS).

As used herein the term "*plasma pyrophosphate (PPi) levels*" refers to the amount of pyrophosphate present in plasma of animals. In certain embodiments, animals include rat, mouse, cat, dog, human, cow and horse. It is necessary to measure PPi in plasma rather than serum because of release from platelets. There are several ways to measure PPi, one of which is by enzymatic assay using uridine-diphosphoglucose (UDPG) pyrophosphorylase (*Lust & Seegmiller, 1976, Clin. Chim. Acta 66:241-249; Cheung & Suhadolnik, 1977, Anal. Biochem. 83:61-63*) with modifications. Typically, normal PPi levels in healthy subjects range from about 1μm to about 3 μM, in some cases between 1-2 μm. Subjects who have defective ENPP1 expression tend to exhibit low PPi levels which range from at least 10% below normal levels, at least 20% below normal levels, at least 30% below normal levels, at least 40% below normal levels, at least 50% below normal levels, at least 60% below normal levels, at least 70% below normal levels, at least 80% below normal levels and combinations thereof. In patients afflicted with GACI, the PPi levels are found to be less than 1 μm and in some cases are below the level of detection. In patients afflicted with PXE, the PPi levels are below 0.5 μm. (*Arterioscler Thromb Vasc Biol. 2014 Sep;34(9):1985-9; Braddock et al., Nat Commun. 2015; 6: 10006.*)

As used herein, the term "*polypeptide*" refers to a polymer composed of amino acid residues, related naturally occurring structural variants, and synthetic non-naturally occurring analogs thereof linked via peptide bonds.

As used herein, the term "*PPi*" refers to pyrophosphate.

As used herein, the term "*prevent" or "prevention*" means no disorder or disease development if none had occurred, or no further disorder or disease development if there had already been development of the disorder or disease. Also considered is the ability of one to prevent some or all of the symptoms associated with the disorder or disease.

"*Sample*" or "*biological sample*" as used herein means a biological material isolated from a subject. The biological sample may contain any biological material suitable for detecting a mRNA, polypeptide or other marker of a physiologic or pathologic process in a subject, and may comprise fluid, tissue, cellular and/or non-cellular material obtained from the individual.

As used herein, "*substantially purified*" refers to being essentially free of other components. For example, a substantially purified polypeptide is a polypeptide that has been

separated from other components with which it is normally associated in its naturally occurring state. Non-limiting embodiments include 95% purity, 99% purity, 99.5% purity, 99.9% purity and 100% purity.

As used herein, the term "*treatment*" or "*treating*" is defined as the application or administration of a therapeutic agent, *i.e.*, a compound useful within the invention (alone or in combination with another pharmaceutical agent), to a patient, or application or administration of a therapeutic agent to an isolated tissue or cell line from a patient (*e.g.*, for diagnosis or *ex vivo* applications), who has a disease or disorder, a symptom of a disease or disorder or the potential to develop a disease or disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve or affect the disease or disorder, the symptoms of the disease or disorder, or the potential to develop the disease or disorder. Such treatments may be specifically tailored or modified, based on knowledge obtained from the field of pharmacogenomics.

The terms "*prevent," "preventing," and "prevention*", as used herein, refer to inhibiting the inception or decreasing the occurrence of a disease in a subject. Prevention may be complete (e.g. the total absence of pathological cells in a subject) or partial. Prevention also refers to a reduced susceptibility to a clinical condition.

As used herein, the term "*wild-type*" refers to a gene or gene product isolated from a naturally occurring source. A wild-type gene is most frequently observed in a population and is thus arbitrarily designed the "normal" or "wild-type" form of the human NPP1 genes. In contrast, the term "*functionally equivalent*" refers to a NPP1 gene or gene product that displays modifications in sequence and/or functional properties (*i.e.*, altered characteristics) when compared to the wild-type gene or gene product. Naturally occurring mutants can be isolated; these are identified by the fact that they have altered characteristics (including altered nucleic acid sequences) when compared to the wild-type gene or gene product.

The term "*functional equivalent variant*", as used herein, relates to a polypeptide substantially homologous to the sequences of ENPP1 (defined above) and that preserves the enzymatic and biological activities of ENPP1. Methods for determining whether a variant preserves the biological activity of the native ENPP1 are widely known to the skilled person and include any of the assays used in the experimental part of said application. Particularly,

functionally equivalent variants of ENPP1 delivered by viral vectors is encompassed by the present invention.

The functionally equivalent variants of ENPP1 are polypeptides substantially homologous to the native ENPP1. The expression "substantially homologous", relates to a protein sequence when said protein sequence has a degree of identity with respect to the ENPP1 sequences described above of at least 80%, at least 85%, at least 90%, at least 91%, at least 92%, at least 93%, at least 94%, at least 95%, at least 96%, at least 97%, at least 98% or at least 99% respectively.

The degree of identity between two polypeptides is determined using computer algorithms and methods that are widely known for the persons skilled in the art. The identity between two amino acid sequences is preferably determined by using the BLASTP algorithm (*BLAST Manual, Altschul, S., et al., NCBI NLM NIH Bethesda, Md. 20894, Altschul, S., et al., J. Mol. Biol. 215: 403-410 (1990)*), though other similar algorithms can also be used. BLAST and BLAST 2.0 are used, with the parameters described herein, to determine percent sequence identity. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information.

"*Functionally equivalent variants*" of ENPP1 may be obtained by replacing nucleotides within the polynucleotide accounting for codon preference in the host cell that is to be used to produce the ENPP1 respectively. Such "codon optimization" can be determined via computer algorithms which incorporate codon frequency tables such as "Human high.cod" for codon preference as provided by the University of Wisconsin Package Version 9.0, Genetics Computer Group, Madison, Wis.

"*About*" as used herein when referring to a measurable value such as an amount, a temporal duration, and the like, is meant to encompass variations of ±10% or ±5%, in certain embodiments ±1-5%, in certain embodiments ±5%, in certain embodiments ±4% , in certain embodiments ±4%, in certain embodiments ±3%, in certain embodiments ±2%, and in certain embodiments ±1% from the specified value (0.2 mg/kg or 0.6 mg/kg or 1.8 mg/kg), as such variations are appropriate to perform the disclosed methods.

The disclosure provides a representative example of protein sequences. The protein sequences described can be converted into nucleic acid sequences by performing revere translation and codon optimization. There are several tools available in art such as Expasy

(https://www.expasy.org/) and bioinformatics servers (http://www.bioinformatics.org)that enable such conversions

Ranges: throughout this disclosure, various aspects according to the invention can be presented in a range format. It should be understood that the description in range format is merely for convenience and brevity and should not be construed as an inflexible limitation on the scope according to the invention. Accordingly, the description of a range should be considered to have specifically disclosed all the possible subranges as well as individual numerical values within that range. For example, description of a range such as from 1 to 6 should be considered to have specifically disclosed subranges such as from 1 to 3, from 1 to 4, from 1 to 5, from 2 to 4, from 2 to 6, from 3 to 6 etc., as well as individual numbers within that range, for example, 1, 2, 2.7, 3, 4, 5, 5.3, and 6. This applies regardless of the breadth of the range.

Preferred methods and materials are described herein, although methods and materials similar or equivalent to those described herein can also be used in the practice or testing of the presently disclosed methods and compositions. All publications, patent applications, patents, and other references mentioned herein are incorporated by reference in their entirety.

ABCC6 Mutation detection

Genomic DNA is isolated from peripheral blood samples of subjects suspected of having ABC66 deficiency using commercially available DNA isolation kits. (*Puregene DNA Isolation Kit; Gentra Systems, Minneapolis, Minnesota, USA*). Control genomic DNA is obtained from the human lymphoblastoid cell line K562 (A*merican Type Culture Collection, Manassas, Virginia, USA*). All DNA samples are adjusted with water to a concentration of 10 ng/μL.

The mutation-detection strategy is based on: (1) identification of the recurrent mutations R1141X and del23–29 by restriction-enzyme digestion; (2) optimised denaturing high-performance liquid chromatography (dHPLC) scanning of PCR products corresponding to all exons in subjects in whom the two recurrent mutations are not identified on both alleles, followed by (3) sequencing of exons with altered dHPLC patterns; and (4) confirmation of novel mutations by restriction-enzyme digestion or resequencing.

Screening for the recurrent mutations R1141X and del23–29 is performed as previously described. (*Compound heterozygosity for a recurrent 16.5-kb Alu-mediated deletion mutation and single-base-pair substitutions in the ABCC6 gene results in pseudoxanthoma elasticum. Ringpfeil F, Nakano A, Uitto J, Pulkkinen L, Am J Hum Genet. 2001 Mar; 68(3):642-52.*) Conditions and primers for generating PCR products spanning all exons of the coding regions and flanking intronic sequences of the ABCC6 gene are identified for optimum dHPLC screening in supplementary table (*J Med Genet. 2007 Oct; 44(10): 621–628.*).

These primers are designed to exclude the pseudogenes homologous to exons 1–4 and 1–931 and to anneal within ~50 bases of the 5′ and 3′ ends of the exon and to exclude known intronic polymorphisms where possible. PCR for dHPLC analysis is performed using 1.5 U Taq polymerase (Qiagen Inc., Valencia, California, USA) mixed with 5 U Optimase Taq polymerase (Transgenomic, Gaithersburg, Maryland, USA) and Q buffer (Qiagen), according to the manufacturer' instructions. PCR reactions contained 200 ng DNA as template and 20 ng of each primer in a final volume of 50 µl. Cycling conditions for all primer pairs were 94°C for 5 min, followed by 41 cycles of 94°C for 1 min, annealing temperature for a particular primer pair (range 55–60°C) for 1 min and 72°C for 1 min, with a final step at 72°C for 5 min.

The PCR products generated using patients with PXE DNA as template are allowed to form heteroduplexes with an equal volume of a PCR product of the same exon amplified from template DNA of the lymphoblastoid control cell line K562. For this purpose, the PCR products were mixed in a 1:1 ratio and denatured at 94°C for 10 min, followed by reannealing at 65°C for 15 min and 37°C for 15 min. The PCR products are then screened by dHPLC (*WAVE; Transgenomic, Gaithersburg, Maryland, USA*) using methods designed to enhance partial denaturation of the PCR products containing mismatched bases (see supplementary table 1of J Med Genet. 2007 Oct; 44(10): 621–628.). PCR products showing pattern shifts are sequenced in both directions in most cases. DNA sequencing is performed on an automated sequencer (ABI Prism 377 or ABI 3100; Perkin-Elmer-Cetus, Foster City, California, USA). Putative mutations are confirmed by restriction-enzyme digestion followed by agarose-gel electrophoresis or by resequencing of a new PCR product when a suitable restriction enzyme was not available. The subjects are then identified as subjects with ABCC6 mutations if they

were found to contain known mutations in ABCC6 genes that affect the activity and or expression level of ABCC6 protein.

DETAILED DESCRIPTION

1. Overview

The disclosure provides for treating, preventing, or reducing the progression rate and/or severity of pathologic calcification and/or ossification or one or more complications of pathologic calcification and/or ossification by administering to a subject ENPP1-FC subcutaneously (SC) at a dose of about 0.2 mg/kg, about 0.6 mg/kg, or about 1.8 mg/kg. With respect to a recited dose of ENPP1-FC, "about" mean a degree of error given the nature or precision of the measurements within ±5 percent (%), ±4%, ±3%, or ±2% or ±1%of the recited dose in mg ENPP1-FC/kg body weight of the subject.

2. Administered Dose of ENPP1 Agent

An ENPP1 agent is administered at a dose of about 0.2 mg/kg, about 0.6 mg/kg, or about 1.8 mg/kg. A medical practitioner will select which of these doses is administered to a given subject, and may be guided by a subject's serum PPi levels, the selected dose being sufficient to restore PPi to normal levels in the subject.

3. ENPP1 Agent

An ENPP1 agent is an ENPP1 polypeptide. ENPP1 polypeptides disclosed herein include naturally occurring polypeptides of the ENPP1 family as well as any variants thereof (including mutants, fragments, fusions, and peptidomimetic forms) that retain a biological activity. The terms "ENPP1" or "ENPP1 polypeptide" refers to ectonucleotide pyrophosphatase/phosphodiesterase 1 proteins (NPP1/ENPP1/PC-1) and ENPP1-related proteins, derived from any species. ENPP1 protein comprises a type II transmembrane glycoprotein that forms a homodimer. Each monomer of the ENPP1 protein comprises a short intracellular N-terminal domain involved in targeting to the plasma membrane, a transmembrane domain, and a large extracellular region comprising several domains. The large extracellular region comprises SMB1 and SMB2 domains, which have been reported to take part in ENPP1 dimerization (R. Gijsbers, H. et al., Biochem. J. 371; 2003: 321–330). Specifically, the SMB domains contain eight cysteine residues, each arranged in four

disulphide bonds, and have been shown to mediate ENPP1 homodimerization through covalent cystine inter- and intramolecular bonds. The protein cleaves a variety of substrates, including phosphodiester bonds of nucleotides and nucleotide sugars and pyrophosphate bonds of nucleotides and nucleotide sugars. ENPP1 protein functions to hydrolyze nucleoside 5' triphosphatase to either corresponding monophosphates and also hydrolyzes diadenosine polyphosphates. ENPP1 proteins play a role in purinergic signaling which is involved in the regulation of cardiovascular, neurological, immunological, musculoskeletal, hormonal, and hematological functions. An exemplary amino acid sequence of the human ENPP1 precursor protein (NCBI accession NP_006199) is shown in Figure 2 (SEQ ID NO: 1). The human ENPP1 precursor protein includes an endogenous ENPP1 signal peptide sequence at the ENPP1 N-terminus. Numbering of amino acids for all ENPP1-related polypeptides described herein is based on the numbering of the human ENPP1 precursor protein sequence provided in Figure 2 unless specifically designated otherwise. In certain embodiments, the ENPP1 precursor protein further comprises an endogenous or heterologous signal peptide sequence. Upon proteolysis, the signal peptide sequence is cleaved from the ENPP1 precursor protein to provide the mature ENPP1 protein. *See*, *e.g.*, Jansen S, et al. J Cell Sci. 2005;118(Pt 14):3081-9. Exemplary signal peptide sequences that can be used with the polypeptides disclosed herein include, but are not limited to, ENPP1 signal peptide sequence, ENPP2 signal peptide sequence, ENPP7 signal peptide sequence, and/or ENPP5 signal peptide sequence. The processed (mature) extracellular ENPP1 polypeptide sequence is shown in Figure 4 (SEQ ID NO: 2).

It is generally known in the art that ENPP1 is well-conserved among vertebrates, with large stretches of the extracellular domain substantially conserved. For example, Figure 5A and Figure 5B depict a multi-sequence alignment of a human ENPP1 extracellular domain compared to various ENPP1 orthologs. ENPP1 binding to various nucleotide triphosphates (*e.g.*, ATP, UTP, GTP, TTP, and CTP), pNP-TMP, 3',5'-cAMP, and 2'-3'-cGAMP is also highly conserved (*see*, *e.g.*, Kato K. et al., Proc Natl Acad Sci USA. 2012;109(42):16876-81 and Mackenzie NC, et al. Bone. 2012;51(5):961-8). Accordingly, from these alignments, it is possible to predict key amino acid positions with the extracellular domain that are important for normal ENPP1 activities as well as to predict amino acid positions that are likely to be tolerant to substitution without significantly altering normal ENPP1 activities. Therefore, an enzymatically active, human ENPP1 polypeptide useful in accordance with the presently disclosed compositions, may include one or more amino acids at corresponding positions

from the sequence of another vertebrate ENPP1, or may include a residue that is similar to that in the human or other vertebrate sequences. Substitutions of one or more amino acids at corresponding positions may include conservative variations or substitutions that are not likely to change the shape of the polypeptide chain or alter normal ENPP1 activities. Examples of conservative variations, or substitutions, include the replacement of one hydrophobic residue such as isoleucine, valine, leucine or methionine for another, or the substitution of one polar residue for another, such as the substitution of arginine for lysine, glutamic for aspartic acid, or glutamine for asparagine. For example, ENPP1 polypeptides include polypeptides derived from the sequence of any known ENPP1 polypeptide having a sequence at least about 80% identical to the sequence of an ENPP1 polypeptide, and preferably at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or greater identity.

4. Enzymatic Activity of ENPP1

ENPP1 proteins have been characterized in the art in terms of structural and biological characteristics. In certain embodiments, soluble ENPP1 proteins disclosed herein comprise pyrophosphatase and/or phosphodiesterase activity. For instance, in some embodiments, the ENPP1 protein binds nucleotide triphosphates (*e.g.*, ATP, UTP, GTP, TTP, and CTP), pNP-TMP, 3',5'-cAMP, and 2'-3'-cGAMP; and converts nucleotide triphosphates into inorganic pyrophosphate [*see*, *e.g.*, Kato K. *et al.*, Proc Natl Acad Sci USA. 2012;109(42):16876-81; Li L, *et al.* Nat Chem Biol. 2014;10(12):1043-8; Jansen S, *et al.* Structure. 2012;20(11):1948-59; and Onyedibe KI, *et al.* Molecules. 2019;24(22)].

"Enzymatically active" or "Biologically active" ENPP1 polypeptides exhibit pyrophosphatase and/or phosphodiesterase activity (*e.g.*, is capable of binding and/or hydrolyzing ATP into AMP and PPi and/or AP3a into ATP). For example, the pyrophosphatase/phosphodiesterase domain of an ENPP1 protein hydrolyzes extracellular nucleotide triphosphates to produce inorganic pyrophosphates (PPi) and is generally soluble. This activity can be measured using a pNP-TMP assay as previously described (Saunders, *et al.,* 2008, Mol. Cancer Ther. 7(10):3352-62; Albright, *et al.,* 2015, Nat Comm. 6:10006). In certain embodiments, the soluble ENPP1 polypeptide has a k_{cat} value for the substrate ATP greater than or equal to about 3.4 (±0.4) s^{-1} $enzyme^{-1}$, wherein the k_{cat} is determined by measuring the rate of hydrolysis of ATP for the polypeptide. In certain embodiments, the soluble ENPP1 polypeptide has a K_M value for the substrate ATP less than or equal to about 2

μM, wherein the K_M is determined by measuring the rate of hydrolysis of ATP for the polypeptide. In addition to the teachings herein, these references provide ample guidance for how to generate soluble ENPP1 proteins that retain one or more biological activities (*e.g.*, conversion of nucleotides into inorganic pyrophosphate).

5. Soluble ENPP1

In one embodiment, the disclosure relates to ENPP1 polypeptides. As described herein, the term soluble ENPP1 polypeptide, includes any naturally occurring extracellular domain of an ENPP1 protein as well as any variants thereof (including mutants, fragments and peptidomimetic forms) that retain a biological activity (*e.g.*, enzymatically active). Examples of soluble ENPP1 polypeptides include, for example, an ENPP1 extracellular domain (SEQ ID NO: 2) as shown in Figure 4. In certain embodiments, the soluble ENPP1 polypeptides further comprise a signal sequence in addition to the extracellular domain of an ENPP1 polypeptide. Exemplary signal sequences include the native signal sequence of an ENPP1 polypeptide, or a signal sequence from another protein, such as a hENPP7 signal sequence. Examples of variant soluble ENPP1 polypeptides are provided in International Patent Application Publication Nos. *WO 2012/125182, WO 2014/126965, WO 2016/187408, WO 2018/027024*, *WO 2020206302* and *WO 2020/047520* the contents of all of which are incorporated herein by reference in their entirety.

6. ENPP1 Fusion Proteins

In some embodiments, the ENPP1 polypeptide is a fusion protein comprising an ENPP1 polypeptide domain and one or more heterologous protein portions (*i.e.*, polypeptide domains heterologous to ENPP1). An amino acid sequence is understood to be heterologous to ENPP1 if it is not uniquely found in the form of ENPP1 represented by SEQ ID NO: 1. In some embodiments, the heterologous protein portion comprises an Fc domain of an immunoglobulin. In some embodiments, the Fc domain of the immunoglobulin is an Fc domain of an IgG1 immunoglobulin. In certain embodiments, the soluble ENPP1 polypeptide is C-terminally fused to the Fc domain of human immunoglobulin 1 (IgGl), human immunoglobulin 2 (IgG2), human immunoglobulin 3 (IgG3), and/or human immunoglobulin 4 (IgG4). In other embodiments, the soluble ENPP1 polypeptide is N-terminally fused to the Fc domain of human immunoglobulin 1 (IgGl), human immunoglobulin 2 (IgG2), human immunoglobulin 3 (IgG3), and/or human immunoglobulin

4 (IgG4). In some embodiments, the presence of an Fc domain improves half-life, solubility, reduces immunogenicity, and increases the activity of the soluble ENPP1 polypeptide. In certain embodiments, portions of the native human IgG proteins (IgG1, IgG2, IgG3, and IgG4), may be used for the Fc portion (*e.g.*, ENPP1-Fc). For instance, the present disclosure provides fusion proteins comprising ENPP1 fused to a polypeptide comprising a constant domain of an immunoglobulin, such as a CH1, CH2, or CH3 domain derived from human IgG1, IgG2, IgG3, and/or IgG4. The Fc fragment may comprise regions of the native IgG such as the hinge region (residues 216- 230 of human IgG1, according to the Rabat numbering system), the entire second constant domain CH2 (residues 231-340), and the third constant domain CH3 (residues 341- 447). As used herein, the term *"ENPP1-Fc construct"* refers to a soluble form of ENPP1 (e.g., the extracellular domain of an ENPP1 polypeptide) recombinantly fused and/or chemically conjugated (including both covalent and non-covalent conjugations) to an FcR binding domain of an IgG molecule (preferably, a human IgG). In certain embodiments, the C-terminus of ENPP1 is fused or conjugated to the N-terminus of the FcR binding domain.

An example of an amino acid sequence that may be used for the Fc portion of human IgG1 (G1Fc) is SEQ ID NO: 6 (Table 1). In part, the disclosure provides polypeptides comprising, consisting essential of, or consisting of amino acid sequences with 70%, 75%, 80%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% identity to SEQ ID NO: 6.

In some embodiments, the heterologous protein portion comprises one or more domains selected from the group consisting of polyhistidine, FLAG tag, Glu-Glu, glutathione S-transferase (GST), thioredoxin, protein A, protein G, an immunoglobulin heavy-chain constant region (Fc), maltose binding protein (MBP), or human serum albumin. A fusion domain may be selected so as to confer a desired property. For example, some fusion domains are particularly useful for isolation of the fusion proteins by affinity chromatography. For the purpose of affinity purification, relevant matrices for affinity chromatography, such as glutathione-, amylase-, and nickel- or cobalt- conjugated resins are used. Many of such matrices are available in "kit" form, such as the Pharmacia GST purification system and the QIAexpress^{TM} system (Qiagen) useful with (HIS_6) fusion partners. As another example, a fusion domain may be selected so as to facilitate detection of the ENPP1 polypeptide. Examples of such detection domains include the various fluorescent

proteins (*e.g.*, GFP) as well as "epitope tags," which are usually short peptide sequences for which a specific antibody is available. Well-known epitope tags for which specific monoclonal antibodies are readily available include FLAG, influenza virus haemagglutinin (HA), and c-myc tags. In some cases, the fusion domains have a protease cleavage site, such as for Factor Xa or thrombin, which allows the relevant protease to partially digest the fusion proteins and thereby liberate the recombinant proteins therefrom. The liberated proteins can then be isolated from the fusion domain by subsequent chromatographic separation.

7. Linkers

In some embodiments, the ENPP1 fusion protein further comprises a linker positioned between the ENPP1 polypeptide domain and the one or more heterologous protein portions (e.g., an Fc immunoglobulin domain). In certain embodiments, the soluble ENPP1 polypeptide is directly or indirectly fused to the Fc domain. In some embodiments, the soluble ENPP1 fusion protein comprises a linker between the Fc domain and the ENPP1 polypeptide. In some embodiments, a linker can be an amino acid spacer including 1-200 amino acids. Suitable peptide spacers are known in the art, and include, for example, peptide linkers containing flexible amino acid residues such as glycine, alanine, and serine. In some embodiments, the linker comprises a polyglycine linker or a Gly-Ser linker. In some embodiments, a spacer can contain motifs, *e.g.*, multiple or repeating motifs, of GA (SEQ ID NO: 21), GS (SEQ ID NO: 22), GG (SEQ ID NO: 23), GGA (SEQ ID NO: 24), GGS (SEQ ID NO: 25), GGG (SEQ ID NO: 26), GGGA (SEQ ID NO: 27), GGGS (SEQ ID NO: 28), GGGG (SEQ ID NO: 29), GGGGA (SEQ ID NO: 30), GGGGS (SEQ ID NO: 31), GGGGG (SEQ ID NO: 32), GGAG (SEQ ID NO: 33), GGSG (SEQ ID NO: 34), AGGG (SEQ ID NO: 35), SGGGG (SEQ ID NO: 36), or SGGG (SEQ ID NO: 37). In some embodiments, a spacer can contain 2 to 12 amino acids including motifs of GA or GS, e.g., GA, GS, GAGA (SEQ ID NO: 38), GSGS (SEQ ID NO: 39), GAGAGA (SEQ ID NO: 40), GSGSGS (SEQ ID NO: 41), GAGAGAGA (SEQ ID NO: 42), GSGSGSGS (SEQ ID NO: 43), GAGAGAGAGA (SEQ ID NO: 44), GSGSGSGSGS (SEQ ID NO: 45), GAGAGAGAGAGA (SEQ ID NO: 46), and GSGSGSGSGSGS (SEQ ID NO: 47). In some embodiments, a spacer can contain 3 to 12 amino acids including motifs of GGA or GGS, e.g., GGA, GGS, GGAGGA (SEQ ID NO: 48), GGSGGS (SEQ ID NO: 49), GGAGGAGGA (SEQ ID NO: 50), GGSGGSGGS (SEQ ID NO: 51), GGAGGAGGAGGA (SEQ ID NO: 52), and GGSGGSGGSGGS (SEQ ID NO: 53). In yet some embodiments, a spacer can contain 4 to 12 amino acids including motifs

of GGAG (SEQ ID NO: 54), GGSG (SEQ ID NO: 55), e.g., GGAG (SEQ ID NO: 56), GGSG (SEQ ID NO: 57), GGAGGGAG (SEQ ID NO: 58), GGSGGGSG (SEQ ID NO: 59), GGAGGGAGGGAG (SEQ ID NO: 60), and GGSGGGSGGGSG (SEQ ID NO: 61). In some embodiments, a spacer can contain motifs of GGGGA (SEQ ID NO: 62) or GGGGS (SEQ ID NO: 63), e.g., GGGGAGGGGAGGGGA (SEQ ID NO: 64) and GGGGSGGGGSGGGGS (SEQ ID NO: 65). In some embodiments of the invention, an amino acid spacer between a heterologous protein portion (*e.g.*, an Fc domain monomer, a wild-type Fc domain, an Fc domain with amino acid substitutions (*e.g.*, one or more substitutions that reduce dimerization), an albumin-binding peptide, a fibronectin domain, or a human serum albumin) and a soluble ENPP1 polypeptide may be GGG, GGGA (SEQ ID NO: 27), GGGG (SEQ ID NO: 29), GGGAG (SEQ ID NO: 66), GGGAGG (SEQ ID NO: 67), or GGGAGGG (SEQ ID NO: 68).

