

CNP VARIANTS, CONJUGATES AND FORMULATIONS THEREOF**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] Priority is claimed to U.S. Provisional Application Nos. 63/597,246 (filed on November 8, 2023); 63/623,961 (filed on January 23, 2024); 63/651,801 (filed on March 24, 2024); 63/660,347 (filed on June 14, 2024); 63/695,491 (filed on September 17, 2024); and 63/711,678 (filed on October 24, 2024), each of which is incorporated herein by reference in entirety.

INCORPORATION BY REFERENCE OF MATERIAL SUBMITTED ELECTRONICALLY

[0002] The Sequence Listing, which is a part of the present disclosure, is submitted concurrently with the specification as a xml file. The name of the xml file containing the Sequence Listing is "70213_Seqlisting.xml", which was created on November 7, 2024 and is 98,392 bytes in size. The subject matter of the Sequence Listing is incorporated herein in its entirety by reference.

FIELD OF THE DISCLOSURE

[0003] The present disclosure, relates, in general, to variants of C-type natriuretic peptide (CNP), pharmaceutical compositions comprising CNP variants and methods of use. The CNP variants are useful as therapeutic agents for the treatment of diseases responsive to CNP, including but not limited to bone-related disorders, such as skeletal dysplasias (e.g., achondroplasia).

BACKGROUND

[0004] C-type natriuretic peptide (CNP) (Biochem. Biophys. Res. Commun., 168: 863-870 (1990) (GenBank Accession No. NP_077720, for the CNP precursor protein, NPPC) (J. Hypertens., 10: 907-912 (1992)) is a small, single chain peptide in a family of peptides (ANP, BNP, CNP) having a 17-amino acid loop structure (Levin et al., N. Engl. J. Med., 339: 863-870 (1998)) and have important roles in multiple biological processes. CNP interacts with natriuretic peptide receptor-B (NPR-B, GC-B) to stimulate the generation of cyclic- guanosine monophosphate (cGMP) (J. Hypertens., 10: 1111-1 114 (1992)). CNP is expressed widely, including in the central nervous system, reproductive tract, bone and endothelium of blood vessels (Hypertension, 49: 419-426 (2007)).

[0005] In humans, CNP is initially produced from the natriuretic peptide precursor C (NPPC) gene as a single chain 126-amino acid pre-pro polypeptide (Biochem. Biophys. Res. Commun., 168: 863-870 (1990)). Removal of the signal peptide yields pro-CNP,

and further cleavage by the endoprotease furin generates an active 53-amino acid peptide (CNP-53), which is secreted and cleaved again by an unknown enzyme to produce the mature 22-amino acid peptide (CNP- 22) (Wu, J. Biol. Chem. 278: 25847-852 (2003)). CNP-53 and CNP-22 differ in their distribution, with CNP-53 predominating in tissues, while CNP-22 is mainly found in plasma and cerebrospinal fluid (J. Alfonzo, Recept. Signal. Transduct. Res., 26: 269-297 (2006)). Both CNP-53 and CNP-22 bind similarly to NPR-B.

[0006] Downstream signaling mediated by cGMP generation influences a diverse array of biological processes that include endochondral ossification. For example, knockout of either CNP or NPR-B in mouse models results in animals having a dwarfed phenotype with shorter long bones and vertebrae. Mutations in human NPR-B that block proper CNP signaling have been identified and result in dwarfism (Olney, *et al*, J. Clin. Endocrinol. Metab. 91(4): 1229-1232 (2006); Bartels, *et al*, Am. J. Hum. Genet. 75: 27-34 (2004)). In contrast, mice engineered to produce elevated levels of CNP display elongated long bones and vertebrae.

[0007] Therapeutic use of CNP (CNP22) has been limited by its short plasma half-life, which has been shown to be 2.6 minutes *in vivo* in humans (J Clin. Endocrinol. Metab., 78: 1428-35 (1994)). A CNP variant having a longer *in vivo* serum half-life and exhibiting similar or improved activity to that of wild-type CNP is important for a sustainable therapeutic strategy.

SUMMARY

[0008] The present disclosure relates to novel variants of C-type natriuretic peptide (CNP) having increased circulating half-life and stability in aqueous media, pharmaceutical compositions comprising such CNP variants, and methods of using such CNP variants to treat disorders responsive to CNP, including but not limited to bone-related disorders such as achondroplasia.

[0009] In various embodiments, the disclosure provides a variant of C-type natriuretic peptide (CNP) selected from the group consisting of
PGQEHPPQARRYRGAQRRGLSRGCFGLKLDRIQSMGSLGC (SEQ ID NO: 5);
PGQEHPPNARKYKGANKKGLSKGCFGLKLDRIQSMGSLGC (SEQ ID NO: 1);
PGQEHPPNARRYRGANRRGLSRGCFGLKLDRIQSMGSLGC (SEQ ID NO: 6); and
PGQEHPPQARRYRGAQRRGLSRGCFGLKLDRIQSMGSLGC (SEQ ID NO: 5).

[0010] In various embodiments, the disclosure provides a variant of C-type natriuretic peptide (CNP) selected from the group consisting of

PGQEHPQARRYRGAQRRGLSRGCFGLKLDRI GMSGLGC (SEQ ID NO: 5);

PGQEHPNARKYKGANKKGLSKGCFGLKLDRI GMSGLGC (SEQ ID NO: 1);

PGQEHPNARRYRGANRRGLSRGCFGLKLDRI GMSGLGC (SEQ ID NO: 6);

PGQEHPQARRYRGAQRRGLSRGCFGLKLDRI GMSGLGC (SEQ ID NO: 5); and

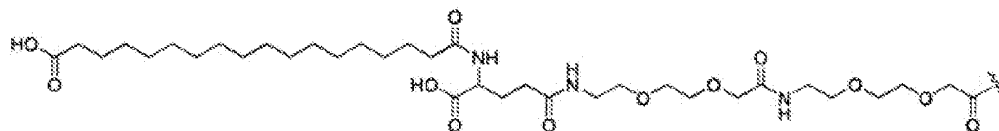
PGQEHPQARKYKGAQKKGLSKGCFGLKLDRI GMSGLGC (SEQ ID NO: 7).

[0011] In various embodiments, the variant peptide further comprises an acetyl group. In various embodiments, the acetyl group is on the N-terminus of the peptide. In various embodiments, the peptide further comprises an OH or an NH₂ group at the C-terminus.

[0012] In various embodiments, the variant peptide comprises a conjugate moiety. In various embodiments, the conjugate moiety is on a residue of the CNP cyclic domain or at a site other than the CNP cyclic domain. In various embodiments, the conjugate moiety is on a lysine residue. In various embodiments, the conjugate moiety comprises one or more acid moieties. In various embodiments, the acid moiety is a hydrophobic acid.

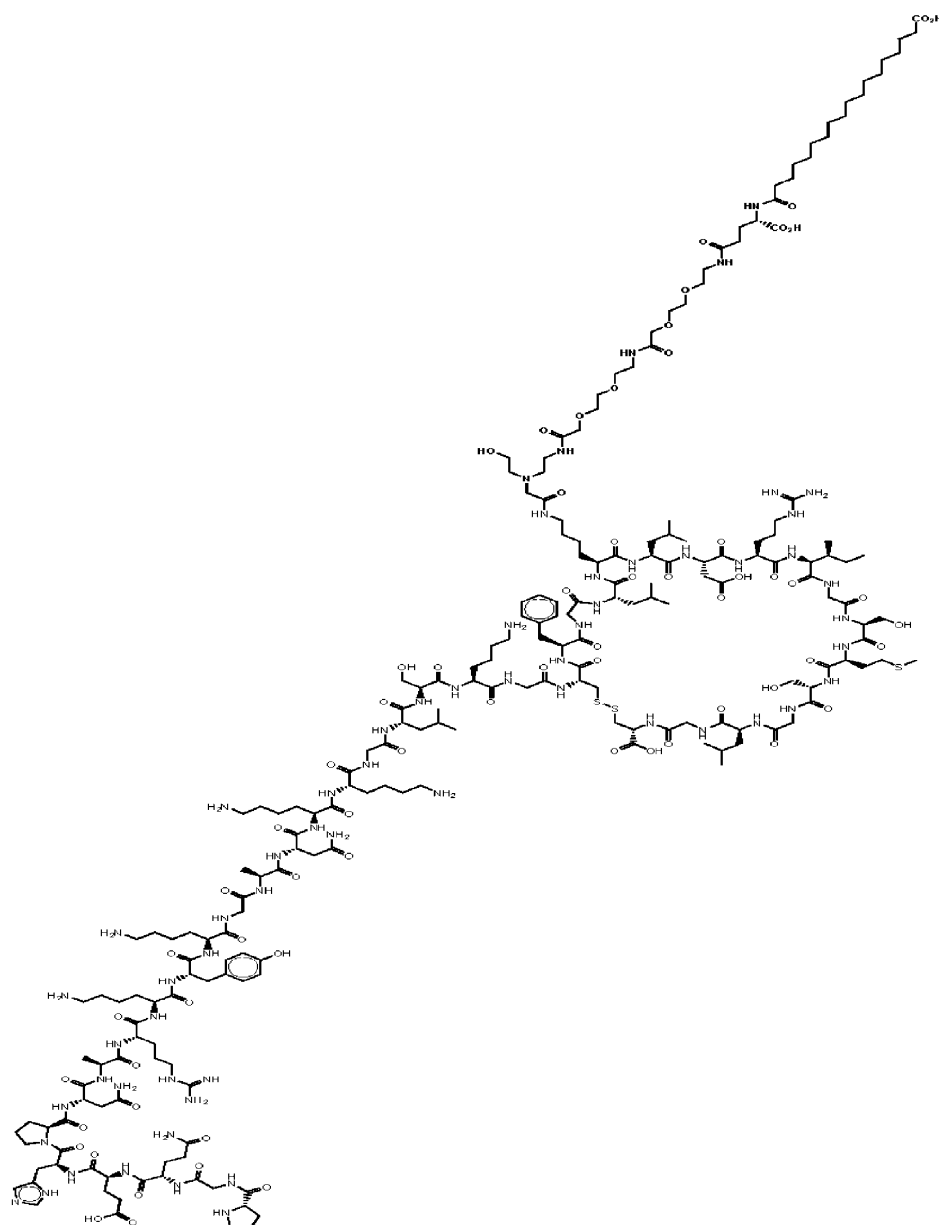
[0013] In various embodiments, the conjugate moiety comprises one or more acid moieties linked to a hydrophilic spacer. In various embodiments, the hydrophilic spacer is any amino acid. In various embodiments, the hydrophilic spacer is gamma glutamic acid (γGlu). In various embodiments, the hydrophilic spacer is OEG (8-amino-3,6-dioxaoctanoic acid). In various embodiments, the hydrophilic spacer is gamma glutamic acid (γGlu) or OEG (8-amino-3,6-dioxaoctanoic acid). In various embodiments, the hydrophilic spacer is gamma glutamic acid (γGlu) linked to one or two or more OEG (8-amino-3,6-dioxaoctanoic acid). In various embodiments, the acid moiety is a fatty acid. Exemplary fatty acids include short chain, medium chain, or long chain fatty acids, or a dicarboxylic fatty acid. In various embodiments, the fatty acid is saturated or unsaturated. Contemplated are C-6 to C-20 fatty acids, including but not limited to, C-6, C-8, C-10, C-12, C-14, C-16, C-18 or C-20 fatty acids, saturated or unsaturated. In various embodiments, the fatty acid is decanoic acid, dodecanoic acid, myristic acid, palmitic acid, stearic acid, arachidic acid, or diacids of the same.