In some embodiments, a spacer can also contain amino acids other than glycine, alanine, and serine, e.g., LIN (SEQ ID NO: 69), TGGGG (SEQ ID NO: 70), AAAL (SEQ ID NO: 71), AAAK (SEQ ID NO: 72), AAAR (SEQ ID NO: 73), EGKSSGSGSESKST (SEQ ID NO: 74), GSAGSAAGSGEF (SEQ ID NO: 75), AEAAAKEAAAKA (SEQ ID NO: 76), KESGSVSSEQLAQFRSLD (SEQ ID NO: 77), GENLYFQSGG (SEQ ID NO: 78), SACYCELS (SEQ ID NO: 79), RSIAT (SEQ ID NO: 80), RPACKIPNDLKQKVMNH (SEQ ID NO: 81), GGSAGGSGSGSSGGSSGASGTGTAGGTGSGSGTGSG (SEQ ID NO: 82), AAANSSIDLISVPVDSR (SEQ ID NO: 83), GGSGGGSEGGGSEGGGSEGGGSEGGGSEGGGSGGGS (SEQ ID NO: 84), NSS (SEQ ID NO: 87), ESS (SEQ ID NO: 88), RQQ (SEQ ID NO: 89), KR (SEQ ID NO: 90), $(R)_m$; m=0-15 (SEQ ID NO: 91), DSSSEEKFLRRIGRFG (SEQ ID NO: 92), EEEEEEEPRGDT (SEQ ID NO: 93), APWHLSSQYSRT (SEQ ID NO: 94), STLPIPHEFSRE (SEQ ID NO: 95), VTKHLNQISQSY (SEQ ID NO: 96), $(E)_m$; m=1-15 (SEQ ID NO: 97), RSGSGGS (SEQ ID NO: 98), $(D)_m$; m=1-15 (SEQ ID NO: 99), LVIMSLGLGLGLGLRK (SEQ ID NO: 100), VIMSLGLGLGLGLRK (SEQ ID NO: 101), IMSLGLGLGLGLRK (SEQ ID NO: 102), MSLGLGLGLGLRK (SEQ ID NO: 103), SLGLGLGLGLRK (SEQ ID NO: 104), LGLGLGLGLRK (SEQ ID NO: 105), GLGLGLGLRK (SEQ ID NO: 106), LGLGLGLRK (SEQ ID NO: 107), GLGLGLRK (SEQ ID NO: 108), LGLGLRK (SEQ ID NO: 109), GLGLRK (SEQ ID NO: 110), LGLRK (SEQ ID NO: 111), GLRK (SEQ ID NO: 112), LRK (SEQ ID NO: 113), RK (SEQ ID NO: 114), or $(K)_m$; m=1-15 (SEQ ID NO: 115). In some embodiments, a spacer can contain motifs, e.g., multiple or repeating motifs, of EAAAK

(SEQ ID NO: 85). In some embodiments, a spacer can contain motifs, e.g., multiple or repeating motifs, of praline-rich sequences such as (XP)n, in which X may be any amino acid (e.g., A, K, or E) and n is from 1-5, and PAPAP(SEQ ID NO: 86).

The length of the peptide spacer and the amino acids used can be adjusted depending on the two proteins involved and the degree of flexibility desired in the final protein fusion polypeptide. The length of the spacer can be adjusted to ensure proper protein folding and avoid aggregate formation.

In some embodiments, different elements of the fusion proteins (*e.g.*, immunoglobulin Fc fusion proteins) may be arranged in any manner that is consistent with desired functionality. For example, a soluble ENPP1 polypeptide domain may be placed C-terminal to a heterologous protein portion, or alternatively, a heterologous protein portion may be placed C-terminal to a soluble ENPP1 polypeptide domain. The soluble ENPP1 polypeptide domain and the heterologous protein portion may be directly or indirectly linked in a fusion protein, and additional domains or amino acid sequences may be included C- or N-terminal to either domain or between the domains. Preferred fusion proteins comprise the amino acid sequence set forth in any one of SEQ ID NOs: 3-5.

In some embodiments, soluble ENPP1 polypeptides of the present disclosure contain one or more heterologous moieties. Optionally, a soluble ENPP1 polypeptide includes one or more heterologous moieties selected from: a glycosylated amino acid, a PEGylated amino acid, a farnesylated amino acid, an acetylated amino acid, a biotinylated amino acid, an amino acid conjugated to a lipid moiety, and an amino acid conjugated to an organic derivatizing agent. In some embodiments, a soluble ENPP1 polypeptide disclosed herein is further modified. Such modifications include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. As a result, the soluble ENPP1 polypeptide may contain non-amino acid elements, such as polyethylene glycols, lipids, polysaccharide or monosaccharide, and phosphates. Effects of such non-amino acid elements on the functionality of a soluble ENPP1 polypeptide may be tested as described herein for other soluble ENPP1 polypeptides. When a polypeptide of the disclosure is produced in cells by cleaving a nascent form of the polypeptide, post-translational processing may also be important for correct folding and/or function of the protein. Different cells (*e.g.*, CHO, HeLa, MDCK, 293, WI38, NIH-3T3 or HEK293) have specific cellular machinery and characteristic mechanisms for such post-translational

activities and may be chosen to ensure the correct modification and processing of the soluble ENPP1 polypeptides.

As used herein, percent "identity" between a polypeptide sequence and a reference sequence, is defined as the percentage of amino acid residues in the polypeptide sequence that are identical to the amino acid residues in the reference sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN, MEGALIGN (DNASTAR), CLUSTALW, or CLUSTAL OMEGA software. In some embodiments, alignment is performed using the CLUSTAL OMEGA software. Those skilled in the art can determine appropriate parameters for aligning sequences, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared.

8. Determining Solubility

In some embodiments, the activity of soluble ENPP1 polypeptides may also be tested in a cell-based or *in vivo* assay. For example, the effect of a soluble ENPP1 polypeptide on the production of inorganic pyrophosphates (PPi) can be measured. Specifically, the pyrophosphatase/phosphodiesterase domain of an ENPP1 protein hydrolyzes extracellular nucleotide triphosphates to produce inorganic pyrophosphates (PPi) and is generally soluble. This activity can be measured using a pNP-TMP assay as well as an HPLC-based ATP hydrolysis assay, as previously described (Saunders, *et al.,* 2008, Mol. Cancer Ther. 7(10):3352-62; Albright, *et al.,* 2015, Nat Comm. 6:10006). The effect of soluble ENPP1 polypeptides on the expression of genes involved in ENPP1 associated diseases such as ARHR2 (*e.g.*, transcription of fibroblast growth factor 23 in osteoblasts and osteoclasts) can be assessed. This may, as needed, be performed in the presence of one or more nucleotide triphosphates or other ENPP1 substrates, and cells may be transfected so as to produce a soluble ENPP1 polypeptide. Likewise, a soluble ENPP1 polypeptide may be administered to a mouse or other animal and effects on ENPP1 associated diseases may be assessed using art-recognized methods.

In some embodiments, ENPP1 polypeptides to be used in accordance with the methods described herein are isolated polypeptides. As used herein, an isolated protein or

polypeptide is one which has been separated from a component of its natural environment. In some embodiments, a polypeptide of the disclosure is purified to greater than 95%, 96%, 97%, 98%, or 99% purity as determined by, for example, electrophoretic (*e.g.*, SDS-PAGE, isoelectric focusing (IEF), capillary electrophoresis) or chromatographic (*e.g.*, ion exchange or reverse phase HPLC) analyses. Methods for assessment of purity are well known in the art [*see*, *e.g.*, Flatman *et al.*, (2007) J. Chromatogr. B 848:79-87]. In some embodiments, soluble ENPP1 polypeptides to be used in accordance with the methods described herein are recombinant polypeptides.

9. ENPP1 Production

ENPP1 polypeptides of the disclosure can be produced by a variety of art-known techniques. For example, polypeptides of the disclosure can be synthesized using standard protein chemistry techniques such as those described in Bodansky, M. Principles of Peptide Synthesis, Springer Verlag, Berlin (1993) and Grant G. A. (ed.), Synthetic Peptides: A User's Guide, W. H. Freeman and Company, New York (1992). In addition, automated peptide synthesizers are commercially available (*e.g.*, Advanced ChemTech Model 396; Milligen/Biosearch 9600). Alternatively, the polypeptides of the disclosure, including fragments or variants thereof, may be recombinantly produced using various expression systems [*e.g.*, E. coli, Chinese Hamster Ovary (CHO) cells, COS cells, baculovirus, Yeast Pichia] as is well known in the art. The protein can be produced in either adherent or suspension cells. In some embodiments, the fusion protein is expressed in CHO cells. To establish stable cell lines the nucleic acid sequence encoding ENPP1 constructs are cloned into an appropriate vector for large scale protein production. In a further embodiment, the modified or unmodified polypeptides of the disclosure may be produced by digestion of recombinantly produced full-length ENPP1 polypeptides by using, for example, a protease, *e.g.*, trypsin, thermolysin, chymotrypsin, pepsin, or paired basic amino acid converting enzyme (PACE). Computer analysis (using commercially available software, *e.g.*, MacVector, Omega, PCGene, Molecular Simulation, Inc.) can be used to identify proteolytic cleavage sites. Alternatively, such polypeptides may be produced from recombinantly generated full-length ENPP1 polypeptides using chemical cleavage (*e.g.*, cyanogen bromide, hydroxylamine, *etc.*).

10. Expression Systems

Many expression systems are known and can be used for the production of ENPP1 fusion protein, including bacteria (for example E. coli and Bacillus subtilis), yeasts (for example *Saccharomyces cerevisiae*, *Kluyveronmyces lactis* and *Pichia pastoris*), filamentous fungi (for example *Aspergillus*), plant cells, animal cells and insect cells. The desired protein can be produced in conventional ways, for example from a coding sequence inserted in the host chromosome or on a free plasmid.

The yeasts can be transformed with a coding sequence for the desired protein in any of the usual ways (*e.g.*, electroporation). Methods for transformation of yeast by electroporation are disclosed in Becker & Guarente, 1990, Methods Enzymol. 194: 182. Successfully transformed cells, *i.e.*, cells that contain a DNA construct of the present disclosure, can be identified by well-known techniques. For example, cells resulting from the introduction of an expression construct can be grown to produce the an ENPP1 polypeptide. Cells can be harvested and lysed and their DNA content examined for the presence of the DNA using a method, such as that described by Southern, 1975, J. Mol. Biol, 98:503 and/or Berent, *et al.*, 1985, Biotech 3:208. Alternatively, the presence of the protein in the supernatant can be detected using antibodies.

Useful yeast plasmid vectors include pRS403—406 and pRS413—416 and are generally available front Stratagene Cloning Systems, La Jolla, CA, USA Plasmids pRS403, pRS404, pRS405 and pRS406 are Yeast Integrating plasmids (Yips) and incorporate the yeast selectable markers I-11S3, TRP1, LEU2 and 1JRA3. Plasmids pRS413— 416 are Yeast Centromere plasmids (YCps).

A variety of methods have been developed to operably link DNA to vectors via complementary cohesive termini. For instance, complementary homopolymer tract can be added to the DNA segment to be inserted to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of joining the DNA segment to vectors. The DNA segment, generated by endonuclease restriction digestion, is treated with bacteriophage T4 DNA polymerase or E. coli DNA polymerase I, which are enzymes that remove protruding, 3'-single-stranded

termini with their 3'-5' -exonucleolytic activities, and fill in recessed 3'-ends with their polymerizing activities.

The combination of these activities thus generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. As a result, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments can be cleaved with an appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Clones of single, stably transfected cells are then established and screened for high expressing clones of the desired ENPP1 fusion protein. Screening of the single cell clones for ENPP1 protein expression can be accomplished in a high-throughput manner in 96 well plates using the synthetic enzymatic substrate pNP-TMP as previously described (Albright, et al., 2015, Nat. Commun. 6:10006). Upon identification of high expressing clones through screening, protein production can be accomplished in shaking flasks or bio-reactors are previously described in Albright, et al., 2015, Nat. Commun. 6:10006.

11. ENPP1 Purification

Purification of ENPP1 can be accomplished using a combination of standard purification techniques known in the art. Following purification, ENPP1-Fc can be dialyzed into PBS supplemented with Zn2+ and Mg2+ (PBSplus) concentrated to between 5 and 7 mg/ml, and frozen at -80 °C in aliquots of 200-500 pl. Aliquots can be thawed immediately prior to use and the specific activity of the solution can be adjusted to 31.25 au/ml (or about 0.7 mg/ml depending on the preparation) by dilution in PBSplus.

12. Route and Frequency of Administration

The polypeptide may be administered acutely or chronically to the subject. In certain embodiments, a second dosage of a soluble ENPP1 polypeptide or ENPP1 fusion polypeptide disclosed herein is administered after a suitable time interval of about after two days, after four days, after a week, or after a month to the subject or even less frequently, such as once every several months or even once a year or less. The frequency of the dose is readily

apparent to the skilled artisan and depends upon any number of factors, such as, but not limited to, the type and severity of the disease being treated, and the type and age of the patient.

A dose amount or frequency may be selected so that the steady state level of plasma PPi is maintained at a constant or steady state level, and/or so as to achieve a continuous level of plasma PPi that is either close to the normal (2-3 μM) level or above (30-50% higher than) normal levels of PPi and does not return to the lower level of PPi that the subject had prior to the administration of first dosage of constructs disclosed herein.

Alternative, the ENPP1 agent may be administered at appropriate time intervals of either every 2 days, or every 4 days, every week or every month so as to achieve a constant level of enzymatic activity of ENPPl.

Alternatively, an ENPP1 agent according to the disclosure is administered at an appropriate time interval of every 2 days, or every 4 days, or every week or every month by monitoring one or more symptoms of a subject's disease or disorder.

Without wishing to be bound by theory, it is believed that maintaining a steady state concentration of plasma PPi at normal levels reduces and/or prevents progression of pathological calcification of subjects.

In certain embodiments, the polypeptide is administered locally, regionally, parenterally or systemically to the subject. In some embodiments, the polypeptide is administered subcutaneously.

As used herein, "parenteral administration" of a formulation includes any route of administration characterized by physical breaching of a tissue of a subject and administration of the ENPP1 agent through the breach in the tissue. Parenteral administration thus includes, but is not limited to, administration of an ENPP1 agent by injection of the composition, by application of the composition through a surgical incision, by application of the composition through a tissue-penetrating non-surgical wound, and the like. In particular, parenteral administration is contemplated to include, but is not limited to, subcutaneous, intravenous, intraperitoneal, intramuscular, intrastemal injection, and kidney dialytic infusion techniques.

The regimen of administration may affect what constitutes an effective amount. For example, several divided dosages, as well as staggered dosages may be administered in a given time period (daily) or sequentially, or the dose may be continuously infused, or may be a bolus injection. Further, selection of a recited dose of an ENPP1 agent may be indicated by the exigencies of the therapeutic or prophylactic situation.

Administration of the compositions of the present disclosure (*e.g.*, soluble ENPP1 polypeptides and fusion proteins thereof) to a patient, such as a mammal (*i.e.*, a human), may be carried out using known procedures, at dosages and for periods of time effective to treat a disease or disorder in the patient. An effective amount of the recited dosages of an ENPP1 agent necessary to achieve a therapeutic effect may vary according to factors such as the activity of the particular compound employed; the time of administration; the rate of excretion of the compound; the duration of the treatment; other drugs, compounds or materials used in combination with the compound; the state of the disease or disorder, age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well-known in the medical arts. Dosage regimens may be adjusted to provide the optimum therapeutic response. A selected dosage is determined based on the biological activity of the therapeutic compound which in turn depends on the half-life and the area under the plasma time of the therapeutic compound curve.

13. Prophylactic Administration

Armed with the disclosure herein, one skilled in the art would thus appreciate that the prevention of a disease or disorder in a subject encompasses administering to a subject an ENPP1 polypeptide as a preventative measure against the disease or disorder.

The relative amounts of the active ingredient (*e.g.*, soluble ENPP1 polypeptides and fusion proteins thereof), the pharmaceutically acceptable carrier, and any additional ingredients in a formulation disclosed herein will vary, depending upon the identity, size, and condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between about 0.1% and about 100% (w/w) active ingredient.

14. Diseases Relating to Low PPi

In some embodiments, the disclosure contemplates methods of reducing or preventing progression of diseases caused by lower levels of plasma PPi in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of the polypeptides disclosed herein to increase the plasma PPi of the subjects to normal (2-3 μM) or above (30-50% higher than) normal levels and then to maintain the plasma PPi at a constant normal or above normal level thereafter. The method further comprises administering additional therapeutic effective amounts at intervals of two days, three days, one week or one month in order to maintain the Plasma PPi of the subject at a constant normal or above normal level to reduce or prevent the progression of pathological calcification or ossification. In certain embodiments, a soluble ENPP1 polypeptide or ENPP1 fusion polypeptide disclosed herein can be used to raise pyrophosphate (PPi) levels in a subject having PPi level lower than normal level (which is around 2μM). In other embodiments, a soluble ENPP1 polypeptide or ENPP1 fusion polypeptide disclosed herein can be used to reduce or prevent progression of pathological calcification or ossification in a subject having PPi levels lower than normal level. In some embodiments, a soluble ENPP1 polypeptide or ENPP1 fusion polypeptide disclosed herein can be used to treat ENPP1 deficiency (*e.g.*, GACI and ARHR2) manifested by a reduction of extracellular PPi concentration in a subject. In certain embodiments, the steady state level of plasma PPi achieved after administration of a first dosage of a soluble ENPP1 polypeptide or ENPP1 fusion polypeptide disclosed herein is maintained for a time period of at least 2 days, at least 4 days, at least a week or at least a month.

In some embodiments, the disclosure contemplates methods of reducing or preventing progression of a disease caused by lower than normal levels of plasma PPi in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a soluble ENPP1 polypeptide or ENPP1 fusion polypeptide disclosed herein (*e.g.*, SEQ ID NOs: 3-5) to increase and/or sustain the plasma PPi of the subjects to a level that is about 90%, 95%, 100%, 105%, 110%, 120%, 130%, 140%, or 150% of the normal PPi level. In certain embodiments, the method further comprises further administration of the polypeptide disclosed herein every two days, three days, one week, or one month in order to maintain the plasma PPi levels at a level that is about 90%, 95%, 100%, 105%, 110%, 120%,

130%, 140%, or 150% of the normal PPi level, thus preventing the progression of pathological calcification or ossification.

15. Treatment/Indications

The recited dosages of an ENPP1 agent disclosed herein may be used in methods of treating, reversing, or preventing progression of diseases associated with an ENPP1 deficiency or an ABCC6 deficiency as disclosed herein.

In an aspect, the disclosure relates to administering to a subject having an ENPP1 deficiency or an ABCC6 deficiency, an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject in order to restore a physiological level of ENPP1 in the plasma or tissues of the subject.

In an aspect, the disclosure relates to administering to a subject having an ENPP1 deficiency or an ABCC6 deficiency, an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject in order to restore a physiological level of Pi and/or PPi in the plasma of the subject. The physiological level of Pi and PPi in human serum (and in mammals generally) is 1-3 mM and 2-3 μM respectively.

In an aspect, the disclosure relates to a method for preventing progression of or reducing vascular calcification in a subject with ENPP1 Deficiency or with ABCC6 deficiency, the method comprising: to thereby prevent the progression of or reduce vascular calcification in the subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce vascular calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing pathological calcification in a subject with ENPP1 Deficiency or with ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce pathological calcification in the subject.

The recited dosages of an ENPP1 agent disclosed herein may be used in methods of treating, reversing, or preventing progression of diseases associated with pathological calcification as disclosed herein.

In an aspect, the disclosure relates to administering to a subject having pathological calcification an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject in order to restore a physiological level of ENPP1 in the plasma or tissues of the subject.

In an aspect, the disclosure relates to administering to a subject having pathological calcification an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject in order to restore a physiological level of Pi and/or PPi in the plasma of the subject. The physiological level of Pi and PPi in human serum (and in mammals generally) is 1-3 mM and 2-3 μM respectively.

In an aspect, the disclosure relates to a method for preventing progression of or reducing vascular calcification in a subject, the method comprising: to thereby prevent the progression of or reduce vascular calcification in the subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce vascular calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing pathological calcification in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce pathological calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing tissue calcification in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing pathological ossification in a subject, the method comprising: administering to the

subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing tissue calcification in a subject with ENPP1 Deficiency or with ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject.

In an aspect, the disclosure relates to a method for preventing the progression of or reducing pathological ossification in a subject with ENPP1 Deficiency or with ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject.

In an aspect, the disclosure relates to a method for increasing circulating pyrophosphate (PPi) in a subject with ENPP1 Deficiency or with ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

In an aspect, the disclosure relates to a method for increasing pyrophosphatase activity in a subject with ENPP1 Deficiency or with ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

In an aspect, the disclosure relates to a method for ameliorating one or more symptoms of ENPP1 Deficiency or ABCC6 deficiency in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby ameliorate one or more symptoms of ENPP1 Deficiency or one or more symptoms of ABCC6 deficiency in the subject.

In an aspect, the disclosure relates to a method for treating a subject with ENPP1 Deficiency or with ABCC6 deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby treat the subject.

In certain aspects, the present disclosure relates to the use of an ENPP1 agent, such as a polypeptide having an amino acid sequences as set forth in, *e.g.*, SEQ ID NOs: 2, 3, 4, and 5.

In certain embodiments, the pathological calcification is selected from the group consisting of kidney and bladder stones, dental pulp stones, gall stones, salivary gland stones, chronic calculous prostatitis, testicular microliths, calcification in hemodialysis patients, atherosclerosis, malacoplakia, scleroderma (systemic sclerosis), calcinosis cutis, calcific aortic stenosis, calcific tenditis, synovitis and arthritis, diffuse interstitial skeletal hyperostosis, juvenile dermatomyositis, Generalized Arterial Calcification of Infancy (GACI), Ossification of the Posterior Longitudinal Ligament (OPLL), hypophosphatemic rickets, autosomal hypophophatemic rickets (ARHR2), osteoarthritis, calcification of atherosclerotic plaques, Chronic Kidney Disease (CKD), End Stage Renal Disease (ESRD), Pseudoxanthoma elasticum (PXE), ankylosing spondylitis, hardening of the arteries, calciphylaxis, and systemic lupus erythematosus.

In certain embodiments, the pathological calcification is selected from the group consisting of Pseudoxanthoma elasticum (PXE) and calcification of atherosclerotic plaques.

In certain embodiments, the pathological calcification is selected from the group consisting of idiopathic infantile arterial calcification (IIAC) and calcification of atherosclerotic plaques.

In some embodiments, the disclosure contemplates methods of reducing or preventing progression of ectopic calcification of soft tissue, including reducing, ameliorating, or preventing vascular calcification, in a subject in need thereof, the method comprising administering to the subject a therapeutically effective amount of a soluble ENPP1 polypeptide or ENPP1 fusion polypeptide disclosed herein.

In some embodiments, the disclosure contemplates methods of reducing or preventing progression of diseases caused by an ENPP1 deficiency (*e.g.*, GACI and ARHR2), the method comprising administering to the subject a therapeutically effective amount of a soluble ENPP1 polypeptide or ENPP1 fusion protein disclosed herein (*e.g.*, SEQ ID NOs: 2, 3, 4, and 5). In some embodiments, the ENPP1 deficiency is GACI. In some embodiments, the ENPP1 deficiency is ARHR2.

In some embodiments, the disclosure contemplates methods of reducing or preventing progression of diseases caused by an ABCC6 deficiency (*e.g.*, PXE), the method comprising administering to the subject a therapeutically effective amount of a soluble ENPP1 polypeptide or ENPP1 fusion protein disclosed herein (*e.g.*, SEQ ID NOs: 2, 3, 4, and 5). In some embodiments, the ABCC6 deficiency is PXE. In some embodiments, subjects having ABCC6 deficiency exhibit symptoms similar to a person diagnosed with GACI or ARHR2.

In some embodiments, the disclosure contemplates methods of reducing or preventing progression of diseases caused by pathological calcification (*e.g.*, GACI and ARHR2), the method comprising administering to the subject a therapeutically effective amount of a soluble ENPP1 polypeptide or ENPP1 fusion protein disclosed herein (*e.g.*, SEQ ID NOs: 2, 9, 10, and 11). In some embodiments, the subject exhibiting pathological calcification has ENPP1 deficiency. In some embodiments subject exhibiting pathological calcification has ABCC6 deficiency.

In certain embodiments, the polypeptide is a secreted product of an ENPP1 precursor protein expressed in a mammalian cell. In other embodiments, the ENPP1 precursor protein comprises a signal peptide sequence and an ENPP1 polypeptide, wherein the ENPP1 precursor protein undergoes proteolytic processing to the polypeptide disclosed herein. In some embodiments, in the ENPP1 precursor protein the signal peptide sequence is conjugated to the ENPP1 polypeptide N-terminus. Upon proteolysis, the signal sequence is cleaved from the ENPP1 precursor protein to provide the ENPP1 polypeptide. In certain embodiments, the signal peptide sequence is selected from the group consisting of ENPP1 signal peptide sequence, ENPP2 signal peptide sequence, ENPP7 signal peptide sequence, and ENPP5 signal peptide sequence.

It will be appreciated by one of skill in the art, when armed with the present disclosure including the methods detailed herein, that the disclosure is not limited to

treatment of a disease or disorder once it is established. Particularly, the symptoms of the disease or disorder need not have manifested to the point of detriment to the subject; indeed, the disease or disorder need not be detected in a subject before treatment is administered. That is, significant pathology from disease or disorder does not have to occur before the present ENPP1 polypeptides may provide benefit.

In certain aspects, the disclosure relates to methods for preventing diseases and disorders in a subject, in that a soluble ENPP1 polypeptide or ENPP1 fusion polypeptide disclosed herein can be administered to a subject prior to the onset of the disease or disorder, thereby preventing the disease or disorder from developing. Therefore, the disclosure relates to methods for preventing or delaying onset, or reducing progression or growth, of a disease or disorder in a subject, comprising administering an ENPP1 polypeptide to a subject prior to detection of the disease or disorder. In certain embodiments, the ENPP1 polypeptide is administered to a subject with a strong family history of the disease or disorder, thereby preventing or delaying onset or progression of the disease or disorder.

16. ENPP1 Polypeptide Sequences

Table 1: Sequences

SEQ ID NO	Sequence	Description
1	1 MERDGCAGGG SRGGEGGRAP REGPAGNGRD RGRSHAAEAP GDPQAAASLL 51 APMDVGEEPL EKAARARTAK DPNTYKVLSL VLSVCVLTTI LGCIFGLKPS 101 CAKEVKSCKG RCFERTFGNC RCDAACVELG NCCLDYQETC IEPEHIWTCN 151 KFRCGEKRLT RSLCACSDDC KDKGDCCINY SSVCQGEKSW VEEPCESINE 201 PQCPAGFETP PTLLFSLDGF RAEYLHTWGG LLPVISKLKK CGTYTKNMRP 251 VYPTKTFPNH YSIVTGLYPE SHGIIDNKMY DPKMNASFSL KSKEKFNPEW 301 YKGEPIWVTA KYQGLKSGTF FWPGSDVEIN GIFPDIYKMY NGSVPFEERI 351 LAVLQWLQLP KDERPHFYTL YLEEPDSSGH SYGPVSSEVI KALQRVDGMV 401 GMLMDGLKEL NLHRCLNLIL ISDHGMEQGS CKKYIYLNKY LGDVKNIKVI 451 YGPAARLRPS DVPDKYYSFN YEGIARNLSC REPNQHFKPY LKHFLPKRLH 501 FAKSDRIEPL TFYLDPQWQL ALNPSERKYC GSGFHGSDNV FSNMQALFVG 551 YGPGFKHGIE ADTFENIEVY NLMCDLLNLT PAPNNGTHGS LNHLLKNPVY 601 TPKHPKEVHP LVQCPFTRNP RDNLGCSCNP SILPIEDFQT QFNLTVAEEK 651 IIKHETLPYG RPRVLQKENT ICLLSQHQFM SGYSQDILMP LWTSYTVDRN 701 DSFSTEDFSN CLYQDFRIPL SPVHKCSFYK NNTKVSYGFL SPPQLNKNSS 751 GIYSEALLTT NIVPMYQSFQ VIWRYFHDTL LRKYAEERNG VNVVSGPVFD 801 FDYDGRCDSL ENLRQKRRVI RNQEILIPTH FFIVLTSCKD TSQTPLHCEN 851 LDTLAFILPH RTDNSESCVH GKHDSSWVEE LLMLHRARIT DVEHITGLSF 901 YQQRKEPVSD ILKLKTHLPT FSQED	Full, unprocessed amino acid sequence of wild-type ENPP1 precursor protein

2	1 PSCAKEVKSC KGRCFERTFG NCRCDAACVE LGNCCLDYQE TCIEPEHIWT 51 CNKFRCGEKR LTRSLCACSD DCKDKGDCCI NYSSVCQGEK SWVEEPCESI 101 NEPQCPAGFE TPPTLLFSLD GFRAEYLHTW GGLLPVISKL KKCGTYTKNM 151 RPVYPTKTFP NHYSIVTGLY PESHGIIDNK MYDPKMNASF SLKSKEKFNP 201 EWYKGEPIWV TAKYQGLKSG TFFWPGSDVE INGIFPDIYK MYNGSVPFEE 251 RILAVLQWLQ LPKDERPHFY TLYLEEPDSS GHSYGPVSSE VIKALQRVDG 301 MVGMLMDGLK ELNLHRCLNL ILISDHGMEQ GSCKKYIYLN KYLGDVKNIK 351 VIYGPAARLR PSDVPDKYYS FNYEGIARNL SCREPNQHFK PYLKHFLPKR 401 LHFAKSDRIE PLTFYLDPQW QLALNPSERK YCGSGFHGSD NVFSNMQALF 451 VGYGPGFKHG IEADTFENIE VYNLMCDLLN LTPAPNNGTH GSLNHLLKNP 501 VYTPKHPKEV HPLVQCPFTR NPRDNLGCSC NPSILPIEDF QTQFNLTVAE 551 EKIIKHETLP YGRPRVLQKE NTICLLSQHQ FMSGYSQDIL MPLWTSYTVD 601 RNDSFSTEDF SNCLYQDFRI PLSPVHKCSF YKNNTKVSYG FLSPPQLNKN 651 SSGIYSEALL TTNIVPMYQS FQVIWRYFHD TLLRKYAEER NGVNVVSGPV 701 FDFDYDGRCD SLENLRQKRR VIRNQEILIP THFFIVLTSC KDTSQTPLHC 751 ENLDTLAFIL PHRTDNSESC VHGKHDSSWV EELLMLHRAR ITDVEHITGL 801 SFYQQRKEPV SDILKLKTHL PTFSQED	The processed (mature) extracellular ENPP1 polypeptide sequence
3	FTAGLKPSCAKEVKSCKGRCFERTFGNCRCDAACVELGNCCLDYQETCIEPEH IWTCNKFRCGEKRLTRSLCACSDDCKDKGDCCINYSSVCQGEKSWVEEPCESINEPQCPA GFETPPTLLFSLDGFRAEYLHTWGGLLPVISKLKKCGTYTKNMRPVYPTKTFPNHYSIVT GLYPESHGIIDNKMYDPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGS DVEINGIFPDIYKMYNGSVPFEERILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGPV SSEVIKALQRVDGMVGMLMDGLKELNLHRCLNLILISDHGMEQGSCKKYIYLNKYLGDVK NIKVIYGPAARLRPSDVPDKYYSFNYEGIARNLSCREPNQHFKPYLKHFLPKRLHFAKSD RIEPLTFYLDPQWQLALNPSERKYCGSGFHGSDNVFSNMQALFVGYGPGFKHGIEADTFE NIEVYNLMCDLLNLTPAPNNGTHGSLNHLLKNPVYTPKHPKEVHPLVQCPFTRNPRDNLG CSCNPSILPIEDFQTQFNLTVAEEKIIKHETLPYGRPRVLQKENTICLLSQHQFMSGYSQ DILMPLWTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPPQL NKNSSGIYSEALLTTNIVPMYQSFQVIWRYFHDTLLRKYAEERNGVNVVSGPVFDFDYDG RCDSLENLRQKRRVIRNQEILIPTHFFIVLTSCKDTSQTPLHCENLDTLAFILPHRTDNS ESCVHGKHDSSWVEELLMLHRARITDVEHITGLSFYQQRKEPVSDILKLKTHLPTFSQED LIN**DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW** **YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS** **KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPV** **LDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK**	ENPP1-linker-hIgG1 Fc construct
4	GLKPSCAKEVKSCKGRCFERTFGNCRCDAACVELGNCCLDYQETCIEPEHIWT CNKFRCGEKRLTRSLCACSDDCKDKGDCCINYSSVCQGEKSWVEEPCESINEPQCPAGFE TPPTLLFSLDGFRAEYLHTWGGLLPVISKLKKCGTYTKNMRPVYPTKTFPNHYSIVTGLY PESHGIIDNKMYDPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVE INGIFPDIYKMYNGSVPFEERILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGPVSSE	ENPP1-linker-hIgG1 Fc construct

	VIKALQRVDGMVGMLMDGLKELNLHRCLNLILISDHGMEQGSCKKYIYLNKYLGDVKNIK VIYGPAARLRPSDVPDKYYSFNYEGIARNLSCREPNQHFKPYLKHFLPKRLHFAKSDRIE PLTFYLDPQWQLALNPSERKYCGSGFHGSDNVFSNMQALFVGYGPGFKHGIEADTFENIE VYNLMCDLLNLTPAPNNGTHGSLNHLLKNPVYTPKHPKEVHPLVQCPFTRNPRDNLGCSC NPSILPIEDFQTQFNLTVAEEKIIKHETLPYGRPRVLQKENTICLLSQHQFMSGYSQDIL MPLWTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPPQLNKN SSGIYSEALLTTNIVPMYQSFQVIWRYFHDTLLRKYAEERNGVNVVSGPVFDFDYDGRCD SLENLRQKRRVIRNQEILIPTHFFIVLTSCKDTSQTPLHCENLDTLAFILPHRTDNSESC VHGKHDSSWVEELLMLHRARITDVEHITGLSFYQQRKEPVSDILKLKTHLPTFSQEDLIN **DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDS DGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK**	
5	PSCAKEVKSCKGRCFERTFGNCRCDAACVELGNCCLDYQETCIEPEHIWTCNK FRCGEKRLTRSLCACSDDCKDKGDCCINYSSVCQGEKSWVEEPCESINEPQCPAGFETPP TLLFSLDGFRAEYLHTWGGLLPVISKLKKCGTYTKNMRPVYPTKTFPNHYSIVTGLYPES HGIIDNKMYDPKMNASFSLKSKEKFNPEWYKGEPIWVTAKYQGLKSGTFFWPGSDVEING IFPDIYKMYNGSVPFEERILAVLQWLQLPKDERPHFYTLYLEEPDSSGHSYGPVSSEVIK ALQRVDGMVGMLMDGLKELNLHRCLNLILISDHGMEQGSCKKYIYLNKYLGDVKNIKVIY GPAARLRPSDVPDKYYSFNYEGIARNLSCREPNQHFKPYLKHFLPKRLHFAKSDRIEPLT FYLDPQWQLALNPSERKYCGSGFHGSDNVFSNMQALFVGYGPGFKHGIEADTFENIEVYN LMCDLLNLTPAPNNGTHGSLNHLLKNPVYTPKHPKEVHPLVQCPFTRNPRDNLGCSCNPS ILPIEDFQTQFNLTVAEEKIIKHETLPYGRPRVLQKENTICLLSQHQFMSGYSQDILMPL WTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFYKNNTKVSYGFLSPPQLNKNSSG IYSEALLTTNIVPMYQSFQVIWRYFHDTLLRKYAEERNGVNVVSGPVFDFDYDGRCDSLE NLRQKRRVIRNQEILIPTHFFIVLTSCKDTSQTPLHCENLDTLAFILPHRTDNSESCVHG KHDSSWVEELLMLHRARITDVEHITGLSFYQQRKEPVSDILKLKTHLPTFSQEDLIN**DKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVE VHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK**	ENPP1-linker-hIgG1 Fc construct
6	DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIE KTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK	Human IgG1 Fc

SEQ ID NO: 7 *(Mouse NPP1- NCBI accession NP_001295256.1)*

1 MERDGDQAGH GPRHGSAGNG RELESPAAAS LLAPMDLGEE PLEKAERARP AKDPNTYKVL

```
 61 SLVLSVCVLT TILGCIFGLK PSCAKEVKSC KGRCFERTFS NCRCDAACVS LGNCCLDFQE
121 TCVEPTHIWT CNKFRCGEKR LSRFVCSCAD DCKTHNDCCI NYSSVCQDKK SWVEETCESI
181 DTPECPAEFE SPPTLLFSLD GFRAEYLHTW GGLLPVISKL KNCGTYTKNM RPMYPTKTFP
241 NHYSIVTGLY PESHGIIDNK MYDPKMNASF SLKSKEKFNP LWYKGQPIWV TANHQEVKSG
301 TYFWPGSDVE IDGILPDIYK VYNGSVPFEE RILAVLEWLQ LPSHERPHFY TLYLEEPDSS
361 GHSHGPVSSE VIKALQKVDR LVGMLMDGLK DLGLDKCLNL ILISDHGMEQ GSCKKYVYLN
421 KYLGDVNNVK VVYGPAARLR PTDVPETYYS FNYEALAKNL SCREPNQHFR PYLKPFLPKR
481 LHFAKSDRIE PLTFYLDPQW QLALNPSERK YCGSGFHGSD NLFSNMQALF IGYGPAFKHG
541 AEVDSFENIE VYNLMCDLLG LIPAPNNGSH GSLNHLLKKP IYNPSHPKEE GFLSQCPIKS
601 TSNDLGCTCD PWIVPIKDFE KQLNLTTEDV DDIYHMTVPY GRPRILLKQH RVCLLQQQQF
661 LTGYSLDLLM PLWASYTFLS NDQFSRDDFS NCLYQDLRIP LSPVHKCSYY KSNSKLSYGF
721 LTPPRLNRVS NHIYSEALLT SNIVPMYQSF QVIWHYLHDT LLQRYAHERN GINVVSGPVF
781 DFDYDGRYDS LEILKQNSRV IRSQEILIPT HFFIVLTSCK QLSETPLECS ALESSAYILP
841 HRPDNIESCT HGKRESSWVE ELLTLHRARV TDVELITGLS FYQDRQESVS ELLRLKTHLP
901 IFSQED
```

SEQ ID NO: 8 *(Cow NPP1- NCBI accession NP_001193141.1)*

```
  1 MERDSCAGGG SRGGEGGRGP REGLAGNGRD PGPGRAAEAS GEPQAAASLL APMDLGEEPL
 61 ERAARARPAK DPNTYKVLSL VLSVCVLTTI LGCIFGLKPS CAKEIKSCKG RCFERTFGNC
121 RCDAACVDLG NCCLDYQETC IEPERIWTCT KFRCGEKRLS RSLCSCSDDC KDKGDCCINH
181 GSVCRGEKSW AEEECDSIDE PQCPAGFETP PTLLFSLDGF RAEYLHTWGG LLPVISKLKT
241 CGTYTKNMRP VYPTKTFPNH YSIVTGLYPE SHGIIDNNIY DPQMNANFAL KNKEKFNPEW
301 YKGEPIWLTA KYQGLKTGTF FWPGSDVKIN GIFPDIYKIY NVSVPFEERI LAILKWLQLP
361 KDERPHFYTL YLEEPDSSGH SYGPVSSEVI RALQRVDNMV GMLMDGLKEL NLHRCLNLIL
421 ISDHGMEQGS CKKYVYLNKY LGDTKDYKVV YGPAARLRPS DVPDKYYSFD YEGIAKNLSC
481 QEPNQHFKPY LKHFLPKRLH FAKNDRIERL TFYLDPQWQL ALNPSERKYC GGGFHGSDNT
541 FLNMQALFIG YGPGFKHSTE VDSFENIEVY NLMCDLLNLT PAPNNGTHGS LNHLLSNPVY
601 TPKHPKEVRP LVQCPFTRAP RESLDCSCDP SILPIVDFQT QLNLTMAEEK TIKRGALPYG
661 RPRVLQNSTV CLLYQHQFVS GYSRDILMPL WTSYTIGRND SFSTEDFSNC LYQDLRIPLS
721 PVHKCSFYKN NAKLSYGLLS PPQLHKGSSQ VYSEALLTTN IVPMYQSFQV IWHYLHGTLL
781 QRYAEERNGL NVVSGPVFDS DYDGRYDSLE TLKQNSKIIR NLEVLIPTHF FLVLTSCKNT
841 SQTPLQCENL DAMAFILPHK TDNSESCAHG KHESLWVEEL LKLHTARITD VEHITGLSFY
901 QERKEPISDI LKLKTHLPTF NQED
```

SEQ ID NO: 9 *(Rabbit NPP1- NCBI accession NP_001162404.1)*

```
  1 MERDGCAGGG SRGGEGGRAP REGPAGNSRD PGRSHAAEAP GNPQAAASLL APMDVGEEPL
 61 EKAARARTAK DPNTYKVLSL VLSVCVLTTI LGCIFGLKPS CAKEVKSCKG RCFERTFGNC
121 RCDAACVELG NCCLDYQETC IEPEHIWTCN KFRCGEKRLT RSLCACSDDC KDQGDCCINY
181 SSVCQGEKSW VEEPCESINE PQCPAGFETP PTLLFSLDGF RAEYLHTWGG LLPVISKLKK
```

241 CGTYTKNMRP VYPTKTFPNH YSIVTGLYPE SHGIIDNKMY DPKMNASFSL KSKEKFNPEW
301 YKGEPIWVTA KYQGLKSGTF FWPGSDVEIN GIFPDIYKMY NGSVPFEERI LAVLQWLQLP
361 KDERPHFYTL YLEEPDSSGH SYGPVSSEVI KALQRVDNMV GMLMDGLKEL NLHRCLNLIL
421 VSDHGMEQGS CKKYIYLNKY LGDVKNIKVI YGPAARLRPS DVPDKYYSFN YEGIARNLSC
481 REPNQHFKPY LKHFLPKRLH FAKSDRIEPL TFYLDPQWQL ALNPSERKYC GSGFHGSDNI
541 FSNMQALFVG YGPGFKHGIE VDTFENIEVY NLMCDLLNLT PAPNNGTHGS LNHLLKNPVY
601 TPKHPKEVHP LIQCPFTRNP RDNLGCSCNP SILPIEDFQT QFNLTVAEEK NIKHETLPYG
661 RPRVLQKKNT ICLLSQHQFM SGYSQDILMP LWTSYTVDRN DSFSTEDFSN CLYQDFRISL
721 SPVHKCSFYK NNTKVSYGFL SPPQLNKNSR GIYSEALLTT NIVPMYQSFQ VIWRYFHDTL
781 LRKYAEERNG VNVVSGPVFD FDYDGRYDSL EILRQKRRVI RNQEILIPTH FFIVLTSCKD
841 ASQTPLHCEN LDTLAFILPH RTDNSESCLH GKHESSWVEE LLMLHRARIT DVEHITGLSF
901 YQQRKEPVSD ILKLKTHLPT FSQED

SEQ ID NO: 10 *(Baboon NPP1- NCBI accession NP_001076211.2)*

1 MERDGCAGGG SQGGGKGGRG PREGLAGNGR DPSHGQASEA PGDPQAAASL LAPMDLGEEP
61 LEKAAGARPA KDPNTYKVLS LVLSVCVLTT ILGCIFGLKP SCAKEVKSCK GRCFERTFGN
121 CRCDVACVDL GNCCLDYQET CIEPERIWTC NKFRCGEKRL SRSLCACSDD CKERGDCCIN
181 YSAVCQGEKS WVEETCENIN EPQCPEGFEM PPTLLFSLDG FRAEYLHTWG GLLPVISKLK
241 KCGTYAKNMR PVYPTKTFPN HYSIVTGLYP ESHGIIDNKM YDPKMNASFS LKSKEKFNPE
301 WYKGEPIWLT AKYQGLRSGT FFWPGSDVKI NGIFPDIYKI YNGSVPFEER ILAILKWLRL
361 PKDERPHFYT LYLEEPDSSG HSYGPVSSEV IKALQRVDNM VGMLMDGLKE LNLHQCLNLI
421 LISDHGMEQG SCKKYIYLNK YLGDTKNIKV IYGPAARLRP SDVPEKYYSF NYENIARNLS
481 CREPNQHFKP YLKHFLPKRL HFAKSDRIEP LTFYLDPQWQ LALSPSERKY CGSGFHGSDN
541 VFSNMQALFV GYGPGFQHGI EVDSFENIEV YNLMCDLLNL TPAPNNGTHG SLNHLLKNPI
601 YTPKHPKEVQ PSVQCPLAGS PRDSLGCSCN PSILPIVDFQ TQFNLTTAEE KNINRASLPY
661 GRPRLLQKKS SVCLLYQHQF VSGYSHDVLM PLWTSYTVNR NDSFSTEDFS NCLYQDLRIS
721 FSPIHNCSFY KNNAKLSYGF LSPPQLSKDS SQIYSEALLT SNIVPMYQSF QVIWRYFHDT
781 LLQRYAEERN SINVVSGPVF DSDYDGRYDS SEALKRNRRV IRNQEILIPT HFFIVITSCK
841 NTSQTPLQCD NLDPLAFILP HRSDNSESCV HEKRESSWIE ELLMMHRARI MDVEHITGLS
901 FYQERKEPVS DILKLKTHLP TVSQED

17. Treatment Protocols

The following protocols may be used as guidance for ENPP1 enzyme replacement therapy at the recited dosages of the ENPP1 agent, as determined to be appropriate by the medical practitioner. Treatment protocols set forth herein rely on the use of abbreviated terms, whose full names are set forth in Table 2.

a. Prohibited Medication or Therapy**

A patient subject to treatment with an ENPP1 agent should discontinue use of oral phosphate and vitamin D3 or analogs prior to the first dose of an ENPP1 agent (e.g., at least 14 days prior to the first dose) and use is prohibited throughout the treatment period. Oral phosphate treatment may be tapered to avoid hypercalciuria. Vitamin D metabolites or analogs may be discontinued without titration. Oral bisphosphonates should be withdrawn 6 months prior to the first dose.

Additional prohibited medications throughout the Treatment Period are:

- bisphosphonates
- anti-FGF23 (e.g. burosumab)
- calcimimetics
- antacids
- systemic corticosteroids
- PTH suppressors

b. Treatment Frequency

An ENPP1 agent is administered at least once or twice bimonthly, at least once or twice monthly, three times monthly, at least once or twice weekly.

The first dose of an ENPP1 agent may be administered on Day 1. On Days 8 to 29 and thereafter, the ENPP1 agent is administered to a subject at a selected dose of ENPP1 agent mg/kg doses twice weekly. The dose may be administered at approximately the same time on each dosing day. The site of injection is alternated, with no site within 2 inches of any prior site of injection within the prior 2 weeks.

A selected dose of an ENPP1 agent of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg is administered SC over a time period determined by a medical practitioner skilled in the field of ENPP1 replacement therapy. The first dose of an ENPP1 agent may be administered on Day 1. After the first dose, a subject may be observed for 7 days to monitor safety and to collect PK samples. On Days 8 to 29, a subject receives a selected dose twice weekly. Administration of an ENPP1 agent at a selected dose is continued as considered appropriate by the medical professional.

A subject may receive 8 doses of an ENPP1 agent over the course of a 29 day period of time, resulting in an exposure of 1.6 mg, 4.8 mg, and 14.4 mg per 29 days, respectively, for dose amounts of 0.2 mg/kg, 0.6 mg/kg, and 1.8 mg/kg.

Like the endogenous ENPP1 enzyme, an ENPP1 agent cleaves ATP to generate AMP and PPi, thereby increasing plasma PPi levels and into AMP which CD73 coverts rapidly to adenosine. Replacement of the endogenous human enzyme is intended to correct the inherent deficiency and allow for improved health and mitigation of clinical complications associated with ENPP1 Deficiency.

c. Evaluation of Treatment

i. Pharmacokinetics

- An ENPP1 agent plasma concentration-time profiles and determination of noncompartmental PK parameters (including Tmax, Cmax, AUClast, AUCtau, AUCinf, T1/2, Cmin, Cl/F and V/F)

- Assess linearity between ascending ENPP1 agent SC doses and PK parameters.

ii. Pharmacodynamics

- Change from baseline for plasma PPi levels, serum phosphate, plasma intact FGF23 levels, TmP/GFR (adjusted to creatinine clearance)
- Assess linearity between ascending ENPP1 agent SC doses and PD parameters
- Correlate the changes in PPi with changes in FGF23
- Correlate the changes in PPi with changes in TmP/GFR
- Correlate the changes in FGF23 with changes in TmP/GFR
    - Exploration of presence and changes of blood and urine biomarkers
    - Plasma and urine creatinine (used to calculate the renal clearance of phosphate)
    - Serum 1,25(OH)2D, plasma ionized and total calcium, parathyroid hormone
    - Bone biomarkers: serum alkaline phosphatase (ALP), bone-specific ALP (BALP), carboxy terminal cross-linked telopeptide of type I collagen (CTx), and procollagen type 1 N-terminal propeptide (P1NP)

iii. Efficacy

In order to assess treatment efficacy, determination of one or more of the following physical parameters may be made prior to and during treatment.

- Baseline Skeletal
  - Bone density using DEXA
  - $Na^{18}F$-PET/HR-pQCT (or HR-CT)
- Baseline arterial and organ calcification
  - $Na^{18}F$-PET/HR-pQCT (or HR-CT)
  - Echocardiogram
- Baseline cardiovascular and peripheral vascular reactivity function
  - Stress Doppler echocardiography
  - ECG
  - Ankle-brachial Index
  - Pulse Wave Velocity
  - Peripheral arterial tonometry (PAT)
  - HRpQCT (or HR-CT) with and without contrast
- Baseline neurological function
  - NIH Stroke Score
  - Neurological exam
- Baseline pulmonary function
  - Standard pulmonary function test
- Baseline performance outcomes
  - 2MWT, 6MWT
  - Handheld dynamometry, grip strength, Range of Motion
  - - Hearing tests: Physical exam and otoscopy, immittance audiometry (tympanometry), Pure Tone Audiometry (PTA), High Frequency Audiometry (HFA)
- Baseline patient, clinician, and caregiver outcomes
  - Patient and Physician Global Impression of Change (CGI-C and CGI-S)
  - Gross Motor Function Classification System - Expanded and Revised
  - PROMIS Pain Interference and pain intensity
    - PROMIS Fatigue, mobility, cognitive impact, upper extremity

- Western Ontario and McMaster University Osteoarthritis Index (WOMAC) Stiffness Score

- Baseline renal calcification as measured by renal ultrasound
- Baseline bone histomorphometry using bone biopsy (optional)
- Baseline optical coherence tomography of aorta, coronary arteries, carotid arteries, and renal arteries and vascular beds.

iv. Safety and Immunogenicity

Safety assessments may be summarized at Baseline and at each observed time point. Safety variables include:

- Incidence, frequency, and severity of adverse events (AEs), treatment-related AEs, and serious adverse events (SAEs)
- Vital signs and weight
- Physical examinations
- Estimated glomerular filtration rate (eGFR)
- Laboratory tests including chemistry, hematology, and urinalysis, including additional biochemical parameters of interest
- Anti-ENPP1-FC antibody testing and dose-limiting toxicities (DLTs)
- Incidence of any anti-drug antibodies (ADA)
- Incidence of TEAEs associated with hypersensitivity reactions
- Concomitant medications
- Electrocardiogram (ECG)

Table 2

2MWT	2-minute walk test
6MWT	6-minute walk test
ADA	anti-drug antibodies
AE	adverse event
ALP	alkaline phosphatase
AMP	adenosine monophosphate
ARHR2	autosomal recessive hypophosphatemic rickets type 2
ATP	adenosine triphosphate
AUC0-t	Area under the plasma concentration-time curve from time zero to the time of last measurable concentration

AUCinf	Area under the plasma concentration-time curve from time zero to infinity
AUCtau	Area under the concentration-time curve over the dosing interval
BALP	bone-specific alkaline phosphatase
CaGI	Caregiver Global Impression
CGI-C	Clinician Global Impression of Change
CL/F	clearance after extravascular administration of drug
Cmax	Maximum plasma concentration
CTx	carboxy terminal cross-linked telopeptide of type I collagen
DSMB	Data Safety Monitoring Board
ECG	Electrocardiogram
eCRF	electronic case report form
ENPP1	ectonucleotide pyrophosphatase/phosphodiesterase 1
EudraCT	European Union Drug Regulating Authorities Clinical Trials
ERT	enzyme replacement therapy
FDA	Food and Drug Administration
FGF23	fibroblast growth factor 23
FIH	first in human
FIP	first in patient
GACI	Generalized Arterial Calcification of Infancy
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMFCS-E and R	Gross Motor Function Classification System - Expanded and Revised
HRqCT	high-resolution quantitative computed tomography
IB	Investigator Brochure
ICF	Informed Consent Form
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICH E6	ICH Harmonised Tripartite Guideline: Guideline for Good Clinical Practice E6
IEC	institutional ethics committee
IND	Investigational New Drug (application)
INZ-701	recombinant human ectonucleotide pyrophosphatase/phosphodiesterase 1 fused to the Fc fragment of IgG1
IRB	Institutional Review Board
MAD	multiple ascending dose
MTD	maximum tolerated dose
NOAEL	no observed adverse effect level

PD	pharmacodynamic(s)
PK	pharmacokinetic(s)
PPi	inorganic pyrophosphate
PROMIS	Patient Reported Outcomes Measurement Information System
PTH	parathyroid hormone
SAE	serious adverse event
SAP	Statistical Analysis Plan
SC	Subcutaneous
T1/2	elimination half-life
Tmax	time to maximum plasma concentration
TEAE	treatment-emergent adverse events
TmP/GFR	tubular maximum reabsorption rate of phosphate adjusted for glomerular filtration rate
V/F	apparent volume of distribution after extravascular administration of drug
WOCBP	women of child-bearing potential
WOMAC	Western Ontario and McMaster University Osteoarthritis Index

EXEMPLIFICATION

The invention now being generally described, it will be more readily understood by reference to the following examples, which are included merely for purposes of illustration of certain embodiments and embodiments of the present invention and are not intended to limit the invention.