[0014] In various embodiments, the acid moiety and the hydrophilic spacer have the structure AEEA-AEEA-γGlu-C18DA. In various embodiments, the acid moiety and the hydrophilic spacer have the structure:



, wherein  represents the point of attachment to a CNP variant. In various embodiments,  represents the point of attachment to a hydrolysable linker, wherein the hydrolysable linker is attached to a CNP variant. In various embodiments, the hydrolysable linker is capable of releasing intact CNP variant.

[0015] In various embodiments, the CNP variant has the structure:



[0016] In various embodiments, the International Union of Pure and Applied Chemistry (IUPAC) name of the CNP variant is

(4R,10S,16S,19S,22S,28S,31S,34S,37S,40S,43S,49S,52R)-52-(2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-1-(L-prolylglycyl- L-glutaminy- L-glutamyl- L-histidyl)pyrrolidine-2-carboxamido)-4-amino-4-oxobutanamido)propanamido)-5-guanidinopentanamido)-6-aminohexanamido)-3-(4-hydroxyphenyl)propanamido)-6-aminohexanamido)acetamido)propanamido)-4-amino-4-oxobutanamido)-6-aminohexanamido)-6-aminohexanamido)acetamido)-4-methylpentanamido)-3-hydroxypropanamido)-6-aminohexanamido)acetamido)-49-benzyl-28-((S)-sec-butyl)-34-(carboxymethyl)-40-((S)-33,51-dicarboxy-8-(2-hydroxyethyl)-6,12,21,30,35-pentaoxo-14,17,23,26-tetraoxa-5,8,11,20,29,34-hexaazahenpentacontyl)-31-(3-guanidinopropyl)-16,22-bis(hydroxymethyl)-10,37,43-trisobutyl-19-(2-(methylthio)ethyl)-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51-hexadeca-1,2-dithia-5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50-hexadecaazacyclotripentacontane-4-carboxylic acid.

[0017] In various embodiments, the chemical formula of the CNP variant is



[0018] In various embodiments, the exact mass of the CNP variant is 4959.61.

[0019] In various embodiments, the molecular weight of the CNP variant is 4962.83.

[0020] In various embodiments, the CNP variant with the conjugate moiety is a component of modified release composition. In various embodiments, the modified release composition is an extended release composition. In various embodiments, the CNP variant comprising a conjugate moiety and hydrolysable linker is capable of releasing the CNP variant, wherein (i) less than about 20% of CNP variant is released by day 1; and (ii) about 90% of the CNP variant is released weekly, or about 90% of the CNP variant is released bi-weekly, or about 90% of the CNP variant is released monthly, at pH 7 to 7.6.

[0021] In various embodiments, (i) less than about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, or about 75% of peptide is released by day 1, at pH 7.0 to 7.6; and (ii) about 90% of peptide is released weekly, or about 90% of peptide is released bi-weekly, or about 90% of peptide is released monthly, at pH 7 to 7.6. It is further contemplated that (i) less than about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, or about 75% of peptide is released by day 1, at pH 7.0 to 7.6; and (ii) about 70%, about 80%, or about 90% of peptide is released weekly; or about 70%, about 80%, or about 90% of peptide is released bi-weekly; or about 70%,

about 80%, or about 90% of peptide is released every three weeks; or about 70%, about 80%, or about 90% of peptide is released monthly, at pH 7 to 7.6; or alternatively ii) about 70%, about 75%, about 80%, about 85%, or about 90% of peptide is released weekly; or about 70%, about 75%, about 80%, about 85%, or about 90% of peptide is released bi-weekly; or about 70%, about 75%, about 80%, about 85%, or about 90% of peptide is released every three weeks; or about 70%, about 75%, about 80%, about 85%, or about 90% of peptide is released monthly, at pH 7 to 7.6.

[0022] In various embodiments, the variant has the structure:

PGQEH PQARRYRGAQRRGLSRGCFGLK(AEEA-AEEA-YGIU-C18DA) LDRIGSMSGLGC (SEQ ID NO: 5), or

AC-PGQEH PQARRYRGAQRRGLSRGCFGLK(AEEA-AEEA-YGIU-C18DA)LDRIGSMSGLGC-OH (SEQ ID NO: 8) .