Example 1. Generation of ENPP1 fusion proteins

One example of an ENPP1 fusion protein is ENPP1-Fc. However, the exemplification of ENPP1-Fc can be applied to other ENNPP1 fusion proteins as set forth herein.

ENPP1-Fc is a recombinant fusion protein that contains the extracellular domains of human ENPP1 (soluble ENPP1) coupled with an Fc fragment of IgG1 (rhENPP1-Fc). The recombinant extracellular domains of ENPP1-Fc contain its catalytic activity and are identical to the native ENPP1 enzyme. ENPP1-Fc is a recombinant human protein produced in CHO cells via a fed batch cell culture process that is free of animal-derived components. The molecular weight of the ENPP1-Fc dimer is approximately 290 kDa; ENPP1-Fc is highly

glycosylated and has a pI of approximately 6.0. Like endogenous ENPP1, the primary substrate for ENPP1-Fc is ATP, which is cleaved to AMP and PPi.

In a specific embodiment, soluble ENPP1 protein was fused to a human Fc domain with a linker via a linker (comprising a leucine, isoleucine, and asparagine). Three ENPP1-Fc constructs are shown in Table 1 as SEQ ID NOs: 3, 4, and 5 as purified from CHO cells.

Purification of ENPP1-Fc could be achieved by a series of column chromatography steps, including, for example, three or more of the following, in any order: protein A chromatography, Q sepharose chromatography, phenylsepharose chromatography, size exclusion chromatography, and cation exchange chromatography. The purification could be completed with viral filtration and buffer exchange. Following purification of the protein, the catalytic activity of the ENPP1-Fc protein could be evaluated using pNP-TMP as a chromogenic substrate.

Example 2: Treatment

ENPP1-FC is administered at one of the following selected doses: 0.2 mg/kg, 0.6 mg/kg, and 1.8 mg/kg. Administration is subcutaneous (SC) at least once or twice bimonthly, at least once or twice monthly, three times monthly, at least once or twice weekly.

The first dose of ENPP1-FC may be administered on Day 1. On Days 8 and thereafter, ENPP1-Fc is administered to a subject at a selected dose of ENPP1 agent mg/kg doses twice weekly. The dose may be administered at approximately the same time on each dosing day. The site of injection is alternated, with no site within 2 inches of any prior site of injection within the prior 2 weeks.

A selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC. The first dose of ENPP1-FC may be administered on Day 1. After the first dose, a subject may be observed for 7 days to monitor safety and to collect PK samples. On Days 8 and thereafter, a subject receives a selected dose twice weekly. Administration of ENPP1-Fc at a selected dose is continued as considered appropriate by the medical professional.

A subject may receive 8 doses of ENPP1-FC over the course of a 29 day period of time, for example, resulting in an exposure of 1.6 mg, 4.8 mg, and 14.4 mg per 29 days,

respectively, for dose amounts of 0.2 mg/kg, 0.6 mg/kg, and 1.8 mg/kg. Or a subject may receive more or less than 8 doses, as considered appropriate by a medical profession.

Like the endogenous ENPP1 enzyme, ENPP1-FC cleaves ATP to generate AMP and PPi, thereby increasing plasma PPi levels and into AMP which CD73 coverts rapidly to adenosine. Replacement of the endogenous human enzyme is intended to correct the inherent deficiency and allow for improved health and mitigation of clinical complications associated with ENPP1 Baseline patient, clinician, and caregiver outcomes.

Example 3: Treatment of a Patient Having an ENPP1 Deficiency

Enpp1-Fc is administered to a patient identified as having an ENPP1 deficiency by subcutaneous injection on Day 1 and twice weekly starting on Day 8 using a select dose as follows.

1	0.2 mg/kg
2	0.6 mg/kg
3	1.8 mg/kg

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. The Patient's response to enzyme replacement is monitored as appropriate, as determined by the medical professional, e.g., by following a reduction in one or more symptoms of ENPP1 deficiency, and/or using guidance provided herein.

Example 4: Treatment of a Patient Diagnosed with GACI

Enpp1 deficiency may mask as GACI. GACI is a rare disease occurring in infants and involving extensive arterial calcification (Albright, et al., 2015, Nature Comm. 10006).

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. GACI Patient response to enzyme replacement is monitored as appropriate, as determined by the medical professional, e.g., by following a reduction in one or more symptoms of GACI, and/or using guidance provided herein.

Example 5: Treatment of Patient Diagnosed with ARHR2

Enpp1 deficiency may mask as ARHR2. ARHR2 is a rare skeletal disorder characterized by low levels of plasma PPi and serum phosphate which can result in rickets, repeated fractures of the long bones, rachitic skeletal deformities and impaired growth and development (Ferreira et al 2014, Moran 1975, Rutsch et al 2008).

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. ARHR2 Patient response to enzyme replacement is monitored as appropriate, as determined by the medical professional, e.g., by following a reduction in one or more symptoms of ARHR2, using guidance provided herein.

The following examples, in the context of the entire specification, provide guidance to determine treatment protocols and efficacy.

Example 6: Treatment of a Patient Having an ABCC6 Deficiency

Enpp1-Fc is administered to a patient identified as having an ABCC6 deficiency by subcutaneous injection on Day 1 and twice weekly starting on Day 8 using a select dose as follows.

1	0.2 mg/kg
2	0.6 mg/kg
3	1.8 mg/kg

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. The Patient's response to enzyme replacement is monitored as appropriate, as determined by the medical professional, e.g., by following a reduction in one or more symptoms of ABCC6 deficiency, and/or using guidance provided herein.

Example 7: Treatment of Patients (female and male) Diagnosed with PXE having an ABCC6 deficiency

A Phase 1/2, Open-Label, Multiple Ascending Dose Study is performed in order to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of INZ-701. This is followed by an Open-Label Long-Term Extension Period in Adults With ABCC6 Deficiency Manifesting as Pseudoxanthoma Elasticum (PXE).

Arm	**Intervention/treatment**
Experimental: INZ-701 The study design is a MAD 3+3 with 3 dose cohorts. The planned doses will be 0.2 mg/kg, 0.6 mg/kg, and 1.8 mg/kg administered via subcutaneous injection twice weekly.	Drug: INZ-701 Recombinant fusion protein that contains the extracellular domains of human ENPP1 coupled with an Fc fragment from an immunoglobulin gamma-1 (IgG1) antibody (rhENPP1-Fc)

Outcome Measures

Measures of Primary and Secondary Outcomes are as follows.

Primary Outcome Measure:

1. Number of Treatment Emergent Adverse Events (TEAEs) [Time Frame: 32 days (Dose Evaluation Period)]

Treatment-emergent AEs are defined as any AE occurring from the first dose of INZ-701 through 30 days after the last dose of INZ-701.

2. Number of Treatment Emergent Adverse Events (TEAEs) [Time Frame: 52 weeks (Day 1 through Safety Follow-up Visit)]

Treatment-emergent AEs are defined as any AE occurring from the first dose of INZ-701 through 30 days after the last dose of INZ-701.

Secondary Outcome Measures:

1. Incidence of Anti-Drug Antibodies (ADAs) [Time Frame: 32 days (Dose Evaluation Period)]

The presence of ADAs will be assessed and, if present, further evaluation will determine specificity and subtypes.

2. Incidence of Anti-Drug Antibodies (ADAs) [Time Frame: 52 weeks (Day 1 through Safety Follow-up Visit)]

The presence of ADAs will be assessed and, if present, further evaluation will determine specificity and subtypes.

3. Area under the Plasma Concentration versus Time Curve (AUC) of INZ-701 [Time Frame: 32 days (Dose Evaluation Period)]

For each subject, variation of concentration of INZ-701 in the plasma will be measured via a series of blood samples obtained throughout the study, comparing the subject's baseline value over time.

4. Maximum Plasma Concentration (Cmax) of INZ-701 [Time Frame: 32 days (Dose Evaluation Period)]

For each subject, the maximum concentration of INZ-701 in the plasma will be measured via a series of blood samples obtained throughout the study, comparing the subject's baseline value over time.

5. Systemic Clearance of INZ-701 [Time Frame: 32 days (Dose Evaluation Period)]

For each subject, clearance of INZ-701 from the body will be measured via a series of blood samples obtained throughout the study, comparing the subject's baseline value over time.

6. Change from Baseline in Plasma Inorganic Pyrophosphate (PPi) Levels [Time Frame: 32 days (Dose Evaluation Period)]

For each subject, plasma PPi will be measured via a series of blood samples obtained throughout the study, comparing the subject's baseline value over time.

7. Change from Baseline in Plasma Inorganic Pyrophosphate (PPi) Levels [Time Frame: 52 weeks (Baseline through Safety Follow-up Visit)].

For each subject, plasma PPi will be measured via a series of blood samples obtained throughout the study, comparing the subject's baseline value over time.

Eligibility Criteria

Ages Eligible for Study: 18 Years to 64 Years

Sexes Eligible for Study: All

Gender Based: No

Accepts Healthy Volunteers: No

Criteria for Inclusion and Exclusion

Inclusion and Exclusion Criteria are as follows.

Inclusion Criteria:

1. Must provide written or electronic consent after the nature of the study has been explained, and prior to any research-related procedures, per ICH GCP

2. Clinical diagnosis of PXE supported by prior genetic identification of biallelic ABCC6 mutations

3. Male or female, 18 to <65 years of age at Screening

4. Plasma PPi < 1300nM at Screening

5. Subjects who are being treated with statins or proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors must be on stable doses for 3 years prior to enrollment through end of study unless the investigator deems, in consultation with the Sponsor, that the change will not confound interpretation of the study data

6. Women of child-bearing potential (WOCBP) must have a negative serum pregnancy test at Screening

7. WOCBP and partners of fertile males who are WOCBP must agree to use 1 highly effective form of contraception and a barrier method from at least 1 month before the first dose of INZ-701 through 30 days after last dose of INZ-701 (greater than 5 half-lives of INZ-701). WOCBP and partners of fertile males who are WOCBP must also agree to not donate ova from the period following the first dose of INZ-701 through 30 days after last dose of INZ-701.

8. Males who are sexually active must agree to use condoms from the period following first dose of INZ-701 through 30 days after the last dose of INZ-701. Males must also agree to not donate sperm from the period following the first dose of INZ-701 through 30 days after last dose of INZ-701.

9. In the opinion of the Investigator, must be willing and able to complete all aspects of the study

10. Agree to provide access to relevant medical records

<u>Exclusion Criteria:</u>

1. In the opinion of the Investigator, presence of any clinically significant disease (outside of those considered associated with the diagnosis of ABCC6 Deficiency) that precludes study participation or may confound interpretation of study results, including known uncontrolled thyroid disease or unrelated connective tissue, bone, mineral, ophthalmologic, or muscle disease

2. Advanced eye disease requiring anti-VEGF treatment at Screening

3. Clinically significant abnormal laboratory result at Screening.

4. Screening laboratory results demonstrating elevations of aspartate aminotransferase, alanine aminotransferase, bilirubin (unless due to Gilbert disorder), eGFR >60, 25-hydroxyvitamin D (25[OH]D) levels <20 ng/mL, parathyroid hormone (PTH) >40% above the upper limit of normal, or significant hyper- or hypocalcemia. Note: Rescreening for certain assessments is permitted as described in the protocol.

5. Known active fungal, bacterial, and/or viral infection including human immunodeficiency virus, hepatitis B virus, hepatitis C virus, or COVID-19 virus. A negative COVID-19 test result is required within 5 days prior to first dose of INZ-701.

6. Malignancy within the last 5 years, except non-melanoma skin cancers or cervical carcinoma in situ.

7. Known intolerance to INZ-701 or any of its excipients.

8. Unable or unwilling to discontinue the use of any prohibited medication (examples include bisphosphonates, calcimimetics, antacids, systemic corticosteroids, pyrophosphate containing medications) as provided in the protocol. Discontinuation should be undertaken only if considered not detrimental and indicated by the subject's treating physician.

9. Receipt of any other investigational new drug within 5 half-lives of the last dose of the other investigational product or from 4 weeks prior to the first dose of INZ-701, whichever is longer, or use of an investigational device, through completion of participation in the study

10. Last symptoms from a COVID-19 vaccination within 14 days prior to the first dose of INZ-701 or as described in Inozyme COVID-19 Vaccine Guidance Document.

11. Subjects who are pregnant, trying to become pregnant, or breastfeeding.

12. Subjects who are trying to father a child.

Example 8: Treatment of a Patient Diagnosed with GACI having ABCC6 deficiency

ABCC6 deficiency may mask as GACI. GACI is a rare disease occurring in infants and involving extensive arterial calcification (Albright, et al., 2015, Nature Comm. 10006). GACI is believed to occur due to a defective ENPP1 protein. Surprisingly, in some instances, GACI phenotype is observed even when the subject has ABCC6 deficiency. Patients with ABCC6 deficiency can be identified by using isolated DNA samples using protocols described in *J Med Genet. 2007 Oct; 44(10): 621–628.* (*Mutation detection in the ABCC6 gene and genotype–phenotype analysis in a large international case series affected by pseudoxanthoma elasticum, Ellen G Pfendner, et al*)

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. GACI Patient (having ABCC6 deficiency) response to enzyme replacement is monitored as appropriate, as determined by the medical professional, e.g., by following a reduction in one or more symptoms of GACI, and/or using guidance provided herein.

Example 9: Treatment of Patient Diagnosed with ARHR2 having ABCC6 deficiency

ABCC6 deficiency may mask as symptoms of ARHR2. ARHR2 is a rare skeletal disorder characterized by low levels of plasma PPi and serum phosphate which can result in rickets, repeated fractures of the long bones, rachitic skeletal deformities and impaired growth and development (Ferreira et al 2014, Moran 1975, Rutsch et al 2008). In some subjects, ARHR2 phenotype is observed when the subject has ABCC6 deficiency. Patients with ABCC6 deficiency can be identified by following the procedure in Example 4.

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. ARHR2 Patient response to enzyme replacement is monitored as appropriate, as determined by the medical professional, e.g., by following a reduction in one or more symptoms of ARHR2, using guidance provided herein.

The following examples, in the context of the entire specification, provide guidance to determine treatment protocols and efficacy.

Example 10: Treatment of Patient Diagnosed with Chronic kidney disease (CKD)

Pathological calcification can manifest as another disease state referred as CKD. Clinical symptoms of chronic kidney diseases include itching, muscle cramps, nausea, lack of appetite, swelling of feet and ankles, sleeplessness and labored breathing. Chronic kidney disease if left untreated tends to progress into End stage renal disease (ESRD). (*Chronic Kidney Disease Diagnosis and Management: A Review, Chen TK, Knicely DH, Grams ME. Chronic Kidney Disease Diagnosis and Management: A Review. JAMA. 2019 Oct 1;322(13):1294-1304.*)

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. CKD Patient response to enzyme replacement is monitored as appropriate, as

determined by the medical professional, e.g., by following a reduction in one or more symptoms of CKD, using guidance provided herein.

The following examples, in the context of the entire specification, provide guidance to determine treatment protocols and efficacy.

Example 11: Treatment of Patient Diagnosed with Ossification of posterior longitudinal ligament (OPLL)

Pathological calcification can manifest as another disease state referred as OPLL. Clinical symptoms and signs caused by OPLL are categorized as: (1) myelopathy, or a spinal cord lesion with motor and sensory disturbance of the upper and lower limbs, spasticity, and bladder dysfunction; (2) cervical radiculopathy, with pain and sensory disturbance of the upper limbs; and (3) axial discomfort, with pain and stiffness around the neck. The most common symptoms in the early stages of OPLL include dysesthesia and tingling sensation in hands, and clumsiness. (*Wu JC, Chen YC, Huang WC. Ossification of the Posterior Longitudinal Ligament in Cervical Spine: Prevalence, Management, and Prognosis. Neurospine. 2018 Mar;15(1):33-41.*)

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. OPLL Patient response to enzyme replacement is monitored as appropriate, as determined by the medical professional, e.g., by following a reduction in one or more symptoms of OPLL, using guidance provided herein.

The following examples, in the context of the entire specification, provide guidance to determine treatment protocols and efficacy.

Example 12: Treatment of Patient Diagnosed with Hereditary Hypophosphatemic Rickets

Pathological calcification can manifest as another disease state referred as Hereditary Hypophosphatemic Rickets (HHR). Clinical symptoms and signs caused by HHR are categorized as: (1) myelopathy, or a spinal cord lesion with motor and sensory disturbance of the upper and lower limbs, spasticity, and bladder dysfunction; (2) cervical radiculopathy, with pain and sensory disturbance of the upper limbs; and (3) axial discomfort, with pain and stiffness around the neck. The most common symptoms of HHR include premature fusion of the skull bones (craniosynostosis) and dental abnormalities. The disorder may also cause

abnormal bone growth where ligaments and tendons attach to joints (enthesopathy). In adults, hypophosphatemia is characterized by a softening of the bones known as osteomalacia. (*Cho HY, Lee BH, Kang JH, Ha IS, Cheong HI, Choi Y. A clinical and molecular genetic study of hypophosphatemic rickets in children. Pediatr Res. 2005 Aug;58(2):329-33.*)

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. HHR Patient response to enzyme replacement is monitored as appropriate, as determined by the medical professional, e.g., by following a reduction in one or more symptoms of HHR, using guidance provided herein.

The following examples, in the context of the entire specification, provide guidance to determine treatment protocols and efficacy.

Example 13: Treatment of Patient Diagnosed with X-linked hypophosphatemia (XLH)

Pathological calcification can manifest as another disease state referred as X-linked hypophosphatemia (XLH). X-linked dominant hypophosphatemic rickets, or X-linked Vitamin D-resistant rickets, is an X-linked dominant form of rickets (or osteomalacia) that differs from most cases of rickets in that vitamin D supplementation does not cure it. It can cause bone deformity including short stature and genu varum (bow leggedness). It is associated with a mutation in the PHEX gene sequence (Xp.22) and subsequent inactivity of the PHEX protein. (*Carpenter TO, Imel EA, Holm IA, Jan de Beur SM, Insogna KL. A clinician's guide to X-linked hypophosphatemia. J Bone Miner Res. 2011 Jul;26(7):1381-8. doi: 10.1002/jbmr.340. Epub 2011 May 2. Erratum in: J Bone Miner Res. 2015 Feb;30(2):394*)

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. XLH Patient response to enzyme replacement is monitored as appropriate, as determined by the medical professional, e.g., by following a reduction in one or more symptoms of XLH, using guidance provided herein.

The following examples, in the context of the entire specification, provide guidance to determine treatment protocols and efficacy.

Example 14: Treatment of Patient Diagnosed with Autosomal Dominant Hypophosphatemic Rickets (ADHR)

Pathological calcification can manifest as another disease state referred as Autosomal Dominant Hypophosphatemic Rickets (ADHR). Clinical symptoms and signs caused by ADHR poorly formed bones (rickets), bone pain, and tooth abscesses. ADHR is caused by a mutation in the fibroblast growth factor 23 (FGF23). ADHR is characterized by impaired mineralization of bone, rickets and/or osteomalacia, suppressed levels of calcitriol (1, 25-dihydroxyvitamin D3), renal phosphate wasting, and low serum phosphate. Mutations in FGF23 render the protein more stable and uncleavable by proteases resulting in enhanced bioactivity of FGF23. The enhanced activity of FGF23 mutants reduce expression of sodium-phosphate co-transporters, NPT2a and NPT2c, on the apical surface of proximal renal tubule cells, resulting in renal phosphate wasting. (*Rowe PS. The wrickkened pathways of FGF23, MEPE and PHEX. Crit Rev Oral Biol Med. 2004 Sep 1;15(5):264-81.*)

ENPP1-Fc is administered at a selected dose of ENPP1-FC is one of 0.2 mg/kg, 0.6 mg/kg, or 1.8 mg/kg SC at least twice weekly for a period of time determined by the medical professional. ADHR Patient response to enzyme replacement is monitored as appropriate, as determined by the medical professional, e.g., by following a reduction in one or more symptoms of ADHR, using guidance provided herein.

The following examples, in the context of the entire specification, provide guidance to determine treatment protocols and efficacy.

Example 15: Measurement of Plasma Inorganic Pyrophosphate

Low plasma PPi levels are a characteristic of ENPP1 Deficiency and are used as an indicator of treatment efficacy. ENPP1-Fc specifically cleaves ATP to generate AMP and PPi. The therapeutic goal of ENPP1 ERT is to normalize extracellular PPi levels and correct clinical abnormalities associated with ENPP1 Deficiency.

PPi is measured by obtaining patient plasma samples. Determined PPi data may be used to adjust dose levels. PPi levels may also serve as the primary PD marker for PK/PD analysis.

The concentration of Pi and PPi in mammals is 1-3 mM and 2-3 μM respectively.

Example 16: Biomarkers Associated with Bone Health

In addition to low plasma PPi, ENPP1 deficient patients are characterized biochemically by low serum phosphate, high urine phosphate, low renal TmP/GFR, normal calcium (Ca), low-normal urine Ca, normal 25-hydroxy Vitamin D (25 OH D), low-normal 1,25(OH)2D, high BAP, high intact FGF23, and normal PTH (IOF 2019).

Biomarkers that may be used as additional determinants of bone health of a treated patient are set forth in Table 3.

Table 3: Clinical Intermediates and Biomarkers

	Laboratory	Sample Type
Primary Pharmacodynamic Markers	Pyrophosphate (PPi)	plasma
	Inorganic phosphate	serum
	FGF23 (intact)	Plasma
	TmP/GFR	serum creatinine, serum phosphate, urine phosphate
	ALP, BALP, CTx, P1NP	serum, plasma, urine

Example 17: Efficacy of Treatment with ENPP1-Fc

Treatment efficacy may be assessed by measuring plasma PPi as well as measuring other plasma analytes, such as FGF23, Pi, FGF23, Pi, TmP/GFR, serum alkaline phosphatase (ALP), bone-specific ALP (BALP), carboxy terminal cross-linked telopeptide of type I collagen (CTx), and procollagen type 1 N-terminal propeptide (P1NP). These analyte measurements may be used as a PD markers associated with ENPP1 Deficiency to determine the efficacy for ENPP1-FC. Changes in these analytes may be described as changes from baseline and in a time-dependent manner over the course of treatment. Dose linearity of PK and PD parameters also may be assessed.

Changes from baseline in plasma PPi levels, FGF23 levels and Urinary phosphorus excretion per creatinine clearance may be analyzed using a t test of paired differences to test the null hypothesis that

Example 18: Drug Concentration Measurements

In addition, blood samples may be obtained from a patient for measurement of ENPP1-FC concentration in plasma and subsequent determination of PK parameters following the first dose (i.e. single dose) and at/after multiple doses (i.e. steady-state).

Example 19: Immunogenicity (Anti-drug Antibodies)

If desired, immunogenicity to ENPP1-Fc may be measured using anti-drug antibodies (ADA). Immunogenicity testing can utilize a multi-tiered approach; if ADA are detected in the initial screen, a confirmatory test may be run to determine specificity. Samples may also be used to assess and further establish assays for specificity confirmation (i.e. titer) and neutralizing antibodies.

Example 20: Pharmacokinetic, Pharmacodynamic, and Exploratory Biomarker Analyses

Pharmacokinetic analysis may be performed on the PK population, and PK parameters of ENPP1-FC may be summarized by treatment with descriptive statistics. Dose linearity of PK and PD parameters may also be assessed. PK/PD analyses, immunogenicity analyses; and exploratory biomarker analyses may be determined.

Example 21: Additional Determinators of Efficacy

Although restoring a normal level of PPi is the primary indicator of efficacy of treatment using NEPP1-Fc, other physical measurements also may be used, if desired to assist in determining treatment efficacy. These include one or more of the following.

1. Radiography and Imaging

X-Rays for Skeletal Severity. Standard X-rays may be obtained to detect rachitic skeletal deformities. Obtain X-rays may be obtained, for example, on the wrists and knees.

DEXA Scan. DEXA scans may be used to evaluate changes in bone density.

Positron Emission Tomography – Computed Tomography. Baseline $Na^{18}F$-PET/HRpQCT (or HR-CT) may be a full body scan done within 1 month of first dose of ENPP1-FC to measure calcification of arteries and organs and skeletal abnormalities at

baseline and for future interventional assessments. The $Na^{18}F$-PET measures bone turnover as well as microcalcification of the arteries. High-resolution quantitative computed tomography (HRQCT) or HR-CT can determine bone microstructure at the non-dominant distal radius and tibia. Standard bone geometric parameters are calculated.

Doppler Echocardiogram. A baseline echocardiogram may be obtained within 3 days prior to a first dose of ENPP1-FC. Doppler echo may be used to measure heart function [LVEF, blood flow] calcification of heart and valves, and arterial stiffness.

Optical Coherence Tomography. Optical coherence tomography may be used to visualize neointimal proliferation.

Peripheral Arterial Tonometry. Peripheral arterial tonometry (PAT) may be used to assess digital pulse wave amplitude (PWA), which corresponds to digital volume variation.