[0023] In various embodiments, the variant is selected from the group consisting of Ac-PGQEH PQARRYRGAQRRGLSRGCFGLKLDRIGSMSGLGC-OH (SEQ ID NO: 8); Ac-PGQEH PNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC-NH₂ (SEQ ID NO: 9); Ac-PGQEH PNARRYRGANRRGLSRGCFGLKLDRIGSMSGLGC-OH (SEQ ID NO: 10); Ac-PGQEH PNARRYRGANRRGLSRGCFGLKLDRIGSMSGLGC-NH₂ (SEQ ID NO: 11); Ac-PGQEH PQARRYRGAQRRGLSRGCFGLKLDRIGSMSGLGC-NH₂ (SEQ ID NO: 12); Ac-PGQEH PQARKYKGAQKKGLSKGCFGLKLDRIGSMSGLGC-NH₂ (SEQ ID NO: 13); and Ac-PGQEH PQARKYKGAQKKGLSKGCFGLKLDRIGSMSGLGC-OH (SEQ ID NO: 14).

[0024] In various embodiments, the variant comprises one or more linker groups. In various embodiments, the linker is on a residue of the CNP cyclic domain or at a site other than the CNP cyclic domain. In various embodiments, the linker is on a lysine residue.

[0025] In various embodiments, the linker is a cleavable or lysable linker. In some instances of those embodiments, the linker is a hydrolysable linker.

[0026] In various embodiments, the CNP variant is attached to the conjugate moiety via the linker. In various embodiments, the linker is attached to the conjugate moiety via the hydrophilic spacer of the conjugate moiety. In various embodiments, the linker is aminoethoxy-2-ethoxy acetic acid (AEEA). In various embodiments, the linker is a bicin-type or peptoid linker, which refers to a linker having a similar cleavage mechanism as bicin (bis-2-hydroxyethylglycinamide), but cleaving instead via an asymmetric N-alkyl peptide, i.e., a peptoid. In various embodiments, the linker is an electronic linker based

on nonenzymatic β -elimination. In various embodiments, the electronic linker comprises an SO_2 moiety. Examples of linkers as illustrated in a CNP conjugate are set out in Figure 1. See also Santi, et.al., Proc Natl Acad Sci USA 109:621 1- 6216, 2012). In various embodiments, cleavage of the cleavable or lysable linker, particularly the hydrolysable linker, releases the CNP variant from the conjugate moiety. An exemplary embodiment where this can occur is depicted in Figure 9.

[0027] In various embodiments, the conjugate moiety is a synthetic polymeric group. In various embodiments, the variant comprises a synthetic polymeric group coupled to the variant through a hydrolysable linker. In various embodiments, the synthetic polymeric group comprises a hydrophilic polymer moiety. In various embodiments, the hydrophilic polymer moiety comprises polyethylene glycol (PEG). In various embodiments, the hydrophilic polymer moiety comprises polyethylene glycol (PEG) having a 6 to 20 atom chain length. For purposes of this conjugate, the synthetic polymeric group is not a peptide.

[0028] In various embodiments, the variant peptide is made synthetically.

[0029] In various embodiments, the variant peptide is stable for 10 days at about 37° C, pH 7.0 to 7.6. In various embodiments, the variant peptide is stable for at least 10 days at about 37° C, pH 7.0 to 7.4. In various embodiments, the variant peptide is stable for at least 10 days at about 37° C, pH 7.2 to 7.6.

[0030] In various embodiments, the variant peptides are stable to deamidation. In various embodiments, the variant peptides are stable to oxidation. In various embodiments, the variant peptides are stable to deamidation, and/or oxidation, or combinations thereof. In various embodiments, methionine is replaced by nor-leucine. In various embodiments, there is little to no detectable deamidation after 10 days.

[0031] In various embodiments, the variant peptide has a half-life of about 10 days at about 37° C, pH 7.0 to 7.6. In various embodiments, the variant peptide has a half-life of about 10 days at about 37° C, pH 7.0 to 7.4. In various embodiments, the variant peptide has a half-life of about 10 days at about 37° C, pH 7.2 to 7.6. In various embodiments, the variant peptide has a half-life of at least 10 days at about 37° C, pH 7.0 to 7.6. In various embodiments, the variant peptide has a half-life of at least 10 days at about 37° C, pH 7.0 to 7.4. In various embodiments, the variant peptide has a half-life of at least 10 days at about 37° C, pH 7.2 to 7.6. In various embodiments, the half-life

is at least about 15 days, 16 days, 17 days, 18 days, 19 days, 20 days, 21 days, 22 days, 23 days, 24 days, 25 days, 30 days or more.

[0032] In various embodiments, the variant peptide has an EC₅₀ from 0.1 to 10 nM in a cGMP assay. In various embodiments, the variant peptide has an EC₅₀ from 0.1 to 25 nM in a cGMP assay.

[0033] In various embodiments, greater than 45% of the variant peptide is detected after 10 days in aqueous media at physiological conditions, e.g., about 37° C, pH 7.0 to 7.6. In various embodiments, greater than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the variant peptide is detected after 10 days in aqueous media at physiological conditions, e.g., about 37° C, pH 7.0 to 7.6.