Renal Ultrasound. Renal ultrasound may be used, for example, within 1 week of starting ENPP1-FC, to measure renal calcification.

Bone Histomorphology and Bone Biopsy. Bone biopsy may be performed as a baseline measurement. Tetracycline loading for 10 days prior to bone biopsy is preferred.

2. Walking Ability. Walk tests may be used as a submaximal exercise measurement to measure functional capacity in ambulatory patients combining cardiopulmonary, neuromuscular, and musculoskeletal functions. The 6 Minute Walk Test (6MWT) was originally developed by the American Thoracic Society (ATS 2002) for use with adults, and is now commonly used in both adult and pediatric populations (Mylius et al 2016), and with children with neuromuscular diseases such as spinal muscular atrophy (Montes et al 2018), Duchenne muscular dystrophy (McDonald et al 2013), and infantile-onset Pompe disease (van der Meijden et al 2018). The 2 Minute Walk Test (2MWT) is included in the NIH Toolbox and is increasingly being used to measure the same properties.

The 6MWT and the 2MWT may be administered to the patient before and during treatment at the discretion of the healthcare provider. If a subject is unable to complete at least the 2MWT at baseline, additional assessments during treatment may be left to a healthcare provider's discretion. Resting heart rate is obtained prior to the test and post-test.

Distance walked in the first 2 minutes of the 6MWT and the full 6 minutes may be recorded. The distances walked in 2 minutes and 6 minutes may be compared to age- and sex-matched normative data (percent predicted values).

3. Dynamometry. Strength may be assessed using dynamometry before and/or during treatment at the discretion of the healthcare provider. Hand-held dynamometry is a direct measurement of strength commonly used in both children and adults. Muscle groups that may be assessed include: shoulder abduction, shoulder flexion, elbow flexion, elbow extension, hip abduction, hip flexion, hip extension, and knee extension. Each muscle group may be measured 2 times bilaterally.

Grip Strength. Grip strength may be measured using a grip strength dynamometer before and/or during treatment at the discretion of the healthcare provider. Equipment and assessor instructions may be standardized across sites. Grip may be assessed bilaterally with 1 practice and 1 maximal force measures taken for each hand and results may be compared to age and gender matched normative data (when available).

Range of Motion. Range of Motion may be assessed using a goniometer, an instrument that tests the angle of joints and measures the degree of movement at a joint. The stationary arm of the goniometer is aligned with the specified bony landmark on the stationary body segment, and the moving arm of the goniometer is aligned with the specified bony landmark of the limb that is moving. The fulcrum of the goniometer is specified for each motion measured using axis of motion and bony landmarks. Range of motion may be assessed for one or more of the following: shoulder abduction, shoulder flexion, elbow flexion, elbow extension, hip abduction, hip flexion, hip extension, and knee extension.

4. Hearing Testing. Moderate hearing loss has been associated with ARHR2 (Brachet et al 2014, Steichen-Gersdorf et al 2015). Baseline hearing may be determined by one or more of: Physical exam and otoscopy, Immittance audiometry (commonly called tympanometry), Pure Tone Audiometry (PTA) with frequencies up to 8 kHz if possible. (If there is a PTA threshold of >15dB, the subject should also undergo bone conduction testing.), High Frequency Audiometry (HFA), with frequencies up to 16 kHz.

5. Clinician Global Impression Scales. The Clinical Global Impression (CGI-S) scales were developed for use in National Institute of Mental Health-sponsored clinical

studies to provide a brief, stand-alone assessment of the clinician's view of the patient's global functioning prior to and after initiating a study medication (Guy 1976). The CGI provides an overall clinician-determined summary measure that considers all available information, including knowledge of the patient's history, psychosocial circumstances, symptoms, behavior, and the impact of the symptoms on the patient's ability to function. The CGI-S may be administered before and/or during treatment at the discretion of the healthcare provider and provides a global assessment of change using a seven-point scale ranging from -3 (severe worsening) to +3 (significant improvement).

6. Gross Motor Function Classification System – Expanded and Revised. The Gross Motor Classification System - Expanded and Revised (GMFCS – E and R) may be administered before and/or during treatment at the discretion of the healthcare provider. The GMFCS – E and R classifies patient-initiated movement with an emphasis on mobility on a scale from 1 to 5.

7. Patient Reported Outcomes Measurement Information Systems. The Patient Reported Outcomes Measurement Information Systems (PROMIS) consists of a variety of questionnaires developed by the National Institutes of Health (NIH) to evaluate physical, mental, and social well–being from the patient perspective (http://www.healthmeasures.net). These questionnaires have been used in clinical studies in people with chronic health conditions such as X-linked hypophosphatemia, arthritis, multiple sclerosis, and neurofibromatosis. Each questionnaire contains 8 to 10 items which are rated by the participant on a 5-point Likert scale ranging from 1 (never) to 5 (always). Scores are summed for each questionnaire, with high scores indicating more of the domain being measured (e.g. more fatigue, more physical function). Raw scores are converted to T-Scores based on a mean of 50 and a standard deviation of 10, allowing comparison of the study sample to the general population. PROMIS Scales may include the Pain Interference (short form 8a), Pain Intensity (version 3a), Physical Function - Upper Extremity (custom short form), Physical Function – Mobility (short form 13a FACIT Fatigue), Fatigue (short form), and Cognitive Impact (short form 8a) and may be administered before and/or during treatment at the discretion of the healthcare provider. These assessments may be completed by the subject without assistance.

8. Caregiver Global Impression Scales. The Caregiver Global Impression of Status may be administered to the patient's caregiver before and/or during treatment at the

discretion of the healthcare provider. The Caregiver Global Impression of Change provides a global assessment of change using a seven-point scale ranging from -3 (severe worsening) to +3 (significant improvement).

9. Western Ontario and McMaster University Osteoarthritis Index. The WOMAC is a patient-reported outcome used to assess activities of daily living, functional mobility, gait, general health, pain, and quality of life in patients with hip or knee pain (www.sralab.org). The assessment consists of 24-items and takes approximately 12 minutes to administer. The WOMAC may be administered before and/or during treatment at the discretion of the healthcare provider. The assessment may be completed by the subject without assistance.

REFERENCES

ATS. American Thoracic Society Statement: Guidelines for the six-minute walk test. AmJRespir, CritCare Med. 2002 2002;166(1):111-7.

Brachet C, Mansbach AL, Clerckx A, Deltenre P, Heinrichs C. Hearing loss is part of the clinical picture of ENPP1 loss of function mutation. Horm Res Paediatr. 2014;81(1):63-6.

CTFG. Recommendations related to contraception and pregnancy testing in clinical trials. 2014 [19 May 2020]; Available from:

http://www.hma.eu/fileadmin/dateien/Human_Medicines/01-

About HMA/Working Groups/CTFG/2014 09 HMA CTFG Contraception.pdf.

Ferreira C, Ziegler S, Gahl W. Generalized Arterial Calcification of Infancy. In: Adam MP, Ardinger HH, Pagon RA, Wallace SE, Bean LJH, Stephens K, et al., editors. GeneReviews((R)). Seattle (WA)2014.

Guy W. The Clinical Global Impression Scale. In: Rush Jr AJ, First MB, Blacker D, editors. Handbook of Psychiatric Measures, 2008. Washington, DC: American Psychiatric Publishing, Inc; 1976. p. 90-2.

IOF. Autosomal Recessive Hypophosphatemic Rickets Type 2 (ARHR2). 2019 [19 May 2020]; Available from: https://www.iofbonehealth.org/osteoporosis-

musculoskeletal-disorders/skeletal-rare-disorders/autosomal-recessive-hypophosphatemi-0.

Mackenzie NC, Huesa C, Rutsch F, MacRae VE. New insights into NPP1 function: lessons from clinical and animal studies. Bone. 2012 Nov;51(5):961-8.

McDonald CM, Henricson EK, Abresch RT, Florence JM, Eagle M, Gappmaier E, et al. The 6-minute walk test and other endpoints in Duchenne muscular dystrophy: longitudinal natural history observations over 48 weeks from a multicenter study. Muscle Nerve. 2013 Sep;48(3):343-56.

Montes J, McDermott MP, Mirek E, Mazzone ES, Main M, Glanzman AM, et al.

Ambulatory function in spinal muscular atrophy: Age-related patterns of progression. PLoS One. 2018;13(6):e0199657.

Moran JJ. Idiopathic arterial calcification of infancy: a clinicopathologic study. Pathol Annu. 1975;10:393-417.

Mylius CF, Paap D, Takken T. Reference value for the 6-minute walk test in children and adolescents: a systematic review. Expert Rev Respir Med. 2016 Dec;10(12):1335-52.

NCI. National Cancer Institute Division of Cancer Treatment and Diagnosis (DCTD), National Cancer Institute (NCI), National Institutes of Health (NIH), Department of Health and Human Services (DHHS). Common Terminology Criteria for Adverse Events V5.0 (CTCAE). Published: November 27, 2017. Available at https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcae_v5_quick_reference 8.5x11.pdf. 2017.

Orriss IR, Arnett TR, Russell RG. Pyrophosphate: a key inhibitor of mineralisation. Curr Opin Pharmacol. 2016 Jun;28:57-68.

Rutsch F, Boyer P, Nitschke Y, Ruf N, Lorenz-Depierieux B, Wittkampf T, et al. Hypophosphatemia, hyperphosphaturia, and bisphosphonate treatment are associated with survival beyond infancy in generalized arterial calcification of infancy. Circ Cardiovasc Genet. 2008 Dec;1(2):133-40.

Steichen-Gersdorf E, Lorenz-Depiereux B, Strom TM, Shaw NJ. Early onset hearing loss in autosomal recessive hypophosphatemic rickets caused by loss of function mutation in ENPP1. J Pediatr Endocrinol Metab. 2015 Jul;28(7-8):967-70.

van der Meijden JC, Kruijshaar ME, Harlaar L, Rizopoulos D, van der Beek N, van der Ploeg AT. Long-term follow-up of 17 patients with childhood Pompe disease treated with enzyme replacement therapy. J Inherit Metab Dis. 2018 Nov;41(6):1205-14.

INCORPORATION BY REFERENCE

All publications and patents mentioned herein are hereby incorporated by reference in their entirety as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

OTHER EMBODIMENTS

While specific embodiments of the subject matter have been discussed, the above specification is illustrative and not restrictive. Many variations will become apparent to those skilled in the art upon review of this specification and the claims below. The full scope of the invention should be determined by reference to the claims, along with their full scope of equivalents, and the specification, along with such variations.

CLAIMS

1. A method for preventing the progression of or reducing vascular calcification in a subject with ENPP1 Deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce vascular calcification in the subject.

2. A method for preventing the progression of or reducing pathological calcification in a subject with ENPP1 Deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce pathological calcification in the subject.

3. A method for preventing the progression of or reducing tissue calcification in a subject with ENPP1 Deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject

4. A method for preventing the progression of or reducing pathological ossification in a subject with ENPP1 Deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject.

5. A method for increasing circulating pyrophosphate (PPi) in a subject with ENPP1 Deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

6. A method for increasing pyrophosphatase activity in a subject with ENPP1 Deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

7. A method for ameliorating one or more symptoms of ENPP1 Deficiency in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2

mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby ameliorate one or more symptoms of ENPP1 Deficiency in the subject.

8. A method for treating a subject with ENPP1 Deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby treat the subject.

9. A method for preventing the progression of or reducing vascular calcification in a subject with pathological calcification, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce vascular calcification in the subject.

10. A method for preventing the progression of or reducing pathological calcification in a subject with pathological calcification, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce pathological calcification in the subject.

11. A method for preventing the progression of or reducing tissue calcification in a subject with pathological calcification, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject.

12. A method for preventing the progression of or reducing pathological ossification in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject.

13. A method for increasing circulating pyrophosphate (PPi) in a subject with pathological calcification, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the

subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

14. A method for increasing pyrophosphatase activity in a subject with pathological calcification, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

15. A method for ameliorating one or more symptoms of pathological calcification in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby ameliorate one or more symptoms of pathological calcification in the subject.

16. A method for treating a subject with pathological calcification, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby treat the subject.

17. A method for preventing the progression of or reducing vascular calcification in a subject with ABCC6 Deficiency or ABCC6 deficient subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce vascular calcification in the subject.

18. A method for preventing the progression of or reducing pathological calcification in a subject with ABCC6 Deficiency or ABCC6 deficient subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce pathological calcification in the subject.

19. A method for preventing the progression of or reducing tissue calcification in a subject with ABCC6 Deficiency or ABCC6 deficient subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce tissue calcification in the subject

20. A method for preventing the progression of or reducing pathological ossification in a subject with ABCC6 Deficiency or ABCC6 deficient subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby prevent the progression of or reduce pathological ossification in the subject.

21. A method for increasing circulating pyrophosphate (PPi) in a subject with ABCC6 Deficiency or ABCC6 deficient subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase circulating PPi in the subject.

22. A method for increasing pyrophosphatase activity in a ABCC6 deficient subject or a subject with ABCC6 Deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby increase pyrophosphatase activity in the subject.

23. A method for ameliorating one or more symptoms of ABCC6 deficient subject or ABCC6 Deficiency in a subject, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby ameliorate one or more symptoms of ABCC6 Deficiency in the subject.

24. A method for treating a ABCC6 deficient subject or a subject with ABCC6 Deficiency, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby treat the subject.

25. A method of treating a subject afflicted with one or more symptoms of PXE, the method comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby treat said subject.

26. A method of treating a subject who is an infant and exhibits abnormal vascular calcification and/or one or more symptoms of ENPP1 deficiency, and/or GACI, the method

comprising: administering to the subject an ENPP1 agent at a dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject, to thereby treat said subject.

27. The method of any one of claims 1-26, wherein the dose is about 0.2 mg per kilogram of the subject.

28. The method of any one of claims 1-26, wherein the dose is about 0.6 mg per kilogram of the subject.

29. The method of any one of claims 1-26, wherein the dose is about 1.8 mg per kilogram of the subject.

30. The method of any one of claims 1-29, wherein the ENPP1 agent is administered at least one time per week.

31. The method of any one of claims 1-30, wherein the ENPP1 agent is administered at least two times per week.

32. The method of any one of claims 1-30, wherein the ENPP1 agent is administered to the subject at least two times per week following an initial dose.

33. The method of any one of claims 1-32, wherein the ENPP1 agent is administered subcutaneously.

34. The method of any one of claims 1-33, wherein the ENPP1 agent is self-administered.

35. The method of any one of claims 1-34, wherein the ENPP1 agent is administered under a dosing regimen comprising: (a) an initial dose of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject and (b) about seven days after the initial dose, twice weekly administration of maintenance doses of the ENPP1 agent of about 0.2 mg per kilogram of the subject, about 0.6 mg per kilogram of the subject, or about 1.8 mg per kilogram of the subject.

36. The method of claim 35, wherein the initial dose and the maintenance doses are the same.

37. The method of any one of claims 1-34, wherein the ENPP1 agent comprises the catalytic domain of ENPP1.

38. The method of any one of claims 1-37, wherein the ENPP1 agent comprises the nuclease domain of ENPP1.

39. The method of any one of claims 1-38, wherein the ENPP1 agent comprises the extracellular domain of ENPP1.

40. The method of any one of claims 1-39, wherein the ENPP1 agent comprises a heterologous moiety.

41. The method of claim 40, wherein the heterologous moiety is a polypeptide.

42. The method of claim 40 or 41, wherein the heterologous moiety increases the circulating half-life of the ENPP1 agent relative to the circulating half-life of the ENPP1 agent lacking the heterologous moiety.

43. The method of claim 41 or 42, wherein the heterologous moiety comprises the Fc region of an immunoglobulin molecule.

44. The method of claim 43, wherein the immunoglobulin molecule is a human immunoglobulin molecule.

45. The method of claim 43 or 44, wherein the immunoglobulin molecule is an IgG1.

46. The method of claim 41 or 42, wherein the heterologous moiety comprises albumin.

47. The method of any one of claims 1-35, wherein the ENPP1 agent comprises amino acid residues 99 (PSCAKE) to 925 (QED) of SEQ ID NO:1 [ENPP1 ECD].

48. The method of any one of claims 1-35, wherein the ENPP1 agent comprises amino acid residues 1 (FTAGLKPSCAKE) to 833 (QED) of SEQ ID NO:3.

49. The method of any one of claims 1-35, wherein the ENPP1 agent comprises the amino acid sequence depicted in SEQ ID NO:5

50. The method of any one of claims 1-35, wherein the ENPP1 agent comprises the amino acid sequence depicted in SEQ ID NO:3.

51. The method of any one of claims 1-35, wherein the ENPP1 agent comprises the amino acid sequence depicted in SEQ ID NO:2.

52. The method of any one of claims 1-35, wherein the ENPP1 agent comprises the amino acid sequence depicted in SEQ ID NO:3 or 4 or 5.

53. The method of any one of claims 1-35, wherein the subject has or is suspected of having generalized arterial calcification of infancy (GACI).

54. The method of any one of claims 1-35, wherein the subject has or is suspected of having autosomal recessive hypophosphatemic rickets type 2 (ARHR2).

55. The method of any one of claims 1-35, wherein the pharmaceutical composition contains as the sole active ingredient around 0.2 to 1.8mg of ENPP1-Fc per kg of weight of the subject.

56. The method of anyone of claims 1-35, wherein the subject has been treated with one or more statins and/or one or more proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors prior to said administration of said ENPP1 agent.

57. The method of anyone of claims 1-35, wherein the subject has been treated with a selected dose or selected doses of one or more statins and/or one or more PCSK9 inhibitors for 3 or more years prior administration of said ENPP1 agent.

58. The method of anyone of claims 1-35, wherein the subject has not been diagnosed with a malignancy prior to said administration of said ENPP1 agent.

59. The method of anyone of claims 1-35, wherein the subject has not been diagnosed with a malignancy within the previous 5 years of said administration of said ENPP1 agent,

60. The method of claim 58, wherein said malignancy excludes non-melanoma skin cancers and cervical carcinoma in situ.

61. The method of any one of claims 1-52, wherein the subject has or is suspected of having Pseudoxanthoma elasticum (PXE)

62. The method of any one of claims 1-52, wherein the subject exhibits symptoms similar to a person having autosomal recessive hypophosphatemic rickets type 2 (ARHR2) or generalized arterial calcification of infancy (GACI).

63. The method of claim 1-35, wherein the pharmaceutical composition contains as the sole active ingredient around 0.2 to 1.8 mg/kg of weight of subject

64. The method of any one of claims 1-52, wherein the subject has or is suspected of having one or more of kidney and bladder stones, dental pulp stones, gall stones, salivary gland stones, chronic calculous prostatitis, testicular microliths, calcification in hemodialysis

patients, atherosclerosis, malacoplakia, scleroderma (systemic sclerosis), calcinosis cutis, calcific aortic stenosis, calcific tenditis, synovitis and arthritis, diffuse interstitial skeletal hyperostosis, juvenile dermatomyositis, Generalized Arterial Calcification of Infancy (GACI), Ossification of the Posterior Longitudinal Ligament (OPLL), autosomal hypophosphatemic rickets (ARHR2), osteoarthritis, calcification of atherosclerotic plaques, Chronic Kidney Disease (CKD), End Stage Renal Disease (ESRD), Pseudoxanthoma elasticum (PXE), ankylosing spondylitis, hardening of the arteries, calciphylaxis, and systemic lupus erythematosus.

65. The method of any one of claims 1-52, wherein said subject exhibits calcification of soft connective tissues.

66. The method of claim 65, wherein said subject exhibits accumulation of deposits of calcium and other minerals (mineralization) in elastic fibers of connective tissues.

67. The method of claim 65, wherein said subject exhibits calcification in the eyes, cardiovascular system, and/or skin.

68. The method of claim 67, wherein said subject exhibits narrowing of the arteries and/or arteriosclerosis.

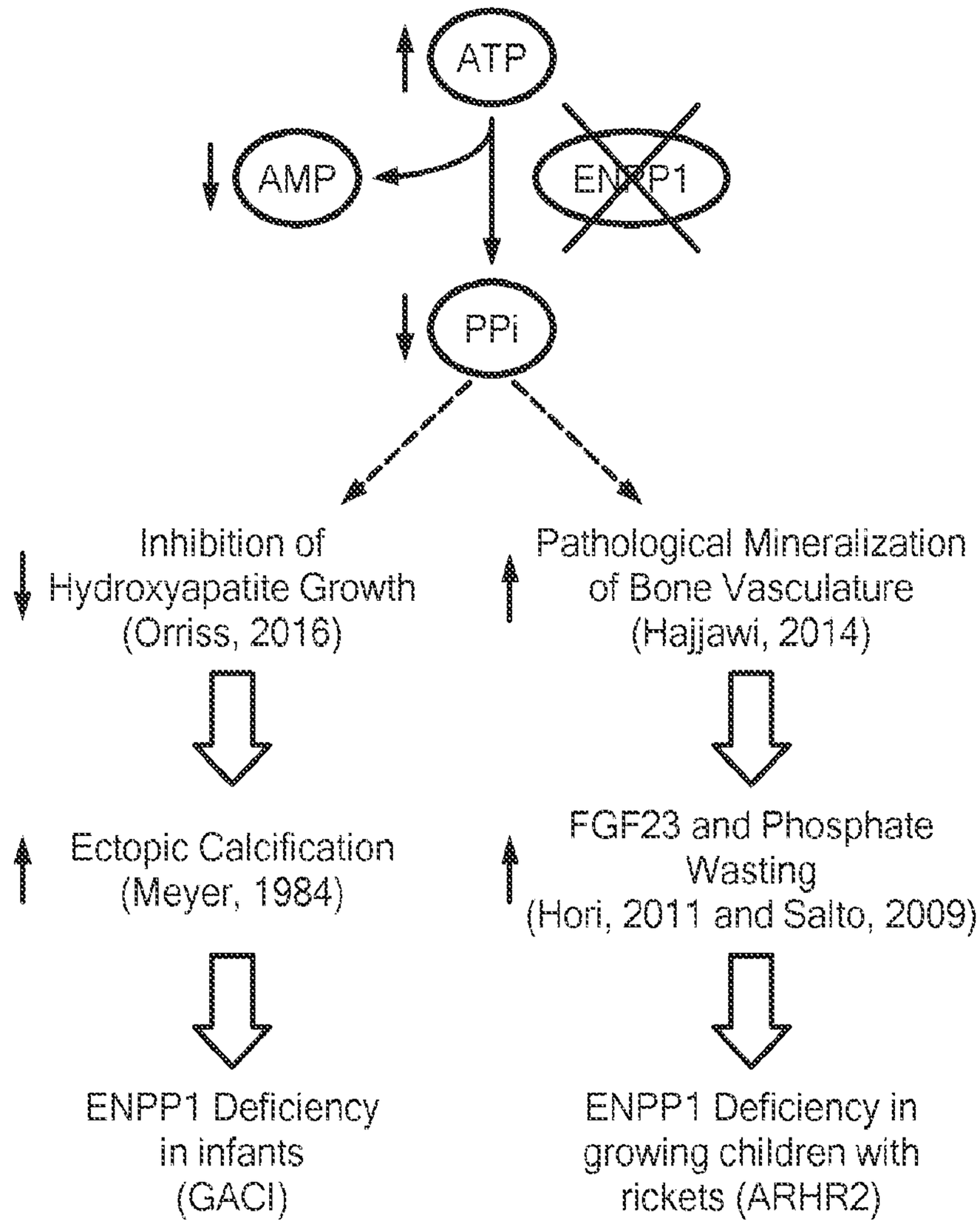
69. The method of treating a subject according to any one of claims 1-52, wherein said subject exhibits ectopic calcification and/or narrow of blood vessels in the lower extremities, and/or claudication.

70. The method of treating a subject according to any one of claims 1-52, wherein said subject exhibits abnormalities in the eyes,

71. The method of claim 70, wherein said eye abnormalities comprising abnormal pigmentation of cells of the retina, and/or angioid streaks, and/or abnormalities in the elastic membrane beneath the retina.

72. The method of treating a subject according to any one of claims 1-52, wherein said subject exhibits choroidal neovascularization

73. The method of treating a subject according to any one of claims 1-52, wherein said subject exhibits visual impairment, vision loss, and/or blindness and also exhibits one or more symptoms of soft tissue calcification.



AMP/ATP = adenosine mono-/tri- phosphate;
ENPP1 = ectonucleotide pyrophosphatase/phosphodiesterase 1;
FGF23 = fibroblast growth factor 23;
PPi = inorganic pyrophosphate
References: Orriss 2016, Meyer 1984, Hajjawi 2014, Hori 2011, Saito 2009

FIGURE 1:

Overview of ENPP1 Deficiency

```
  1 MERDGCAGGG SRGGEGGRAP REGPAGNGRD RGRSHAAEAP GDPQAAASLL
 51 APMDVGEEPL EKAARARTAK DPNTYKVLSL VLSVCVLTTI LGCIFGLKPS
101 CAKEVKSCKG RCFERTFGNC RCDAACVELG NCCLDYQETC IEPEHIWTCN
151 KFRCGEKRLT RSLCACSDDC KDKGDCCINY SSVCQGEKSW VEEPCESINE
201 PQCPAGFETP PTLLFSLDGF RAEYLHTWGG LLPVISKLKK CGTYTKNMRP
251 VYPTKTFPNH YSIVTGLYPE SHGIIDNKMY DPKMNASFSL KSKEKFNPEW
301 YKGEPIWVTA KYQGLKSGTF FWPGSDVEIN GIFPDIYKMY NGSVPFEERI
351 LAVLQWLQLP KDERPHFYTL YLEEPDSSGH SYGPVSSEVI KALQRVDGMV
401 GMLMDGLKEL NLHRCLNLIL ISDHGMEQGS CKKYIYLNKY LGDVKNIKVI
451 YGPAARLRPS DVPDKYYSFN YEGIARNLSC REPNQHFKPY LKHFLPKRLH
501 FAKSDRIEPL TFYLDPQWQL ALNPSERKYC GSGFHGSDNV FSNMQALFVG
551 YGPGFKHGIE ADTFENIEVY NLMCDLLNLT PAPNNGTHGS LNHLLKNPVY
601 TPKHPKEVHP LVQCPFTRNP RDNLGCSCNP SILPIEDFQT QFNLTVAEEK
651 IIKHETLPYG RPRVLQKENT ICLLSQHQFM SGYSQDILMP LWTSYTVDRN
701 DSFSTEDFSN CLYQDFRIPL SPVHKCSFYK NNTKVSYGFL SPPQLNKNSS
751 GIYSEALLTT NIVPMYQSFQ VIWRYFHDTL LRKYAEERNG VNVVSGPVFD
801 FDYDGRCDSL ENLRQKRRVI RNQEILIPTH FFIVLTSCKD TSQTPLHCEN
851 LDTLAFILPH RTDNSESCVH GKHDSSWVEE LLMLHRARIT DVEHITGLSF
901 YQQRKEPVSD ILKLKTHLPT FSQED (SEQ ID NO: 1)
```

FIGURE 2

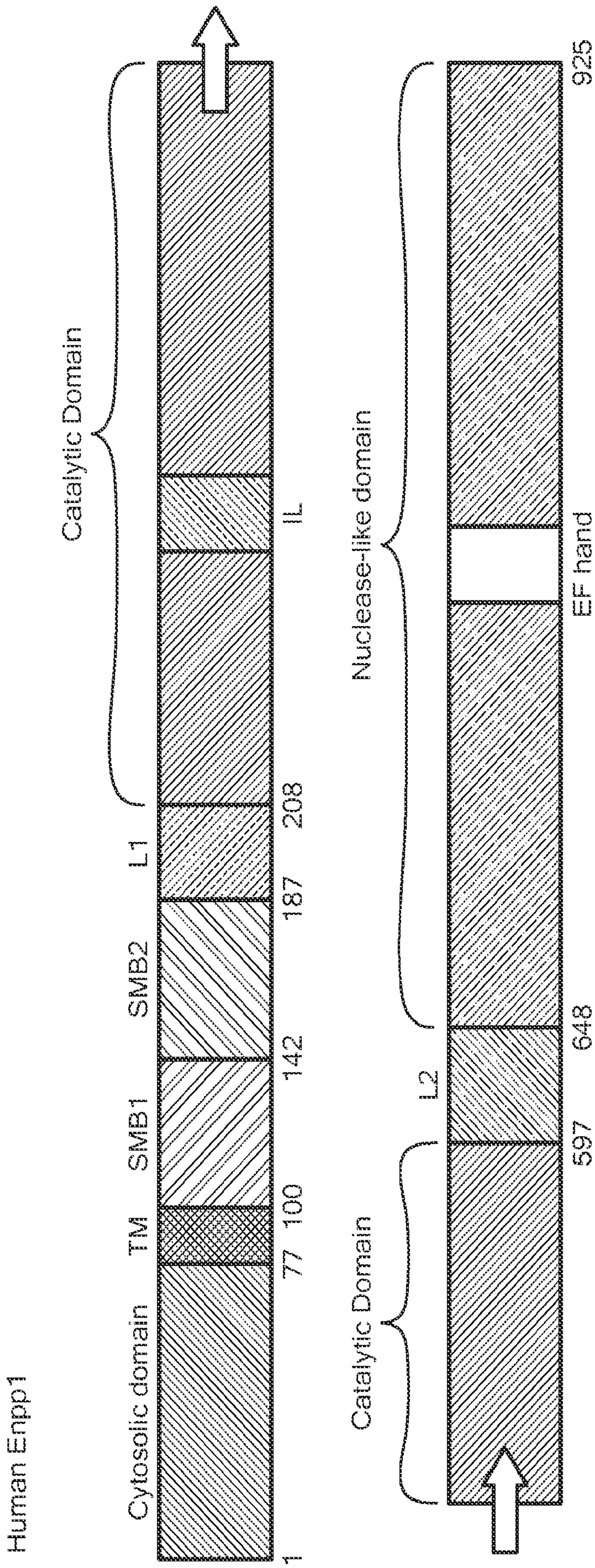

Human Enpp1
Cytosolic domain
TM
SMB1
SMB2
L1
IL
Nuclease-like domain
L2
EF hand
Catalytic Domain
1
77
100
142
187
208
597
648
925

FIGURE 3

```
  1 PSCAKEVKSC KGRCFERTFG NCRCDAACVE LGNCCLDYQE TCIEPEHIWT
 51 CNKFRCGEKR LTRSLCACSD DCKDKGDCCI NYSSVCQGEK SWVEEPCESI
101 NEPQCPAGFE TPPTLLFSLD GFRAEYLHTW GGLLPVISKL KKCGTYTKNM
151 RPVYPTKTFP NHYSIVTGLY PESHGIIDNK MYDPKMNASF SLKSKEKFNP
201 EWYKGEPIWV TAKYQGLKSG TFFWPGSDVE INGIFPDIYK MYNGSVPFEE
251 RILAVLQWLQ LPKDERPHFY TLYLEEPDSS GHSYGPVSSE VIKALQRVDG
301 MVGMLMDGLK ELNLHRCLNL ILISDHGMEQ GSCKKYIYLN KYLGDVKNIK
351 VIYGPAARLR PSDVPDKYYS FNYEGIARNL SCREPNQHFK PYLKHFLPKR
401 LHFAKSDRIE PLTFYLDPQW QLALNPSERK YCGSGFHGSD NVFSNMQALF
451 VGYGPGFKHG IEADTFENIE VYNLMCDLLN LTPAPNNGTH GSLNHLLKNP
501 VYTPKHPKEV HPLVQCPFTR NPRDNLGCSC NPSILPIEDF QTQFNLTVAE
551 EKIIKHETLP YGRPRVLQKE NTICLLSQHQ FMSGYSQDIL MPLWTSYTVD
601 RNDSFSTEDF SNCLYQDFRI PLSPVHKCSF YKNNTKVSYG FLSPPQLNKN
651 SSGIYSEALL TTNIVPMYQS FQVIWRYFHD TLLRKYAEER NGVNVVSGPV
701 FDFDYDGRCD SLENLRQKRR VIRNQEILIP THFFIVLTSC KDTSQTPLHC
751 ENLDTLAFIL PHRTDNSESC VHGKHDSSWV EELLMLHRAR ITDVEHITGL
801 SFYQQRKEPV SDILKLKTHL PTFSQED (SEQ ID NO: 2)
```

FIGURE 4

```
Mouse     WVEETCESIDTPECPAEFESPPTLLFSLDGFRAEYLHTWGGLLPVISKLKNCGTYTKNMR   60
Cow       WAEEECDSIDEPQCPAGFETPPTLLFSLDGFRAEYLHTWGGLLPVISKLKTCGTYTKNMR   60
Rabbit    WVEETCENINEPQCPEGFEMPPTLLFSLDGFRAEYLHTWGGLLPVISKLKKCGTYAKNMR   60
Human     WVEEPCESINEPQCPAGFETPPTLLFSLDGFRAEYLHTWGGLLPVISKLKKCGTYTKNMR   60
Baboon    WVEEPCESINEPQCPAGFETPPTLLFSLDGFRAEYLHTWGGLLPVISKLKKCGTYTKNMR   60
          *.** *:.*: *:**  ** ******************************.****:****

Mouse     PMYPTKTFPNHYSIVTGLYPESHGIIDNKMYDPKMNASFSLKSKEKFNPLWYKGQPIWVT   120
Cow       PVYPTKTFPNHYSIVTGLYPESHGIIDNNIYDPQMNANFALKNKEKFNPEWYKGEPIWLT   120
Rabbit    PVYPTKTFPNHYSIVTGLYPESHGIIDNKMYDPKMNASFSLKSKEKFNPEWYKGEPIWLT   120
Human     PVYPTKTFPNHYSIVTGLYPESHGIIDNKMYDPKMNASFSLKSKEKFNPEWYKGEPIWVT   120
Baboon    PVYPTKTFPNHYSIVTGLYPESHGIIDNKMYDPKMNASFSLKSKEKFNPEWYKGEPIWVT   120
          *:*****************************:***:***.*:**.****** ****:***:*

Mouse     ANHQEVKSGTYFWPGSDVEIDGILPDIYKVYNGSVPFEERILAVLEWLQLPSHERPHFYT   180
Cow       AKYQGLKTGTFFWPGSDVKINGIFPDIYKIYNVSVPFEERILAILKWLQLPKDERPHFYT   180
Rabbit    AKYQGLRSGTFFWPGSDVKINGIFPDIYKIYNGSVPFEERILAILKWLRLPKDERPHFYT   180
Human     AKYQGLKSGTFFWPGSDVEINGIFPDIYKMYNGSVPFEERILAVLQWLQLPKDERPHFYT   180
Baboon    AKYQGLKSGTFFWPGSDVEINGIFPDIYKMYNGSVPFEERILAVLQWLQLPKDERPHFYT   180
          *:** :::**:*******:*:**:*****:** **********:*:**:**..*******

Mouse     LYLEEPDSSGHSHGPVSSEVIKALQKVDRLVGMLMDGLKDLGLDKCLNLILISDHGMEQG   240
Cow       LYLEEPDSSGHSYGPVSSEVIRALQRVDNMVGMLMDGLKELNLHRCLNLILISDHGMEQG   240
Rabbit    LYLEEPDSSGHSYGPVSSEVIKALQRVDNMVGMLMDGLKELNLHQCLNLILISDHGMEQG   240
Human     LYLEEPDSSGHSYGPVSSEVIKALQRVDGMVGMLMDGLKELNLHRCLNLILISDHGMEQG   240
Baboon    LYLEEPDSSGHSYGPVSSEVIKALQRVDNMVGMLMDGLKELNLHRCLNLILVSDHGMEQG   240
          ************:********:***:** :*********:*.*.:******:********

Mouse     SCKKYVYLNKYLGDVNNVKVVYGPAARLRPTDVPETYYSFNYEALAKNLSCREPNQHFRP   300
Cow       SCKKYVYLNKYLGDTKDYKVVYGPAARLRPSDVPDKYYSFDYEGIAKNLSCQEPNQHFKP   300
Rabbit    SCKKYIYLNKYLGDTKNIKVIYGPAARLRPSDVPEKYYSFNYENIARNLSCREPNQHFKP   300
Human     SCKKYIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYYSFNYEGIARNLSCREPNQHFKP   300
Baboon    SCKKYIYLNKYLGDVKNIKVIYGPAARLRPSDVPDKYYSFNYEGIARNLSCREPNQHFKP   300
          *****:********.:: **:*********:***:.****:** :*:****:******:*

Mouse     YLKPFLPKRLHFAKSDRIEPLTFYLDPQWQLALNPSERKYCGSGFHGSDNLFSNMQALFI   360
Cow       YLKHFLPKRLHFAKNDRIERLTFYLDPQWQLALNPSERKYCGGGFHGSDNTFLNMQALFI   360
Rabbit    YLKHFLPKRLHFAKSDRIEPLTFYLDPQWQLALSPSERKYCGSGFHGSDNVFSNMQALFV   360
Human     YLKHFLPKRLHFAKSDRIEPLTFYLDPQWQLALNPSERKYCGSGFHGSDNVFSNMQALFV   360
Baboon    YLKHFLPKRLHFAKSDRIEPLTFYLDPQWQLALNPSERKYCGSGFHGSDNIFSNMQALFV   360
          *** **********.**** **************.********.******* * ******:
```

FIGURE 5A

```
Mouse     GYGPAFKHGAEVDSFENIEVYNLMCDLLGLIPAPNNGSHGSLNHLLKKPIYNPSHPKEEG   420
Cow       GYGPGFKHSTEVDSFENIEVYNLMCDLLNLTPAPNNGTHGSLNHLLSNPVYTPKHPKEVR   420
Rabbit    GYGPGFQHGIEVDSFENIEVYNLMCDLLNLTPAPNNGTHGSLNHLLKNPIYTPKHPKEVQ   420
Human     GYGPGFKHGIEADTFENIEVYNLMCDLLNLTPAPNNGTHGSLNHLLKNPVYTPKHPKEVH   420
Baboon    GYGPGFKHGIEVDTFENIEVYNLMCDLLNLTPAPNNGTHGSLNHLLKNPVYTPKHPKEVH   420
          ****.*:*. *.*;***************.* ******:********.:*:*.*.****

Mouse     FLSQCPIKST-SNDLGCTCDPWIVPIKDFEKQLNLTTEDVDDIYHMTVPYGRPRILLKQH   479
Cow       PLVQCPFTRAPRESLDCSCDPSILPIVDFQTQLNLTMAEEKTIKRGALPYGRPRVLQN-S   479
Rabbit    PSVQCPLAGSPRDSLGCSCNPSILPIVDFQTQFNLTTAEEKNINRASLPYGRPRLLQKKS   480
Human     PLVQCPFTRNPRDNLGCSCNPSILPIEDFQTQFNLTVAEEKIIKHETLPYGRPRVLQKEN   480
Baboon    PLIQCPFTRNPRDNLGCSCNPSILPIEDFQTQFNLTVAEEKNIKHETLPYGRPRVLQKKN   480
             ***:     :.*.*:*:* *:** **:.*:***  : . * : ::******:* :


Mouse     RVCLLQQQQFLTGYSLDLLMPLWASYTFLSNDQFSRDDFSNCLYQDLRIPLSPVHKCSYY   539
Cow       TVCLLYQHQFVSGYSRDILMPLWTSYTIGRNDSFSTEDFSNCLYQDLRIPLSPVHKCSFY   539
Rabbit    SVCLLYQHQFVSGYSHDVLMPLWTSYTVNRNDSFSTEDFSNCLYQDLRISFSPIHNCSFY   540
Human     TICLLSQHQFMSGYSQDILMPLWTSYTVDRNDSFSTEDFSNCLYQDFRIPLSPVHKCSFY   540
Baboon    TICLLSQHQFMSGYSQDILMPLWTSYTVDRNDSFSTEDFSNCLYQDFRISLSPVHKCSFY   540
           :*** *:**:*** *:*****:***.  **.** :**********:** :**:*:**:*

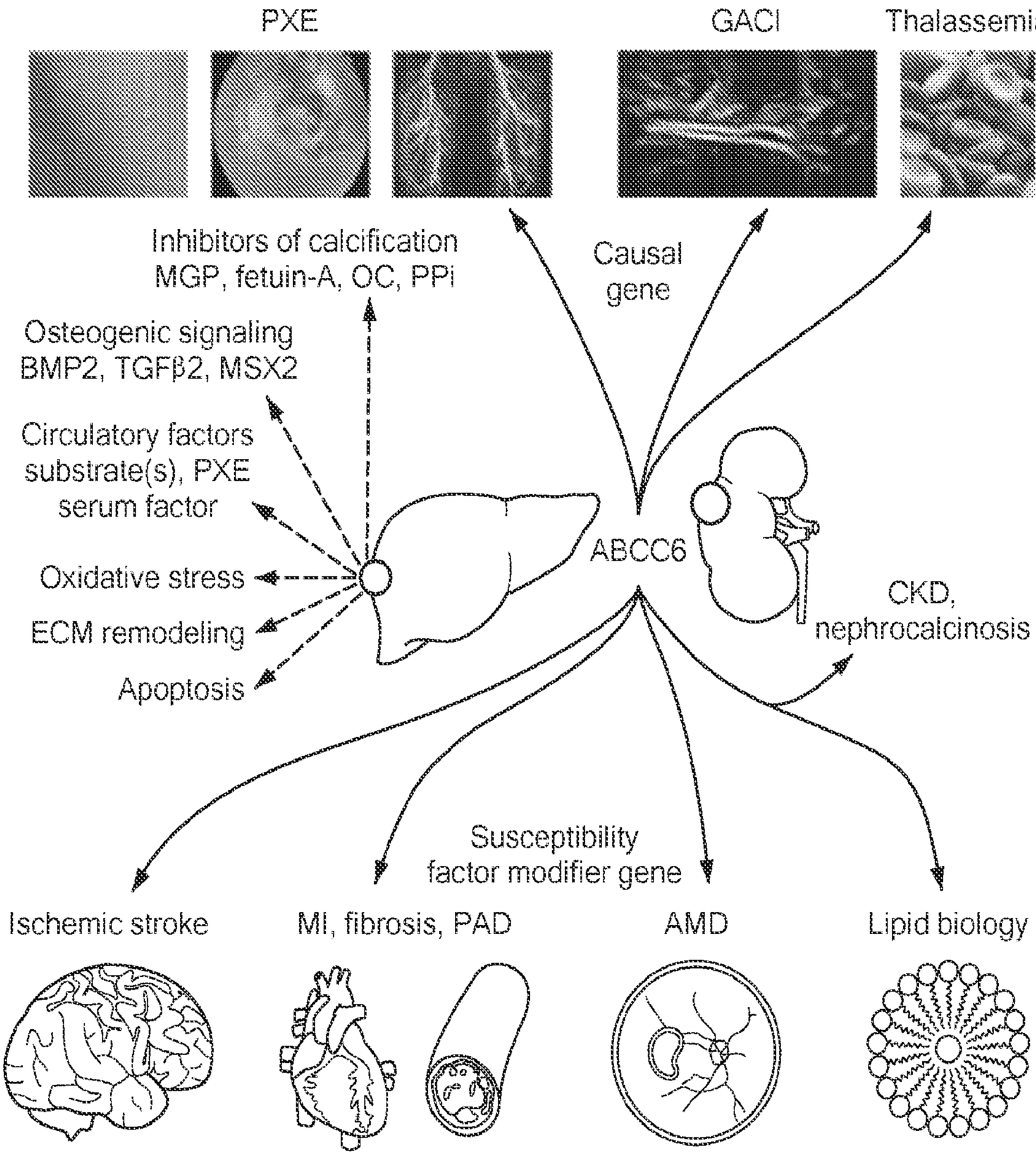

Mouse     KSNSKLSYGFLTPPRLNRVSNHIYSEALLTSNIVPMYQSFQVIWHYLHDTLLQRYAHERN   599
Cow       KNNAKLSYGLLSPPQLHKGSSQVYSEALLTTNIVPMYQSFQVIWHYLHGTLLQRYAEERN   599
Rabbit    KNNAKLSYGFLSPPQLSKDSSQIYSEALLTSNIVPMYQSFQVIWRYFHDTLLQRYAEERN   600
Human     KNNTKVSYGFLSPPQLNKNSSGIYSEALLTTNIVPMYQSFQVIWRYFHDTLLRKYAEERN   600
Baboon    KNNTKVSYGFLSPPQLNKNSRGIYSEALLTTNIVPMYQSFQVIWRYFHDTLLRKYAEERN   600
          *.*:*:***:*:**:* : *   :*******:**************:*:*.***::**.***


Mouse     GINVVSGPVFDFDYDGRYDSLEILKQNSRVIRSQEILIPTHFFIVLTSCKQLSETPLECS   659
Cow       GLNVVSGPVFDSDYDGRYDSLETLKQNSKIIRNLEVLIPTHFFLVLTSCKNTSQTPLQCE   659
Rabbit    SINVVSGPVFDSDYDGRYDSSEALKRNRRVIRNQEILIPTHFFIVITSCKNTSQTPLQCD   660
Human     GVNVVSGPVFDFDYDGRCDSLENLRQKRRVIRNQEILIPTHFFIVLTSCKDTSQTPLHCE   660
Baboon    GVNVVSGPVFDFDYDGRYDSLEILRQKRRVIRNQEILIPTHFFIVLTSCKDASQTPLHCE   660
          .:********* ***** ** * *::: ::**. *:*******:*:****: *:***.*.


Mouse      ALESSAYILPHRPDNIESCTHGKRESSWVEELLTLHRARVTDVELITGLSFYQDRQESVS  719
Cow        NLDAMAFILPHKTDNSESCAHGKHESLWVEELLKLHTARITDVEHITGLSFYQERKEPIS  719
Rabbit     NLDPLAFILPHRSDNSESCVHEKRESSWIEELLMMHRARIMDVEHITGLSFYQERKEPVS  720
Human      NLDTLAFILPHRTDNSESCVHGKHDSSWVEELLMLHRARITDVEHITGLSFYQQRKEPVS  720
Baboon     NLDTLAFILPHRTDNSESCLHGKHESSWVEELLMLHRARITDVEHITGLSFYQQRKEPVS  720
            *:  *:****; ** *** * *:;* *:**** :* **: *** ********:*:* ;*


Mouse      ELLRLKTHLPIFSQED    735
Cow        DILKLKTHLPTFNQED    735
Rabbit     DILKLKTHLPTVSQED    736
Human      DILKLKTHLPTFSQED    736
Baboon     DILKLKTHLPTFSQED    736
           ::*:****** ..***
```

FIGURE 5B



Pseudoxanthoma elasticum (PXE), Generalized arterial calcification of infancy (GACI), Myocardial infarction (MI), Cardiac fibrosis, Peripheral artery disease (PAD), Age-related macular degeneration (AMD), Chronic kidney disease (CKD), Nephrocalcinosis, and Dyslipidemia. BMP2: Bone morphogenetic protein 2; MGP: Matrix Gla protein; MSX2: Muscle segment homeobox 2; OC: Osteocalcin; PPi: inorganic pyrophosphate; TGF: Transforming growth factor

Reference: De Vilder EY, Hosen MJ, Vanakker OM, The ABCC6 Transporter as a Paradigm for Networking from an Orphan Disease to Complex Disorders. Biomed Res Int. 2015;2015:648569

FIGURE 6:

Overview of ABCC6 Deficiency

SEQUENCE LISTING

<110> Inozyme Pharma, Inc.
Huertas, Pedro
Wenkert, Deborah

<120> Treatment of ENPP1 Deficiency and ABCC6 Deficiency

<130> 4427-10902

<140> Not Yet Assigned
<141> 2021-11-19

<150> 63/116,086
<151> 2020-11-19

<150> 63/116,106
<151> 2020-11-19

<150> 63/116,093
<151> 2020-11-19

<150> 63/219,229
<151> 2021-07-07

<150> 63/237,351
<151> 2021-08-26

<160> 115

<170> PatentIn version 3.5

<210> 1
<211> 925
<212> PRT
<213> Homo sapiens

<400> 1

Met Glu Arg Asp Gly Cys Ala Gly Gly Gly Ser Arg Gly Gly Glu Gly
1 5 10 15

Gly Arg Ala Pro Arg Glu Gly Pro Ala Gly Asn Gly Arg Asp Arg Gly
20 25 30

Arg Ser His Ala Ala Glu Ala Pro Gly Asp Pro Gln Ala Ala Ala Ser
35 40 45

Leu Leu Ala Pro Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala Ala
50 55 60