[0034] In various embodiments, greater than 45% of the variant peptide is detected after 10 days in aqueous media at 37° C, pH 7.4. In various embodiments, greater than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the variant peptide is detected after 10 days in aqueous media at 37° C, pH 7.4.

[0035] In various embodiments, greater than 45% of the variant peptide is detected after 10 days in plasma at physiological conditions, e.g., about 37° C, pH 7.0 to 7.6. In various embodiments, greater than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the variant peptide is detected after 10 days in plasma at physiological conditions, e.g., about 37° C, pH 7.0 to 7.6.

[0036] In various embodiments, greater than 45% of the variant peptide is detected after 10 days in plasma at 37° C, pH 7.4. In various embodiments, greater than 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of the variant peptide is detected after 10 days in plasma at 37° C, pH 7.4.

[0037] In various embodiments, the variant peptide is conjugated to a lipid, fatty acid, hydrophilic spacer, or linker, or optionally combinations thereof. In various embodiments, the linker is a hydrophilic polymer moiety. In various embodiments, the hydrophilic polymer moiety is a synthetic hydrophilic polymer moiety.

[0038] In various embodiments, the variant peptide has a longer half-life compared to Pro-Gly-CNP37. In various embodiments, the variant peptide has a longer half-life compared to CNP-22. In various embodiments, the variant peptide has a longer half-life compared to Pro-Gly-CNP37 and/or CNP-22. In various embodiments, the variant peptide has a longer half-life compared to Pro-Gly-CNP37 and/or CNP-22 in vitro and/or in vivo. In various embodiments, the CNP prodrug composition exhibits lower C_{max} and

higher AUC compared to free drug, e.g., an equivalent composition lacking an acid moiety, a spacer, and a hydrolysable linker.

[0039] The disclosure further provides a pharmaceutical composition comprising a CNP variant described herein, and a pharmaceutically acceptable excipient, carrier or diluent.

[0040] In various embodiments, the composition is a lyophilized formulation prepared from a formulation that comprises a citric acid/citrate buffer or an acetic acid/acetate buffer having a pH from about 4 to about 6. In various embodiments, the lyophilized formulation is prepared from a formulation that further comprises an isotonicity-adjusting agent or a bulking agent selected from the group consisting of mannitol, sucrose, sorbitol, trehalose, polysorbate 80, and combinations thereof. In various embodiments, the lyophilized formulation is prepared from a formulation that further comprises an antioxidant selected from the group consisting of methionine, ascorbic acid, salt forms of ascorbic acid, thioglycerol, and combinations thereof. In various embodiments, the CNP variant composition is supplied as a lyophilized powder for reconstitution from 0.8 mg to 10 mg. In various embodiments, the CNP variant composition is supplied as a 0.8-mg or 2-mg lyophilized, preservative-free powder for reconstitution.

[0041] In various embodiments, the composition is a lyophilized formulation prepared from a formulation that comprises a histidine buffer (including salts thereof, solvates thereof, and solvates of salts thereof) or an L-histidine buffer having a pH from about 4 to about 6. In various embodiments, the lyophilized formulation is prepared from a formulation that further comprises an isotonicity-adjusting agent or a bulking agent selected from the group consisting of mannitol, sucrose, sorbitol, trehalose, polysorbate 80, and combinations thereof. In various embodiments, the lyophilized formulation is prepared from a formulation that further comprises an antioxidant selected from the group consisting of methionine, ascorbic acid, salt forms of ascorbic acid, thioglycerol, and combinations thereof. In various embodiments, the CNP variant composition is supplied as a lyophilized powder for reconstitution from 1 mg to 300 mg. For example, in some embodiments the CNP variant composition is supplied as a lyophilized powder in an amount of 10 to 290 mg (e.g., 25 to 250 mg, 50 to 200 mg, and 75 to 150 mg). In some embodiments, the CNP variant composition is supplied as a lyophilized powder in an amount of 10 to 50 mg (e.g., 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, and 50 mg). In various embodiments, the CNP variant composition is supplied as a 13 mg or 39 mg lyophilized, preservative-free powder for reconstitution. Also contemplated herein are formulations comprising (a) a CNP