Arg Ala Arg Thr Ala Lys Asp Pro Asn Thr Tyr Lys Val Leu Ser Leu
65 70 75 80

Val Leu Ser Val Cys Val Leu Thr Thr Ile Leu Gly Cys Ile Phe Gly
85 90 95

Leu Lys Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg Cys

```
            100                 105                 110

Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val Glu
        115                 120                 125

Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro Glu
    130                 135                 140

His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu Thr
145                 150                 155                 160

Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp Cys
                165                 170                 175

Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val Glu
            180                 185                 190

Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe Glu
        195                 200                 205

Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr
    210                 215                 220

Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Lys
225                 230                 235                 240

Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr
                245                 250                 255

Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His
            260                 265                 270

Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe
        275                 280                 285

Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly Glu
    290                 295                 300

Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr Phe
305                 310                 315                 320

Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp Ile
                325                 330                 335

Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala
            340                 345                 350

Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr
        355                 360                 365
```

```
Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro
    370                 375                 380

Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val Asp Gly Met Val
385                 390                 395                 400

Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys Leu
                405                 410                 415

Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys
            420                 425                 430

Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val Lys Asn Ile Lys
        435                 440                 445

Val Ile Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro Asp
    450                 455                 460

Lys Tyr Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg Asn Leu Ser Cys
465                 470                 475                 480

Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu Pro
                485                 490                 495

Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile Glu Pro Leu Thr Phe
            500                 505                 510

Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro Ser Glu Arg Lys
        515                 520                 525

Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn Val Phe Ser Asn Met
    530                 535                 540

Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys His Gly Ile Glu
545                 550                 555                 560

Ala Asp Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu
                565                 570                 575

Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn
            580                 585                 590

His Leu Leu Lys Asn Pro Val Tyr Thr Pro Lys His Pro Lys Glu Val
        595                 600                 605

His Pro Leu Val Gln Cys Pro Phe Thr Arg Asn Pro Arg Asp Asn Leu
    610                 615                 620
```

```
Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu Asp Phe Gln Thr
625                 630                 635                 640

Gln Phe Asn Leu Thr Val Ala Glu Glu Lys Ile Ile Lys His Glu Thr
                645                 650                 655

Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Lys Glu Asn Thr Ile Cys
            660                 665                 670

Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr Ser Gln Asp Ile Leu
        675                 680                 685

Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn Asp Ser Phe Ser
    690                 695                 700

Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe Arg Ile Pro Leu
705                 710                 715                 720

Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn Thr Lys Val Ser
                725                 730                 735

Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn Ser Ser Gly Ile
            740                 745                 750

Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln Ser
        755                 760                 765

Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu Leu Arg Lys Tyr
    770                 775                 780

Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Val Phe Asp
785                 790                 795                 800

Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn Leu Arg Gln Lys
                805                 810                 815

Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe Phe
            820                 825                 830

Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr Pro Leu His Cys
        835                 840                 845

Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His Arg Thr Asp Asn
    850                 855                 860

Ser Glu Ser Cys Val His Gly Lys His Asp Ser Ser Trp Val Glu Glu
865                 870                 875                 880
```

```
Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val Glu His Ile Thr
                885                 890                 895


Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile Leu
            900                 905                 910


Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu Asp
        915                 920                 925


<210>  2
<211>  827
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  ENPP1 Polypeptide

<400>  2

Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg Cys Phe Glu
1               5                   10                  15


Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val Glu Leu Gly
            20                  25                  30


Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro Glu His Ile
        35                  40                  45


Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu Thr Arg Ser
    50                  55                  60


Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp Cys Cys Ile
65                  70                  75                  80


Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val Glu Glu Pro
                85                  90                  95


Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe Glu Thr Pro
            100                 105                 110


Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr Leu His
        115                 120                 125


Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Lys Cys Gly
    130                 135                 140


Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr Phe Pro
145                 150                 155                 160


Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His Gly Ile
                165                 170                 175
```

```
Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe Ser Leu
            180                 185                 190

Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly Glu Pro Ile
        195                 200                 205

Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr Phe Phe Trp
    210                 215                 220

Pro Gly Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp Ile Tyr Lys
225                 230                 235                 240

Met Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala Val Leu
                245                 250                 255

Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr Thr Leu
            260                 265                 270

Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro Val Ser
        275                 280                 285

Ser Glu Val Ile Lys Ala Leu Gln Arg Val Asp Gly Met Val Gly Met
    290                 295                 300

Leu Met Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys Leu Asn Leu
305                 310                 315                 320

Ile Leu Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys Lys Tyr
                325                 330                 335

Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val Lys Asn Ile Lys Val Ile
            340                 345                 350

Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro Asp Lys Tyr
        355                 360                 365

Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg Asn Leu Ser Cys Arg Glu
    370                 375                 380

Pro Asn Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu Pro Lys Arg
385                 390                 395                 400

Leu His Phe Ala Lys Ser Asp Arg Ile Glu Pro Leu Thr Phe Tyr Leu
                405                 410                 415

Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro Ser Glu Arg Lys Tyr Cys
            420                 425                 430
```

```
Gly Ser Gly Phe His Gly Ser Asp Asn Val Phe Ser Asn Met Gln Ala
        435                 440                 445

Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys His Gly Ile Glu Ala Asp
    450                 455                 460

Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu Leu Asn
465                 470                 475                 480

Leu Thr Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn His Leu
                485                 490                 495

Leu Lys Asn Pro Val Tyr Thr Pro Lys His Pro Lys Glu Val His Pro
            500                 505                 510

Leu Val Gln Cys Pro Phe Thr Arg Asn Pro Arg Asp Asn Leu Gly Cys
        515                 520                 525

Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu Asp Phe Gln Thr Gln Phe
    530                 535                 540

Asn Leu Thr Val Ala Glu Glu Lys Ile Ile Lys His Glu Thr Leu Pro
545                 550                 555                 560

Tyr Gly Arg Pro Arg Val Leu Gln Lys Glu Asn Thr Ile Cys Leu Leu
                565                 570                 575

Ser Gln His Gln Phe Met Ser Gly Tyr Ser Gln Asp Ile Leu Met Pro
            580                 585                 590

Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn Asp Ser Phe Ser Thr Glu
        595                 600                 605

Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe Arg Ile Pro Leu Ser Pro
    610                 615                 620

Val His Lys Cys Ser Phe Tyr Lys Asn Asn Thr Lys Val Ser Tyr Gly
625                 630                 635                 640

Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn Ser Ser Gly Ile Tyr Ser
                645                 650                 655

Glu Ala Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln Ser Phe Gln
            660                 665                 670

Val Ile Trp Arg Tyr Phe His Asp Thr Leu Leu Arg Lys Tyr Ala Glu
        675                 680                 685

Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Val Phe Asp Phe Asp
```

```
    690                 695                 700

Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn Leu Arg Gln Lys Arg Arg
705                 710                 715                 720

Val Ile Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe Phe Ile Val
                725                 730                 735

Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr Pro Leu His Cys Glu Asn
            740                 745                 750

Leu Asp Thr Leu Ala Phe Ile Leu Pro His Arg Thr Asp Asn Ser Glu
        755                 760                 765

Ser Cys Val His Gly Lys His Asp Ser Ser Trp Val Glu Glu Leu Leu
    770                 775                 780

Met Leu His Arg Ala Arg Ile Thr Asp Val Glu His Ile Thr Gly Leu
785                 790                 795                 800

Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile Leu Lys Leu
                805                 810                 815

Lys Thr His Leu Pro Thr Phe Ser Gln Glu Asp
            820                 825


<210>  3
<211>  1063
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  ENPP1 Polypeptide

<400>  3

Phe Thr Ala Gly Leu Lys Pro Ser Cys Ala Lys Glu Val Lys Ser Cys
1               5                   10                  15

Lys Gly Arg Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala
            20                  25                  30

Ala Cys Val Glu Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys
        35                  40                  45

Ile Glu Pro Glu His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu
    50                  55                  60

Lys Arg Leu Thr Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp
65                  70                  75                  80
```

```
Lys Gly Asp Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys
                85                  90                  95

Ser Trp Val Glu Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro
            100                 105                 110

Ala Gly Phe Glu Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe
        115                 120                 125

Arg Ala Glu Tyr Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser
    130                 135                 140

Lys Leu Lys Lys Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr
145                 150                 155                 160

Pro Thr Lys Thr Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr
                165                 170                 175

Pro Glu Ser His Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met
            180                 185                 190

Asn Ala Ser Phe Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp
        195                 200                 205

Tyr Lys Gly Glu Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys
    210                 215                 220

Ser Gly Thr Phe Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile
225                 230                 235                 240

Phe Pro Asp Ile Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu
                245                 250                 255

Arg Ile Leu Ala Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg
            260                 265                 270

Pro His Phe Tyr Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His
        275                 280                 285

Ser Tyr Gly Pro Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val
    290                 295                 300

Asp Gly Met Val Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu
305                 310                 315                 320

His Arg Cys Leu Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln
                325                 330                 335

Gly Ser Cys Lys Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val
```

```
            340                 345                 350

Lys Asn Ile Lys Val Ile Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser
        355                 360                 365

Asp Val Pro Asp Lys Tyr Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg
    370                 375                 380

Asn Leu Ser Cys Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys
385                 390                 395                 400

His Phe Leu Pro Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile Glu
                405                 410                 415

Pro Leu Thr Phe Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro
            420                 425                 430

Ser Glu Arg Lys Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn Val
        435                 440                 445

Phe Ser Asn Met Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys
    450                 455                 460

His Gly Ile Glu Ala Asp Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu
465                 470                 475                 480

Met Cys Asp Leu Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His
                485                 490                 495

Gly Ser Leu Asn His Leu Leu Lys Asn Pro Val Tyr Thr Pro Lys His
            500                 505                 510

Pro Lys Glu Val His Pro Leu Val Gln Cys Pro Phe Thr Arg Asn Pro
        515                 520                 525

Arg Asp Asn Leu Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu
    530                 535                 540

Asp Phe Gln Thr Gln Phe Asn Leu Thr Val Ala Glu Glu Lys Ile Ile
545                 550                 555                 560

Lys His Glu Thr Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Lys Glu
                565                 570                 575

Asn Thr Ile Cys Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr Ser
            580                 585                 590

Gln Asp Ile Leu Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn
        595                 600                 605
```

```
Asp Ser Phe Ser Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe
    610                 615                 620

Arg Ile Pro Leu Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn
625                 630                 635                 640

Thr Lys Val Ser Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn
                645                 650                 655

Ser Ser Gly Ile Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro
            660                 665                 670

Met Tyr Gln Ser Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu
        675                 680                 685

Leu Arg Lys Tyr Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly
    690                 695                 700

Pro Val Phe Asp Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn
705                 710                 715                 720

Leu Arg Gln Lys Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro
                725                 730                 735

Thr His Phe Phe Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr
            740                 745                 750

Pro Leu His Cys Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His
        755                 760                 765

Arg Thr Asp Asn Ser Glu Ser Cys Val His Gly Lys His Asp Ser Ser
    770                 775                 780

Trp Val Glu Glu Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val
785                 790                 795                 800

Glu His Ile Thr Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val
                805                 810                 815

Ser Asp Ile Leu Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu
            820                 825                 830

Asp Leu Ile Asn Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro
        835                 840                 845

Glu Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys
    850                 855                 860
```

```
Asp Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val
865                 870                 875                 880


Asp Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp
                885                 890                 895


Gly Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr
            900                 905                 910


Asn Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp
        915                 920                 925


Trp Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu
    930                 935                 940


Pro Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg
945                 950                 955                 960


Glu Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys
                965                 970                 975


Asn Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp
            980                 985                 990


Ile Ala Val Glu Trp Glu Ser Asn  Gly Gln Pro Glu Asn  Asn Tyr Lys
        995                 1000                1005


Thr Thr  Pro Pro Val Leu Asp  Ser Asp Gly Ser Phe  Phe Leu Tyr
    1010                1015                1020


Ser Lys  Leu Thr Val Asp Lys  Ser Arg Trp Gln Gln  Gly Asn Val
    1025                1030                1035


Phe Ser  Cys Ser Val Met His  Glu Ala Leu His Asn  His Tyr Thr
    1040                1045                1050


Gln Lys  Ser Leu Ser Leu Ser  Pro Gly Lys
    1055                1060


<210>  4
<211>  1060
<212>  PRT
<213>  Artificial Sequence

<220>
<223>  ENPP1 Polypeptide

<400>  4

Gly Leu Lys Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg
1               5                   10                  15
```

```
Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val
            20                  25                  30

Glu Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro
        35                  40                  45

Glu His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu
    50                  55                  60

Thr Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp
65                  70                  75                  80

Cys Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val
                85                  90                  95

Glu Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe
            100                 105                 110

Glu Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu
        115                 120                 125

Tyr Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys
    130                 135                 140

Lys Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys
145                 150                 155                 160

Thr Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser
                165                 170                 175

His Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser
            180                 185                 190

Phe Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly
        195                 200                 205

Glu Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr
    210                 215                 220

Phe Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp
225                 230                 235                 240

Ile Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu
                245                 250                 255

Ala Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe
            260                 265                 270
```

```
Tyr Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly
        275                 280                 285

Pro Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val Asp Gly Met
    290                 295                 300

Val Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys
305                 310                 315                 320

Leu Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys
                325                 330                 335

Lys Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val Lys Asn Ile
            340                 345                 350

Lys Val Ile Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro
        355                 360                 365

Asp Lys Tyr Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg Asn Leu Ser
    370                 375                 380

Cys Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu
385                 390                 395                 400

Pro Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile Glu Pro Leu Thr
                405                 410                 415

Phe Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro Ser Glu Arg
            420                 425                 430

Lys Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn Val Phe Ser Asn
        435                 440                 445

Met Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys His Gly Ile
    450                 455                 460

Glu Ala Asp Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp
465                 470                 475                 480

Leu Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu
                485                 490                 495

Asn His Leu Leu Lys Asn Pro Val Tyr Thr Pro Lys His Pro Lys Glu
            500                 505                 510

Val His Pro Leu Val Gln Cys Pro Phe Thr Arg Asn Pro Arg Asp Asn
        515                 520                 525
```

```
Leu Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu Asp Phe Gln
    530                 535                 540

Thr Gln Phe Asn Leu Thr Val Ala Glu Glu Lys Ile Ile Lys His Glu
545                 550                 555                 560

Thr Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Lys Glu Asn Thr Ile
                565                 570                 575

Cys Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr Ser Gln Asp Ile
            580                 585                 590

Leu Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn Asp Ser Phe
        595                 600                 605

Ser Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe Arg Ile Pro
    610                 615                 620

Leu Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn Thr Lys Val
625                 630                 635                 640

Ser Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn Ser Ser Gly
                645                 650                 655

Ile Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln
            660                 665                 670

Ser Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu Leu Arg Lys
        675                 680                 685

Tyr Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Val Phe
    690                 695                 700

Asp Phe Asp Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn Leu Arg Gln
705                 710                 715                 720

Lys Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe
                725                 730                 735

Phe Ile Val Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr Pro Leu His
            740                 745                 750

Cys Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His Arg Thr Asp
        755                 760                 765

Asn Ser Glu Ser Cys Val His Gly Lys His Asp Ser Ser Trp Val Glu
    770                 775                 780

Glu Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val Glu His Ile
```

```
785                 790                 795                 800

Thr Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile
                805                 810                 815

Leu Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu Asp Leu Ile
            820                 825                 830

Asn Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu
        835                 840                 845

Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu
    850                 855                 860

Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser
865                 870                 875                 880

His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu
                885                 890                 895

Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr
            900                 905                 910

Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn
        915                 920                 925

Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro
    930                 935                 940

Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln
945                 950                 955                 960

Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val
                965                 970                 975

Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val
            980                 985                 990

Glu Trp Glu Ser Asn Gly Gln Pro  Glu Asn Asn Tyr Lys  Thr Thr Pro
        995                 1000                1005

Pro Val  Leu Asp Ser Asp Gly  Ser Phe Phe Leu Tyr  Ser Lys Leu
    1010                1015                1020

Thr Val  Asp Lys Ser Arg Trp  Gln Gln Gly Asn Val  Phe Ser Cys
    1025                1030                1035

Ser Val  Met His Glu Ala Leu  His Asn His Tyr Thr  Gln Lys Ser
    1040                1045                1050
```

Leu Ser Leu Ser Pro Gly Lys
    1055   1060

<210> 5
<211> 1057
<212> PRT
<213> Artificial Sequence

<220>
<223> ENPP1 Polypeptide

<400> 5

Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg Cys Phe Glu
1   5   10   15

Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val Glu Leu Gly
            20   25   30

Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro Glu His Ile
        35   40   45

Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu Thr Arg Ser
    50   55   60

Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp Cys Cys Ile
65   70   75   80

Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val Glu Glu Pro
                85   90   95

Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe Glu Thr Pro
            100   105   110

Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr Leu His
        115   120   125

Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Lys Cys Gly
    130   135   140

Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr Phe Pro
145   150   155   160

Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His Gly Ile
                165   170   175

Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe Ser Leu
            180   185   190

Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly Glu Pro Ile

```
        195                 200                 205

Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr Phe Phe Trp
    210                 215                 220

Pro Gly Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp Ile Tyr Lys
225                 230                 235                 240

Met Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala Val Leu
                245                 250                 255

Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr Thr Leu
            260                 265                 270

Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro Val Ser
        275                 280                 285

Ser Glu Val Ile Lys Ala Leu Gln Arg Val Asp Gly Met Val Gly Met
    290                 295                 300

Leu Met Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys Leu Asn Leu
305                 310                 315                 320

Ile Leu Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys Lys Tyr
                325                 330                 335

Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val Lys Asn Ile Lys Val Ile
            340                 345                 350

Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro Asp Lys Tyr
        355                 360                 365

Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg Asn Leu Ser Cys Arg Glu
    370                 375                 380

Pro Asn Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu Pro Lys Arg
385                 390                 395                 400

Leu His Phe Ala Lys Ser Asp Arg Ile Glu Pro Leu Thr Phe Tyr Leu
                405                 410                 415

Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro Ser Glu Arg Lys Tyr Cys
            420                 425                 430

Gly Ser Gly Phe His Gly Ser Asp Asn Val Phe Ser Asn Met Gln Ala
        435                 440                 445

Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys His Gly Ile Glu Ala Asp
    450                 455                 460
```

```
Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu Leu Asn
465                 470                 475                 480

Leu Thr Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn His Leu
                485                 490                 495

Leu Lys Asn Pro Val Tyr Thr Pro Lys His Pro Lys Glu Val His Pro
            500                 505                 510

Leu Val Gln Cys Pro Phe Thr Arg Asn Pro Arg Asp Asn Leu Gly Cys
        515                 520                 525

Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu Asp Phe Gln Thr Gln Phe
    530                 535                 540

Asn Leu Thr Val Ala Glu Glu Lys Ile Ile Lys His Glu Thr Leu Pro
545                 550                 555                 560

Tyr Gly Arg Pro Arg Val Leu Gln Lys Glu Asn Thr Ile Cys Leu Leu
                565                 570                 575

Ser Gln His Gln Phe Met Ser Gly Tyr Ser Gln Asp Ile Leu Met Pro
            580                 585                 590

Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn Asp Ser Phe Ser Thr Glu
        595                 600                 605

Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe Arg Ile Pro Leu Ser Pro
    610                 615                 620

Val His Lys Cys Ser Phe Tyr Lys Asn Asn Thr Lys Val Ser Tyr Gly
625                 630                 635                 640

Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn Ser Ser Gly Ile Tyr Ser
                645                 650                 655

Glu Ala Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln Ser Phe Gln
            660                 665                 670

Val Ile Trp Arg Tyr Phe His Asp Thr Leu Leu Arg Lys Tyr Ala Glu
        675                 680                 685

Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Val Phe Asp Phe Asp
    690                 695                 700

Tyr Asp Gly Arg Cys Asp Ser Leu Glu Asn Leu Arg Gln Lys Arg Arg
705                 710                 715                 720
```

```
Val Ile Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe Phe Ile Val
                725                 730                 735

Leu Thr Ser Cys Lys Asp Thr Ser Gln Thr Pro Leu His Cys Glu Asn
            740                 745                 750

Leu Asp Thr Leu Ala Phe Ile Leu Pro His Arg Thr Asp Asn Ser Glu
        755                 760                 765

Ser Cys Val His Gly Lys His Asp Ser Ser Trp Val Glu Glu Leu Leu
    770                 775                 780

Met Leu His Arg Ala Arg Ile Thr Asp Val Glu His Ile Thr Gly Leu
785                 790                 795                 800

Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile Leu Lys Leu
                805                 810                 815

Lys Thr His Leu Pro Thr Phe Ser Gln Glu Asp Leu Ile Asn Asp Lys
            820                 825                 830

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
        835                 840                 845

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
    850                 855                 860

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
865                 870                 875                 880

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
                885                 890                 895

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
            900                 905                 910

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
        915                 920                 925

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
    930                 935                 940

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
945                 950                 955                 960

Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr
                965                 970                 975
```

```
Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
            980                 985                 990


Ser Asn Gly Gln Pro Glu Asn Asn  Tyr Lys Thr Thr Pro  Pro Val Leu
        995                 1000                1005


Asp Ser  Asp Gly Ser Phe Phe  Leu Tyr Ser Lys Leu  Thr Val Asp
    1010                1015                1020


Lys Ser  Arg Trp Gln Gln Gly  Asn Val Phe Ser Cys  Ser Val Met
    1025                1030                1035


His Glu  Ala Leu His Asn His  Tyr Thr Gln Lys Ser  Leu Ser Leu
    1040                1045                1050


Ser Pro  Gly Lys
    1055
```

<210> 6
<211> 227
<212> PRT
<213> Artificial Sequence

<220>
<223> human IgG1 Fc

<400> 6

```
Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
1               5                   10                  15


Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            20                  25                  30


Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        35                  40                  45


Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    50                  55                  60


His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
65                  70                  75                  80


Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                85                  90                  95


Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            100                 105                 110


Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
        115                 120                 125
```

```
Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
    130                 135                 140

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
145                 150                 155                 160

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
                165                 170                 175

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
            180                 185                 190

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
        195                 200                 205

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
    210                 215                 220

Pro Gly Lys
225
```

<210> 7
<211> 906
<212> PRT
<213> Mus musculus

<400> 7

```
Met Glu Arg Asp Gly Asp Gln Ala Gly His Gly Pro Arg His Gly Ser
1               5                   10                  15

Ala Gly Asn Gly Arg Glu Leu Glu Ser Pro Ala Ala Ala Ser Leu Leu
            20                  25                  30

Ala Pro Met Asp Leu Gly Glu Glu Pro Leu Glu Lys Ala Glu Arg Ala
        35                  40                  45

Arg Pro Ala Lys Asp Pro Asn Thr Tyr Lys Val Leu Ser Leu Val Leu
    50                  55                  60

Ser Val Cys Val Leu Thr Thr Ile Leu Gly Cys Ile Phe Gly Leu Lys
65                  70                  75                  80

Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg Cys Phe Glu
                85                  90                  95

Arg Thr Phe Ser Asn Cys Arg Cys Asp Ala Ala Cys Val Ser Leu Gly
            100                 105                 110

Asn Cys Cys Leu Asp Phe Gln Glu Thr Cys Val Glu Pro Thr His Ile
```

```
        115                 120                 125

Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu Ser Arg Phe
    130                 135                 140

Val Cys Ser Cys Ala Asp Asp Cys Lys Thr His Asn Asp Cys Cys Ile
145                 150                 155                 160

Asn Tyr Ser Ser Val Cys Gln Asp Lys Lys Ser Trp Val Glu Glu Thr
                165                 170                 175

Cys Glu Ser Ile Asp Thr Pro Glu Cys Pro Ala Glu Phe Glu Ser Pro
            180                 185                 190

Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr Leu His
        195                 200                 205

Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Asn Cys Gly
    210                 215                 220

Thr Tyr Thr Lys Asn Met Arg Pro Met Tyr Pro Thr Lys Thr Phe Pro
225                 230                 235                 240

Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His Gly Ile
                245                 250                 255

Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe Ser Leu
            260                 265                 270

Lys Ser Lys Glu Lys Phe Asn Pro Leu Trp Tyr Lys Gly Gln Pro Ile
        275                 280                 285

Trp Val Thr Ala Asn His Gln Glu Val Lys Ser Gly Thr Tyr Phe Trp
    290                 295                 300

Pro Gly Ser Asp Val Glu Ile Asp Gly Ile Leu Pro Asp Ile Tyr Lys
305                 310                 315                 320

Val Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala Val Leu
                325                 330                 335

Glu Trp Leu Gln Leu Pro Ser His Glu Arg Pro His Phe Tyr Thr Leu
            340                 345                 350

Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser His Gly Pro Val Ser
        355                 360                 365

Ser Glu Val Ile Lys Ala Leu Gln Lys Val Asp Arg Leu Val Gly Met
    370                 375                 380
```

```
Leu Met Asp Gly Leu Lys Asp Leu Gly Leu Asp Lys Cys Leu Asn Leu
385                 390                 395                 400

Ile Leu Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys Lys Tyr
                405                 410                 415

Val Tyr Leu Asn Lys Tyr Leu Gly Asp Val Asn Asn Val Lys Val Val
            420                 425                 430

Tyr Gly Pro Ala Ala Arg Leu Arg Pro Thr Asp Val Pro Glu Thr Tyr
        435                 440                 445

Tyr Ser Phe Asn Tyr Glu Ala Leu Ala Lys Asn Leu Ser Cys Arg Glu
    450                 455                 460

Pro Asn Gln His Phe Arg Pro Tyr Leu Lys Pro Phe Leu Pro Lys Arg
465                 470                 475                 480

Leu His Phe Ala Lys Ser Asp Arg Ile Glu Pro Leu Thr Phe Tyr Leu
                485                 490                 495

Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro Ser Glu Arg Lys Tyr Cys
            500                 505                 510

Gly Ser Gly Phe His Gly Ser Asp Asn Leu Phe Ser Asn Met Gln Ala
        515                 520                 525

Leu Phe Ile Gly Tyr Gly Pro Ala Phe Lys His Gly Ala Glu Val Asp
    530                 535                 540

Ser Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu Leu Gly
545                 550                 555                 560

Leu Ile Pro Ala Pro Asn Asn Gly Ser His Gly Ser Leu Asn His Leu
                565                 570                 575

Leu Lys Lys Pro Ile Tyr Asn Pro Ser His Pro Lys Glu Glu Gly Phe
            580                 585                 590

Leu Ser Gln Cys Pro Ile Lys Ser Thr Ser Asn Asp Leu Gly Cys Thr
        595                 600                 605

Cys Asp Pro Trp Ile Val Pro Ile Lys Asp Phe Glu Lys Gln Leu Asn
    610                 615                 620

Leu Thr Thr Glu Asp Val Asp Asp Ile Tyr His Met Thr Val Pro Tyr
625                 630                 635                 640
```

```
Gly Arg Pro Arg Ile Leu Leu Lys Gln His Arg Val Cys Leu Leu Gln
                645                 650                 655

Gln Gln Gln Phe Leu Thr Gly Tyr Ser Leu Asp Leu Leu Met Pro Leu
            660                 665                 670

Trp Ala Ser Tyr Thr Phe Leu Ser Asn Asp Gln Phe Ser Arg Asp Asp
        675                 680                 685

Phe Ser Asn Cys Leu Tyr Gln Asp Leu Arg Ile Pro Leu Ser Pro Val
    690                 695                 700

His Lys Cys Ser Tyr Tyr Lys Ser Asn Ser Lys Leu Ser Tyr Gly Phe
705                 710                 715                 720

Leu Thr Pro Pro Arg Leu Asn Arg Val Ser Asn His Ile Tyr Ser Glu
                725                 730                 735

Ala Leu Leu Thr Ser Asn Ile Val Pro Met Tyr Gln Ser Phe Gln Val
            740                 745                 750

Ile Trp His Tyr Leu His Asp Thr Leu Leu Gln Arg Tyr Ala His Glu
        755                 760                 765

Arg Asn Gly Ile Asn Val Val Ser Gly Pro Val Phe Asp Phe Asp Tyr
    770                 775                 780

Asp Gly Arg Tyr Asp Ser Leu Glu Ile Leu Lys Gln Asn Ser Arg Val
785                 790                 795                 800

Ile Arg Ser Gln Glu Ile Leu Ile Pro Thr His Phe Phe Ile Val Leu
                805                 810                 815

Thr Ser Cys Lys Gln Leu Ser Glu Thr Pro Leu Glu Cys Ser Ala Leu
            820                 825                 830

Glu Ser Ser Ala Tyr Ile Leu Pro His Arg Pro Asp Asn Ile Glu Ser
        835                 840                 845

Cys Thr His Gly Lys Arg Glu Ser Ser Trp Val Glu Glu Leu Leu Thr
    850                 855                 860

Leu His Arg Ala Arg Val Thr Asp Val Glu Leu Ile Thr Gly Leu Ser
865                 870                 875                 880

Phe Tyr Gln Asp Arg Gln Glu Ser Val Ser Glu Leu Leu Arg Leu Lys
                885                 890                 895
```

```
Thr His Leu Pro Ile Phe Ser Gln Glu Asp
            900                 905


<210>  8
<211>  924
<212>  PRT
<213>  Bos taurus

<400>  8

Met Glu Arg Asp Ser Cys Ala Gly Gly Gly Ser Arg Gly Gly Glu Gly
1               5                   10                  15


Gly Arg Gly Pro Arg Glu Gly Leu Ala Gly Asn Gly Arg Asp Pro Gly
            20                  25                  30


Pro Gly Arg Ala Ala Glu Ala Ser Gly Glu Pro Gln Ala Ala Ala Ser
        35                  40                  45


Leu Leu Ala Pro Met Asp Leu Gly Glu Glu Pro Leu Glu Arg Ala Ala
    50                  55                  60


Arg Ala Arg Pro Ala Lys Asp Pro Asn Thr Tyr Lys Val Leu Ser Leu
65                  70                  75                  80


Val Leu Ser Val Cys Val Leu Thr Thr Ile Leu Gly Cys Ile Phe Gly
                85                  90                  95


Leu Lys Pro Ser Cys Ala Lys Glu Ile Lys Ser Cys Lys Gly Arg Cys
            100                 105                 110


Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val Asp
        115                 120                 125


Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro Glu
    130                 135                 140


Arg Ile Trp Thr Cys Thr Lys Phe Arg Cys Gly Glu Lys Arg Leu Ser
145                 150                 155                 160


Arg Ser Leu Cys Ser Cys Ser Asp Asp Cys Lys Asp Lys Gly Asp Cys
                165                 170                 175


Cys Ile Asn His Gly Ser Val Cys Arg Gly Glu Lys Ser Trp Ala Glu
            180                 185                 190


Glu Glu Cys Asp Ser Ile Asp Glu Pro Gln Cys Pro Ala Gly Phe Glu
        195                 200                 205


Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr
    210                 215                 220
```

```
Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Thr
225                 230                 235                 240

Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr
                245                 250                 255

Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His
            260                 265                 270

Gly Ile Ile Asp Asn Asn Ile Tyr Asp Pro Gln Met Asn Ala Asn Phe
        275                 280                 285

Ala Leu Lys Asn Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly Glu
    290                 295                 300

Pro Ile Trp Leu Thr Ala Lys Tyr Gln Gly Leu Lys Thr Gly Thr Phe
305                 310                 315                 320

Phe Trp Pro Gly Ser Asp Val Lys Ile Asn Gly Ile Phe Pro Asp Ile
                325                 330                 335

Tyr Lys Ile Tyr Asn Val Ser Val Pro Phe Glu Glu Arg Ile Leu Ala
            340                 345                 350

Ile Leu Lys Trp Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr
        355                 360                 365

Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro
    370                 375                 380

Val Ser Ser Glu Val Ile Arg Ala Leu Gln Arg Val Asp Asn Met Val
385                 390                 395                 400

Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys Leu
                405                 410                 415

Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys
            420                 425                 430

Lys Tyr Val Tyr Leu Asn Lys Tyr Leu Gly Asp Thr Lys Asp Tyr Lys
        435                 440                 445

Val Val Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro Asp
    450                 455                 460

Lys Tyr Tyr Ser Phe Asp Tyr Glu Gly Ile Ala Lys Asn Leu Ser Cys
465                 470                 475                 480
```

```
Gln Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu Pro
                485                 490                 495

Lys Arg Leu His Phe Ala Lys Asn Asp Arg Ile Glu Arg Leu Thr Phe
            500                 505                 510

Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro Ser Glu Arg Lys
        515                 520                 525

Tyr Cys Gly Gly Gly Phe His Gly Ser Asp Asn Thr Phe Leu Asn Met
    530                 535                 540

Gln Ala Leu Phe Ile Gly Tyr Gly Pro Gly Phe Lys His Ser Thr Glu
545                 550                 555                 560

Val Asp Ser Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu
                565                 570                 575

Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn
            580                 585                 590

His Leu Leu Ser Asn Pro Val Tyr Thr Pro Lys His Pro Lys Glu Val
        595                 600                 605

Arg Pro Leu Val Gln Cys Pro Phe Thr Arg Ala Pro Arg Glu Ser Leu
    610                 615                 620

Asp Cys Ser Cys Asp Pro Ser Ile Leu Pro Ile Val Asp Phe Gln Thr
625                 630                 635                 640

Gln Leu Asn Leu Thr Met Ala Glu Glu Lys Thr Ile Lys Arg Gly Ala
                645                 650                 655

Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Asn Ser Thr Val Cys Leu
            660                 665                 670

Leu Tyr Gln His Gln Phe Val Ser Gly Tyr Ser Arg Asp Ile Leu Met
        675                 680                 685

Pro Leu Trp Thr Ser Tyr Thr Ile Gly Arg Asn Asp Ser Phe Ser Thr
    690                 695                 700

Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Leu Arg Ile Pro Leu Ser
705                 710                 715                 720

Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn Ala Lys Leu Ser Tyr
                725                 730                 735
```

```
Gly Leu Leu Ser Pro Pro Gln Leu His Lys Gly Ser Ser Gln Val Tyr
            740                 745                 750

Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln Ser Phe
        755                 760                 765

Gln Val Ile Trp His Tyr Leu His Gly Thr Leu Leu Gln Arg Tyr Ala
    770                 775                 780

Glu Glu Arg Asn Gly Leu Asn Val Val Ser Gly Pro Val Phe Asp Ser
785                 790                 795                 800

Asp Tyr Asp Gly Arg Tyr Asp Ser Leu Glu Thr Leu Lys Gln Asn Ser
                805                 810                 815

Lys Ile Ile Arg Asn Leu Glu Val Leu Ile Pro Thr His Phe Phe Leu
            820                 825                 830

Val Leu Thr Ser Cys Lys Asn Thr Ser Gln Thr Pro Leu Gln Cys Glu
        835                 840                 845

Asn Leu Asp Ala Met Ala Phe Ile Leu Pro His Lys Thr Asp Asn Ser
    850                 855                 860

Glu Ser Cys Ala His Gly Lys His Glu Ser Leu Trp Val Glu Glu Leu
865                 870                 875                 880

Leu Lys Leu His Thr Ala Arg Ile Thr Asp Val Glu His Ile Thr Gly
                885                 890                 895

Leu Ser Phe Tyr Gln Glu Arg Lys Glu Pro Ile Ser Asp Ile Leu Lys
            900                 905                 910

Leu Lys Thr His Leu Pro Thr Phe Asn Gln Glu Asp
        915                 920


<210>  9
<211>  925
<212>  PRT
<213>  Oryctolagus cuniculus

<400>  9

Met Glu Arg Asp Gly Cys Ala Gly Gly Gly Ser Arg Gly Gly Glu Gly
1               5                   10                  15

Gly Arg Ala Pro Arg Glu Gly Pro Ala Gly Asn Ser Arg Asp Pro Gly
            20                  25                  30

Arg Ser His Ala Ala Glu Ala Pro Gly Asn Pro Gln Ala Ala Ala Ser
        35                  40                  45
```

```
Leu Leu Ala Pro Met Asp Val Gly Glu Glu Pro Leu Glu Lys Ala Ala
    50                  55                  60

Arg Ala Arg Thr Ala Lys Asp Pro Asn Thr Tyr Lys Val Leu Ser Leu
65                  70                  75                  80

Val Leu Ser Val Cys Val Leu Thr Thr Ile Leu Gly Cys Ile Phe Gly
                85                  90                  95

Leu Lys Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg Cys
            100                 105                 110

Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Ala Ala Cys Val Glu
        115                 120                 125

Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro Glu
    130                 135                 140

His Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu Thr
145                 150                 155                 160

Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Asp Gln Gly Asp Cys
                165                 170                 175

Cys Ile Asn Tyr Ser Ser Val Cys Gln Gly Glu Lys Ser Trp Val Glu
            180                 185                 190

Glu Pro Cys Glu Ser Ile Asn Glu Pro Gln Cys Pro Ala Gly Phe Glu
        195                 200                 205

Thr Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu Tyr
    210                 215                 220

Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys Lys
225                 230                 235                 240

Cys Gly Thr Tyr Thr Lys Asn Met Arg Pro Val Tyr Pro Thr Lys Thr
                245                 250                 255

Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser His
            260                 265                 270

Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser Phe
        275                 280                 285

Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly Glu
    290                 295                 300
```

```
Pro Ile Trp Val Thr Ala Lys Tyr Gln Gly Leu Lys Ser Gly Thr Phe
305                 310                 315                 320

Phe Trp Pro Gly Ser Asp Val Glu Ile Asn Gly Ile Phe Pro Asp Ile
                325                 330                 335

Tyr Lys Met Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu Ala
            340                 345                 350

Val Leu Gln Trp Leu Gln Leu Pro Lys Asp Glu Arg Pro His Phe Tyr
        355                 360                 365

Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly Pro
    370                 375                 380

Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val Asp Asn Met Val
385                 390                 395                 400

Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu His Arg Cys Leu
                405                 410                 415

Asn Leu Ile Leu Val Ser Asp His Gly Met Glu Gln Gly Ser Cys Lys
            420                 425                 430

Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Val Lys Asn Ile Lys
        435                 440                 445

Val Ile Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro Asp
    450                 455                 460

Lys Tyr Tyr Ser Phe Asn Tyr Glu Gly Ile Ala Arg Asn Leu Ser Cys
465                 470                 475                 480

Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu Pro
                485                 490                 495

Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile Glu Pro Leu Thr Phe
            500                 505                 510

Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Asn Pro Ser Glu Arg Lys
        515                 520                 525

Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn Ile Phe Ser Asn Met
    530                 535                 540

Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe Lys His Gly Ile Glu
545                 550                 555                 560
```

```
Val Asp Thr Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp Leu
                565                 570                 575

Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu Asn
            580                 585                 590

His Leu Leu Lys Asn Pro Val Tyr Thr Pro Lys His Pro Lys Glu Val
        595                 600                 605

His Pro Leu Ile Gln Cys Pro Phe Thr Arg Asn Pro Arg Asp Asn Leu
    610                 615                 620

Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile Glu Asp Phe Gln Thr
625                 630                 635                 640

Gln Phe Asn Leu Thr Val Ala Glu Glu Lys Asn Ile Lys His Glu Thr
                645                 650                 655

Leu Pro Tyr Gly Arg Pro Arg Val Leu Gln Lys Lys Asn Thr Ile Cys
            660                 665                 670

Leu Leu Ser Gln His Gln Phe Met Ser Gly Tyr Ser Gln Asp Ile Leu
        675                 680                 685

Met Pro Leu Trp Thr Ser Tyr Thr Val Asp Arg Asn Asp Ser Phe Ser
    690                 695                 700

Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Phe Arg Ile Ser Leu
705                 710                 715                 720

Ser Pro Val His Lys Cys Ser Phe Tyr Lys Asn Asn Thr Lys Val Ser
                725                 730                 735

Tyr Gly Phe Leu Ser Pro Pro Gln Leu Asn Lys Asn Ser Arg Gly Ile
            740                 745                 750

Tyr Ser Glu Ala Leu Leu Thr Thr Asn Ile Val Pro Met Tyr Gln Ser
        755                 760                 765

Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu Leu Arg Lys Tyr
    770                 775                 780

Ala Glu Glu Arg Asn Gly Val Asn Val Val Ser Gly Pro Val Phe Asp
785                 790                 795                 800

Phe Asp Tyr Asp Gly Arg Tyr Asp Ser Leu Glu Ile Leu Arg Gln Lys
                805                 810                 815

Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe Phe
```

```
            820                 825                 830

Ile Val Leu Thr Ser Cys Lys Asp Ala Ser Gln Thr Pro Leu His Cys
        835                 840                 845

Glu Asn Leu Asp Thr Leu Ala Phe Ile Leu Pro His Arg Thr Asp Asn
    850                 855                 860

Ser Glu Ser Cys Leu His Gly Lys His Glu Ser Ser Trp Val Glu Glu
865                 870                 875                 880

Leu Leu Met Leu His Arg Ala Arg Ile Thr Asp Val Glu His Ile Thr
                885                 890                 895

Gly Leu Ser Phe Tyr Gln Gln Arg Lys Glu Pro Val Ser Asp Ile Leu
            900                 905                 910

Lys Leu Lys Thr His Leu Pro Thr Phe Ser Gln Glu Asp
        915                 920                 925
```

<210> 10
<211> 925
<212> PRT
<213> Papio hamadryas

<400> 10

```
Met Glu Arg Asp Gly Cys Ala Gly Gly Gly Ser Gln Gly Gly Gly Lys
1               5                   10                  15

Gly Gly Arg Gly Pro Arg Glu Gly Leu Ala Gly Asn Gly Arg Asp Pro
            20                  25                  30

Ser His Gly Gln Ala Ser Glu Ala Pro Gly Asp Pro Gln Ala Ala Ala
        35                  40                  45

Ser Leu Leu Ala Pro Met Asp Leu Gly Glu Glu Pro Leu Glu Lys Ala
    50                  55                  60

Ala Gly Ala Arg Pro Ala Lys Asp Pro Asn Thr Tyr Lys Val Leu Ser
65                  70                  75                  80

Leu Val Leu Ser Val Cys Val Leu Thr Thr Ile Leu Gly Cys Ile Phe
                85                  90                  95

Gly Leu Lys Pro Ser Cys Ala Lys Glu Val Lys Ser Cys Lys Gly Arg
            100                 105                 110

Cys Phe Glu Arg Thr Phe Gly Asn Cys Arg Cys Asp Val Ala Cys Val
        115                 120                 125
```

```
Asp Leu Gly Asn Cys Cys Leu Asp Tyr Gln Glu Thr Cys Ile Glu Pro
    130                 135                 140

Glu Arg Ile Trp Thr Cys Asn Lys Phe Arg Cys Gly Glu Lys Arg Leu
145                 150                 155                 160

Ser Arg Ser Leu Cys Ala Cys Ser Asp Asp Cys Lys Glu Arg Gly Asp
                165                 170                 175

Cys Cys Ile Asn Tyr Ser Ala Val Cys Gln Gly Glu Lys Ser Trp Val
            180                 185                 190

Glu Glu Thr Cys Glu Asn Ile Asn Glu Pro Gln Cys Pro Glu Gly Phe
        195                 200                 205

Glu Met Pro Pro Thr Leu Leu Phe Ser Leu Asp Gly Phe Arg Ala Glu
    210                 215                 220

Tyr Leu His Thr Trp Gly Gly Leu Leu Pro Val Ile Ser Lys Leu Lys
225                 230                 235                 240

Lys Cys Gly Thr Tyr Ala Lys Asn Met Arg Pro Val Tyr Pro Thr Lys
                245                 250                 255

Thr Phe Pro Asn His Tyr Ser Ile Val Thr Gly Leu Tyr Pro Glu Ser
            260                 265                 270

His Gly Ile Ile Asp Asn Lys Met Tyr Asp Pro Lys Met Asn Ala Ser
        275                 280                 285

Phe Ser Leu Lys Ser Lys Glu Lys Phe Asn Pro Glu Trp Tyr Lys Gly
    290                 295                 300

Glu Pro Ile Trp Leu Thr Ala Lys Tyr Gln Gly Leu Arg Ser Gly Thr
305                 310                 315                 320

Phe Phe Trp Pro Gly Ser Asp Val Lys Ile Asn Gly Ile Phe Pro Asp
                325                 330                 335

Ile Tyr Lys Ile Tyr Asn Gly Ser Val Pro Phe Glu Glu Arg Ile Leu
            340                 345                 350

Ala Ile Leu Lys Trp Leu Arg Leu Pro Lys Asp Glu Arg Pro His Phe
        355                 360                 365

Tyr Thr Leu Tyr Leu Glu Glu Pro Asp Ser Ser Gly His Ser Tyr Gly
    370                 375                 380
```

```
Pro Val Ser Ser Glu Val Ile Lys Ala Leu Gln Arg Val Asp Asn Met
385                 390                 395                 400

Val Gly Met Leu Met Asp Gly Leu Lys Glu Leu Asn Leu His Gln Cys
                405                 410                 415

Leu Asn Leu Ile Leu Ile Ser Asp His Gly Met Glu Gln Gly Ser Cys
            420                 425                 430

Lys Lys Tyr Ile Tyr Leu Asn Lys Tyr Leu Gly Asp Thr Lys Asn Ile
        435                 440                 445

Lys Val Ile Tyr Gly Pro Ala Ala Arg Leu Arg Pro Ser Asp Val Pro
    450                 455                 460

Glu Lys Tyr Tyr Ser Phe Asn Tyr Glu Asn Ile Ala Arg Asn Leu Ser
465                 470                 475                 480

Cys Arg Glu Pro Asn Gln His Phe Lys Pro Tyr Leu Lys His Phe Leu
                485                 490                 495

Pro Lys Arg Leu His Phe Ala Lys Ser Asp Arg Ile Glu Pro Leu Thr
            500                 505                 510

Phe Tyr Leu Asp Pro Gln Trp Gln Leu Ala Leu Ser Pro Ser Glu Arg
        515                 520                 525

Lys Tyr Cys Gly Ser Gly Phe His Gly Ser Asp Asn Val Phe Ser Asn
    530                 535                 540

Met Gln Ala Leu Phe Val Gly Tyr Gly Pro Gly Phe Gln His Gly Ile
545                 550                 555                 560

Glu Val Asp Ser Phe Glu Asn Ile Glu Val Tyr Asn Leu Met Cys Asp
                565                 570                 575

Leu Leu Asn Leu Thr Pro Ala Pro Asn Asn Gly Thr His Gly Ser Leu
            580                 585                 590

Asn His Leu Leu Lys Asn Pro Ile Tyr Thr Pro Lys His Pro Lys Glu
        595                 600                 605

Val Gln Pro Ser Val Gln Cys Pro Leu Ala Gly Ser Pro Arg Asp Ser
    610                 615                 620

Leu Gly Cys Ser Cys Asn Pro Ser Ile Leu Pro Ile Val Asp Phe Gln
625                 630                 635                 640

Thr Gln Phe Asn Leu Thr Thr Ala Glu Glu Lys Asn Ile Asn Arg Ala
```

```
                645                 650                 655

Ser Leu Pro Tyr Gly Arg Pro Arg Leu Leu Gln Lys Lys Ser Ser Val
            660                 665                 670

Cys Leu Leu Tyr Gln His Gln Phe Val Ser Gly Tyr Ser His Asp Val
        675                 680                 685

Leu Met Pro Leu Trp Thr Ser Tyr Thr Val Asn Arg Asn Asp Ser Phe
    690                 695                 700

Ser Thr Glu Asp Phe Ser Asn Cys Leu Tyr Gln Asp Leu Arg Ile Ser
705                 710                 715                 720

Phe Ser Pro Ile His Asn Cys Ser Phe Tyr Lys Asn Asn Ala Lys Leu
                725                 730                 735

Ser Tyr Gly Phe Leu Ser Pro Pro Gln Leu Ser Lys Asp Ser Ser Gln
            740                 745                 750

Ile Tyr Ser Glu Ala Leu Leu Thr Ser Asn Ile Val Pro Met Tyr Gln
        755                 760                 765

Ser Phe Gln Val Ile Trp Arg Tyr Phe His Asp Thr Leu Leu Gln Arg
    770                 775                 780

Tyr Ala Glu Glu Arg Asn Ser Ile Asn Val Val Ser Gly Pro Val Phe
785                 790                 795                 800

Asp Ser Asp Tyr Asp Gly Arg Tyr Asp Ser Ser Glu Ala Leu Lys Arg
                805                 810                 815

Asn Arg Arg Val Ile Arg Asn Gln Glu Ile Leu Ile Pro Thr His Phe
            820                 825                 830

Phe Ile Val Ile Thr Ser Cys Lys Asn Thr Ser Gln Thr Pro Leu Gln
        835                 840                 845

Cys Asp Asn Leu Asp Pro Leu Ala Phe Ile Leu Pro His Arg Ser Asp
    850                 855                 860

Asn Ser Glu Ser Cys Val His Glu Lys Arg Glu Ser Ser Trp Ile Glu
865                 870                 875                 880

Glu Leu Leu Met Met His Arg Ala Arg Ile Met Asp Val Glu His Ile
                885                 890                 895

Thr Gly Leu Ser Phe Tyr Gln Glu Arg Lys Glu Pro Val Ser Asp Ile
            900                 905                 910
```

Leu Lys Leu Lys Thr His Leu Pro Thr Val Ser Gln Glu
915 920 925

<210> 11

<400> 11
000

<210> 12

<400> 12
000

<210> 13

<400> 13
000

<210> 14

<400> 14
000

<210> 15

<400> 15
000

<210> 16

<400> 16
000

<210> 17

<400> 17
000

<210> 18

<400> 18
000

<210> 19

<400> 19
000

<210> 20

<400> 20
000

<210> 21

<400> 21
000

<210> 22

<400> 22
000

<210> 23

<400> 23
000

<210> 24

<400> 24
000

<210> 25

<400> 25
000

<210> 26

<400> 26
000

<210> 27
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 27

Gly Gly Gly Ala
1

<210> 28
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 28

Gly Gly Gly Ser
1

<210> 29
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 29

Gly Gly Gly Gly
1

<210> 30
<211> 5

<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 30

Gly Gly Gly Gly Ala
1   5

<210> 31
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 31

Gly Gly Gly Gly Ser
1   5

<210> 32
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 32

Gly Gly Gly Gly Gly
1   5

<210> 33
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 33

Gly Gly Ala Gly
1

<210> 34
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 34

Gly Gly Ser Gly

1

<210> 35
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 35

Ala Gly Gly Gly
1

<210> 36
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 36

Ser Gly Gly Gly Gly
1   5

<210> 37
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 37

Ser Gly Gly Gly
1

<210> 38
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 38

Gly Ala Gly Ala
1

<210> 39
<211> 4
<212> PRT
<213> Artificial Sequence

<220>

<223> Linker

<400> 39

Gly Ser Gly Ser
1

<210> 40
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 40

Gly Ala Gly Ala Gly Ala
1   5

<210> 41
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 41

Gly Ser Gly Ser Gly Ser
1   5

<210> 42
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 42

Gly Ala Gly Ala Gly Ala Gly Ala
1   5

<210> 43
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 43

Gly Ser Gly Ser Gly Ser Gly Ser
1   5

<210> 44

<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 44

Gly Ala Gly Ala Gly Ala Gly Ala Gly Ala
1   5   10

<210> 45
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 45

Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
1   5   10

<210> 46
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 46

Gly Ala Gly Ala Gly Ala Gly Ala Gly Ala Gly Ala
1   5   10

<210> 47
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 47

Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser Gly Ser
1   5   10

<210> 48
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 48

Gly Gly Ala Gly Gly Ala
1   5

<210> 49
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 49

Gly Gly Ser Gly Gly Ser
1   5

<210> 50
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 50

Gly Gly Ala Gly Gly Ala Gly Gly Ala
1   5

<210> 51
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 51

Gly Gly Ser Gly Gly Ser Gly Gly Ser
1   5

<210> 52
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 52

Gly Gly Ala Gly Gly Ala Gly Gly Ala Gly Gly Ala
1   5   10

<210> 53
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 53

Gly Gly Ser Gly Gly Ser Gly Gly Ser Gly Gly Ser
1   5   10

<210> 54
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 54

Gly Gly Ala Gly
1

<210> 55
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 55

Gly Gly Ser Gly
1

<210> 56
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 56

Gly Gly Ala Gly
1

<210> 57
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 57

Gly Gly Ser Gly
1

<210> 58
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 58

Gly Gly Ala Gly Gly Gly Ala Gly
1   5

<210> 59
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 59

Gly Gly Ser Gly Gly Gly Ser Gly
1   5

<210> 60
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 60

Gly Gly Ala Gly Gly Gly Ala Gly Gly Gly Ala Gly
1   5   10

<210> 61
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 61

Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Gly
1   5   10

<210> 62
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 62

Gly Gly Gly Gly Ala
1   5

<210> 63
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 63

Gly Gly Gly Gly Ser
1   5

<210> 64
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 64

Gly Gly Gly Gly Ala Gly Gly Gly Gly Ala Gly Gly Gly Gly Ala
1   5   10   15

<210> 65
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 65

Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser Gly Gly Gly Gly Ser
1   5   10   15

<210> 66
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 66

Gly Gly Gly Ala Gly
1   5

<210> 67
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 67

Gly Gly Gly Ala Gly Gly
1   5

<210> 68
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 68

Gly Gly Gly Ala Gly Gly Gly
1   5

<210> 69

<400> 69
000

<210> 70
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 70

Thr Gly Gly Gly Gly
1   5

<210> 71
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 71

Ala Ala Ala Leu
1

<210> 72
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 72

Ala Ala Ala Lys
1

<210> 73
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 73

Ala Ala Ala Arg
1

<210> 74
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 74

Glu Gly Lys Ser Ser Gly Ser Gly Ser Glu Ser Lys Ser Thr
1   5   10

<210> 75
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 75

Gly Ser Ala Gly Ser Ala Ala Gly Ser Gly Glu Phe
1   5   10

<210> 76
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 76

Ala Glu Ala Ala Ala Lys Glu Ala Ala Ala Lys Ala
1   5   10

<210> 77
<211> 18
<212> PRT

<213> Artificial Sequence

<220>
<223> Linker

<400> 77

Lys Glu Ser Gly Ser Val Ser Ser Glu Gln Leu Ala Gln Phe Arg Ser
1   5   10   15

Leu Asp

<210> 78
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 78

Gly Glu Asn Leu Tyr Phe Gln Ser Gly Gly
1   5   10

<210> 79
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 79

Ser Ala Cys Tyr Cys Glu Leu Ser
1   5

<210> 80
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 80

Arg Ser Ile Ala Thr
1   5

<210> 81
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 81

Arg Pro Ala Cys Lys Ile Pro Asn Asp Leu Lys Gln Lys Val Met Asn
1   5   10   15

His

<210> 82
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 82

Gly Gly Ser Ala Gly Gly Ser Gly Ser Gly Ser Ser Gly Gly Ser Ser
1   5   10   15

Gly Ala Ser Gly Thr Gly Thr Ala Gly Gly Thr Gly Ser Gly Ser Gly
            20   25   30

Thr Gly Ser Gly
        35

<210> 83
<211> 17
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 83

Ala Ala Ala Asn Ser Ser Ile Asp Leu Ile Ser Val Pro Val Asp Ser
1   5   10   15

Arg

<210> 84
<211> 36
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 84

Gly Gly Ser Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly
1   5   10   15

Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser Glu Gly Gly Gly Ser

20 25 30

Gly Gly Gly Ser
35

<210> 85
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 85

Glu Ala Ala Ala Lys
1 5

<210> 86
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 86

Pro Ala Pro Ala Pro
1 5

<210> 87

<400> 87
000

<210> 88

<400> 88
000

<210> 89

<400> 89
000

<210> 90

<400> 90
000

<210> 91
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<220>

<221> MISC_FEATURE
<223> Features section of the listing that any one or all of amino
       acids 1-15 can either be present or absent

<400> 91

Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg Arg
1   5   10   15

<210> 92
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 92

Asp Ser Ser Ser Glu Glu Lys Phe Leu Arg Arg Ile Gly Arg Phe Gly
1   5   10   15

<210> 93
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 93

Glu Glu Glu Glu Glu Glu Glu Pro Arg Gly Asp Thr
1   5   10

<210> 94
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 94

Ala Pro Trp His Leu Ser Ser Gln Tyr Ser Arg Thr
1   5   10

<210> 95
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 95

Ser Thr Leu Pro Ile Pro His Glu Phe Ser Arg Glu
1   5   10

<210> 96
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 96

Val Thr Lys His Leu Asn Gln Ile Ser Gln Ser Tyr
1   5   10

<210> 97
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<220>
<221> MISC_FEATURE
<223> Features section of the listing that any one or all of amino
       acids 2-15 can either be present or absent

<400> 97

Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu Glu
1   5   10   15

<210> 98
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 98

Arg Ser Gly Ser Gly Gly Ser
1   5

<210> 99
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<220>
<221> MISC_FEATURE
<223> Features section of the listing that any one or all of amino
       acids 2-15 can either be present or absent

<400> 99

Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp Asp
1   5   10   15

<210> 100
<211> 16
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 100

Leu Val Ile Met Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys
1   5   10   15

<210> 101
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 101

Val Ile Met Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys
1   5   10   15

<210> 102
<211> 14
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 102

Ile Met Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys
1   5   10

<210> 103
<211> 13
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 103

Met Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys
1   5   10

<210> 104
<211> 12
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 104

Ser Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys
1   5   10

<210> 105
<211> 11
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 105

Leu Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys
1   5   10

<210> 106
<211> 10
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 106

Gly Leu Gly Leu Gly Leu Gly Leu Arg Lys
1   5   10

<210> 107
<211> 9
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 107

Leu Gly Leu Gly Leu Gly Leu Arg Lys
1   5

<210> 108
<211> 8
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 108

Gly Leu Gly Leu Gly Leu Arg Lys
1   5

<210> 109
<211> 7
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 109

Leu Gly Leu Gly Leu Arg Lys
1   5

<210> 110
<211> 6
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 110

Gly Leu Gly Leu Arg Lys
1   5

<210> 111
<211> 5
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 111

Leu Gly Leu Arg Lys
1   5

<210> 112
<211> 4
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<400> 112

Gly Leu Arg Lys
1

<210> 113

<400> 113
000

<210> 114

<400> 114
000

<210> 115
<211> 15
<212> PRT
<213> Artificial Sequence

<220>
<223> Linker

<220>
<221> MISC_FEATURE
<223> Features section of the listing that any one or all of amino
       acids 2-15 can either be present or absent

<400> 115

Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys Lys
1   5   10   15