variant peptide described herein and (b) one or more components selected from the group consisting of a buffering agent, an isotonicity agent, a stabilizer and an anti-adsorbent agent. In particularly preferred embodiments, buffering agents employed in the formulations may be L-histidine, histidine monohydrochloride monohydrate, or a combination of the two. In yet other preferred embodiments, isotonicity agents employed in the formulations of the present invention may be trehalose dihydrate, D-mannitol, or a combination of the two. In other preferred embodiments, the stabilizer employed in the formulations of the present invention is L-methionine. In yet other preferred embodiments, the anti-adsorbent agent employed in the formulations of the present invention is polysorbate 80. In various embodiments, the formulations of the present invention are lyophilized, in liquid form, or in liquid form that has been reconstituted from a previously lyophilized form. In certain embodiments, the formulations of the present invention are preservative-free and, optionally, may be contained within a type 1 untreated borosilicate glass vial. Optionally, the formulations of the present invention have a pH in the range of between about 5.0 and about 6.0 (e.g., 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, and 6.0). In some embodiments, the disclosed formulations have a pH of about 5.5. In other embodiments, the formulations of the present invention comprise a CNP variant peptide as described herein, L-histidine, histidine monohydrochloride monohydrate, trehalose dihydrate, D-mannitol, L-methionine and polysorbate 80. In certain preferred embodiments, the CNP variant peptide is present at a concentration between about 10.0 mg/ml and about 30.0 mg/ml, the L-histidine is present at a concentration between about 0.18 mg/ml and about 0.50 mg/ml, the histidine monohydrochloride monohydrate is present at a concentration between about 0.75 mg/ml and about 2.26 mg/ml, the trehalose dihydrate is present at a concentration between about 30 mg/ml and about 70 mg/ml, the D-mannitol is present at a concentration between about 10 mg/ml and about 20.0 mg/ml, the L-methionine is present at a concentration between about 0.5 mg/ml and about 1.5 mg/ml and the polysorbate 80 is present at a concentration between about 0.01 mg/ml and about 0.1 mg/ml. In other embodiments, a particularly preferred embodiments, the CNP variant is present at a concentration of about 10.0 mg/ml or 30 mg/ml, the L-histidine is present at a concentration of about 0.347 mg/ml, the histidine monohydrochloride monohydrate is present at a concentration of about 1.627 mg/ml, the trehalose dihydrate is present at a concentration of about 58.00 mg/ml, the D-mannitol is present at a concentration of about 15.0 mg/ml, the L-methionine is present at a concentration of about 0.73 mg/ml and the polysorbate 80 is present at a concentration of about 0.05 mg/ml. In other embodiments, the CNP variant peptide is present at a concentration between about 0.4 mg/ml and about 3.5 mg/ml, the citric acid monohydrate is present at a concentration

between about 0.15 mg/ml and about 0.40 mg/ml, the sodium citrate dihydrate is present at a concentration between about 0.5 mg/ml and about 1.5 mg/ml, the trehalose dihydrate is present at a concentration between about 30 mg/ml and about 70 mg/ml, the D-mannitol is present at a concentration between about 10 mg/ml and about 20.0 mg/ml, the L-methionine is present at a concentration between about 0.5 mg/ml and about 1.5 mg/ml and the polysorbate 80 is present at a concentration between about 0.01 mg/ml and about 0.1 mg/ml. In another embodiment, the CNP variant is present at a concentration of about 0.8 mg/ml, 2.0 mg/ml, or 5.0 mg/ml, the citric acid monohydrate is present at a concentration of about 0.28 mg/ml, the sodium citrate dihydrate is present at a concentration of about 1.08 mg/ml, the trehalose dihydrate is present at a concentration of about 58.01 mg/ml, the D-mannitol is present at a concentration of about 15.0 mg/ml, the L-methionine is present at a concentration of about 0.73 mg/ml and the polysorbate 80 is present at a concentration of about 0.05 mg/ml.

[0042] In various embodiments, the composition is an extended release composition.

[0043] In various embodiments, the CNP variant is PGQEHNPARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (Pro-Gly-CNP-37) (BMN 111) (SEQ ID NO: 1).

[0044] Also provided is a method of treating a bone-related disorder or skeletal dysplasia in a subject in need thereof comprising administering to the subject a composition comprising a CNP variant as described herein.

[0045] In various embodiments, the bone-related disorder or skeletal dysplasia is selected from the group consisting of osteoarthritis, hypophosphatemic rickets, achondroplasia, hypochondroplasia, short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis imperfecta, achondrogenesis, chondrodysplasia punctata, homozygous achondroplasia, campomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis imperfecta, short-rib polydactyly syndromes, rhizomelic type of chondrodysplasia punctata, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenita, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia.

[0046] In various embodiments, the bone-related disorder or skeletal dysplasia is selected from the group consisting of osteoarthritis, hypophosphatemic rickets, achondroplasia, hypochondroplasia, short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis imperfecta, achondrogenesis, chondrodysplasia punctata, homozygous achondroplasia, campomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis imperfecta, short-rib polydactyly syndromes, rhizomelic type of chondrodysplasia punctata, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenita, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, spondyloepimetaphyseal dysplasia and osteoporosis.

[0047] In various embodiments the CNP variants are useful as an adjunct or alternative to growth hormone for treating idiopathic short stature and other skeletal dysplasias.

[0048] In various embodiments, the bone-related disorder, skeletal dysplasia or short stature disorder results from an NPR2 mutation, SHOX mutation (Turner's syndrome/Leri Weill), or PTPN11 mutations (Noonan's syndrome).

[0049] In various embodiments, the bone-related disorder, skeletal dysplasia or short stature disorder results from an NPR2 mutation, SHOX mutation (Turner's syndrome/Leri Weill), or PTPN11 mutations (Noonan's syndrome), or insulin growth factor 1 receptor (IGF1R).

[0050] In various embodiments, the CNP variants are useful to treat growth plate disorders and short stature, including familial short stature, dominant familial short stature which is also known as dominant inherited short stature, or idiopathic short stature. In various embodiments, the short stature or growth plate disorder is a result of a mutation in collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), Indian hedgehog (IHH), PTPN11, NPR2, NPPC, or FGFR3.

[0051] In various embodiments, the CNP variants are useful to treat growth plate disorders and short stature, including familial short stature, dominant familial short stature which is also known as dominant inherited short stature, or idiopathic short stature. In various embodiments, the short stature or growth plate disorder is a result of a mutation in

collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), Indian hedgehog (IHH), PTPN11, SHOX, NPR2, NPPC, or FGFR3.

[0052] In various embodiments, the growth plate disorder or short stature is associated with one or more mutations in a gene associated with a RASopathy.

[0053] In various embodiments, the bone-related disorder, skeletal dysplasia or short stature disorder results from a RASopathy. In various embodiments, the RASopathy is Noonan syndrome, Costello syndrome, Cardiofaciocutaneous syndrome, Neurofibromatosis Type 1, or LEOPARD syndrome.

[0054] In one embodiment, the RASopathy is hereditary gingival fibromatosis type 1.

[0055] In various embodiments, the CNP variants are useful to treat growth plate disorders and short stature, including familial short stature, dominant familial short stature which is also known as dominant inherited short stature, or idiopathic short stature. In various embodiments, the short stature or growth plate disorder is a result of a mutation in collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), indian hedgehog (IHH), PTPN11, NPR2, NPPC, FGFR3, or insulin growth factor 1 receptor (IGF1R).

[0056] In various embodiments, the CNP variants are useful to treat growth plate disorders and short stature, including familial short stature, dominant familial short stature which is also known as dominant inherited short stature, or idiopathic short stature. In various embodiments, the short stature or growth plate disorder is a result of a mutation in collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), indian hedgehog (IHH), PTPN11, SHOX, NPR2, NPPC, FGFR3, or insulin growth factor 1 receptor (IGF1R).

[0057] In various embodiments, the growth plate disorder or short stature is associated with one or more mutations in a gene associated with a RASopathy.

[0058] In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of less than -1.0, -1.5, -2.0, -2.5, or -3.0, and having at least one parent with a height SDS of less than -1.0, -1.5, -2.0 or -2.5, optionally wherein the second parent has height within the normal range. In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of less than -1.0, -1.5, -2.0, -2.25, -2.5, or -3.0, and having at least one parent with a height SDS of less than -1.0, -1.5, -2.0, -2.25 or -2.5, optionally wherein the second parent has height within the normal range. In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of between -2.0 to -

3.0. In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of between -2.0 to -2.5. In various embodiments, the short stature is associated with one or more mutations in a gene associated with short stature, such as, collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), Indian hedgehog (IHH), PTPN11, NPR2, NPPC, FGFR3, or insulin growth factor 1 receptor (IGF1R), or combinations thereof. In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of between -2.0 to -2.5. In various embodiments, the short stature is associated with one or more mutations in a gene associated with short stature, such as, collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), Indian hedgehog (IHH), PTPN11, SHOX, NPR2, NPPC, FGFR3, or insulin growth factor 1 receptor (IGF1R), or combinations thereof. In various embodiments, the growth plate disorder or short stature is associated with one or more mutations in a gene associated with a RASopathy.

[0059] In various embodiments, the short stature is a result of mutations in multiple genes as determined by polygenic risk score (PRS). In various embodiments, the subject has a mutation in NPR2 and a low PRS. In various embodiments, the subject has a mutation in FGFR3 and a low PRS. In various embodiments, the subject has a mutation in NPR2 and a low PRS. In various embodiments, the subject has a mutation in IGF1R and a low PRS. In various embodiments, the subject has a mutation in NPPC and a low PRS. In various embodiments, the subject has a mutation in SHOX and a low PRS. In various embodiments, the subject has one or more mutation in one or more of FGFR3, IGF1R, NPPC, NPR2 and SHOX, and a low PRS. In various embodiments, the PRS is 1 or 2. In various embodiments, the PRS is 1. In various embodiments, the PRS is 2. Polygenic risk scores (PRS) were calculated for height as described in Example 4. PRS 1 refers to the lowest height, PRS 5 the tallest height.

[0060] Also provided is a method of elongating a bone or increasing long bone growth in a subject in need thereof, comprising administering to the subject a composition as described herein, and wherein the administering elongates a bone or increases long bone growth.

[0061] In various embodiments, the composition is administered subcutaneously, intradermally, intraarticularly, orally, or intramuscularly.

[0062] In various embodiments, the composition provides an extended release composition.

[0063] In various embodiments, the composition is administered once every 5 days, once a week, once every two weeks, once every three weeks, once every 4 weeks, once every 6 weeks, once every two months, once every three months or once every six months.

[0064] In various embodiments, the administration increases the annualized growth velocity (AGV) in the subject at 12 months, optionally compared to baseline or to a normal control. In various embodiments, the AGV in the subject increases over 1 year or over 2 years, or more.

[0065] In various embodiments, the administration improves the height Z score at 12 months, optionally compared to baseline or to a normal control.

[0066] In various embodiments, the subject is greater than 3 years old. In various embodiments, the subject is between 3 and 17 years old. In various embodiments, the subject has open epiphyses.

[0067] In various embodiments, the composition is administered at a dose from about 5 µg/kg to 500 µg/kg or from about 15 µg/kg to 350 µg/kg. In various embodiments, the CNP Prodrug is administered at a dose from about 5 µg/kg to 500 µg/kg, from about 15 µg/kg to 350 µg/kg, from about 25 µg/kg to 300 µg/kg, from about 50 µg/kg to 250 µg/kg or from about 75 µg/kg to 200 µg/kg. In various embodiments, the CNP variant is administered at a dose of about 15 µg/kg, 20 µg/kg, 25 µg/kg, 30 µg/kg, 35 µg/kg, 40 µg/kg, 45 µg/kg, 50 µg/kg, 60 µg/kg, 70 µg/kg, 75 µg/kg, 80 µg/kg, 90 µg/kg, 100 µg/kg, 125 µg/kg, 150 µg/kg, 175 µg/kg, 200 µg/kg, 225 µg/kg, 250 µg/kg, 275 µg/kg, 300 µg/kg, 325 µg/kg, 350 µg/kg, 400 µg/kg, 450 µg/kg, or 500 µg/kg.

[0068] In various embodiments, the administration does not result in cardiovascular (CV) side effects. In various embodiments, the CV side effect is change in systemic blood pressure, mean arterial pressure, systolic and/or diastolic blood pressures, pulse pressure or heart rate. In various embodiments, subjects receiving a CNP Prodrug to treat skeletal dysplasia as described herein have reduced or none of the cardiovascular (CV) side effects such as changes in systemic blood pressures (mean arterial pressure, systolic and diastolic blood pressures, pulse pressure) and heart rate observed with administration of non-prodrug CNP variant.

[0069] In various embodiments, the CNP variant is PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP-37) (SEQ ID NO: 1).

[0070] In various embodiments, the peptide further comprises an acetyl group. In various embodiments, the acetyl group is on the N-terminus of the peptide. In various embodiments, the peptide further comprises an OH or an NH₂ group at the C-terminus. In various embodiments, the variant comprises one or more linker groups as described herein. In various embodiments, the linker is a hydrolysable linker.

[0071] In various embodiments, the disclosure provides a method of elongating a bone or increasing long bone growth in a subject in need thereof, comprising administering to the subject a composition comprising a CNP variant described herein, and wherein the administering elongates a bone or increases long bone growth.

[0072] In various embodiments, the composition is administered subcutaneously, intradermally, intraarticularly, orally, or intramuscularly.

[0073] In various embodiments, the composition is administered once daily, once weekly, once every two weeks, once every three weeks, once every 4 weeks, once every 6 weeks, once every two months, once every three months or once every six months.

[0074] In various embodiments, the composition is an extended release composition.

[0075] Further contemplated is a method of treating a CNP-responsive condition or disorder, comprising administering a CNP variant or composition as described herein to a subject, and monitoring the level of at least one bone- or cartilage-associated biomarker in the subject, wherein an increase in the level of the at least one bone- or cartilage-associated biomarker indicates a therapeutic effect of the CNP peptide or variant on the subject or the condition or disorder.

[0076] Further contemplated is a method of overcoming cell growth arrest induced by a constitutively active mutant fibroblast growth factor receptor 3 (FGFR-3) comprising contacting a cell expressing the constitutively active FGFR-3 with a CNP variant or a composition as described herein.

[0077] Further contemplated is a method of stimulating cGMP production in a cell expressing natriuretic peptide receptor B (NPR-B) comprising contacting the cell expressing NPR-B with a CNP variant or a composition as described herein.

[0078] In various embodiments, the method further comprises adjusting the amount or frequency of administration of the CNP peptide or variant as described herein, wherein i) the amount or frequency of administration of the CNP peptide or variant is increased if the level of the at least one bone- or cartilage-associated biomarker is below a target level; or ii) the amount or frequency of administration of the CNP peptide or variant is decreased if the level of the at least one bone- or cartilage-associated biomarker is above a target level.

[0079] In various embodiments, the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, Collagen Type I C-Telopeptide (CTx), osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of

type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, collagen X, and alkaline phosphatase.

[0080] In various embodiments, the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, Collagen Type I C-Telopeptide (CTx), osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, collagen X, CXM (noncollagenous 1 (NC1) domain of type X collagen), NTproCNP, alkaline phosphatase, N-terminal collagen type I pro-peptide, bone-specific alkaline phosphatase, amino-terminal propeptide of type I collagen/procollagen type I N-propeptide (PINP), cross-linked N-telopeptide of type I collagen (NTx) tartrate-resistant acid phosphatase 5b (TRAP-5b), transcriptomics readouts, e.g., from PAXgene® RNA, and CNP-variant bioactivity. Cartilage- and bone-associated biomarkers can be measured in any appropriate biological sample, including but not limited to tissues, blood, serum, plasma, cerebrospinal fluid, synovial fluid and urine. In some embodiments, the biomarkers are measured in blood, plasma, serum or urine from subjects undergoing efficacy/pharmacodynamic *in vivo* studies and/or from the conditioned media of *ex vivo* studies.

[0081] In various embodiments, the CNP variant is PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (Pro-Gly-CNP-37) (SEQ ID NO: 1).

[0082] In various embodiments, the peptide further comprises an acetyl group. In various embodiments, the acetyl group is on the N-terminus of the peptide. In various embodiments, the acetyl group is on an amino acid side chain within the peptide sequence. In various embodiments, the peptide further comprises an OH or an NH₂ group at the C-terminus. In various embodiments, the variant comprises one or more linker groups as described herein. In various embodiments, the linker is a hydrolysable linker.

[0083] Also provided is a method of making a CNP variant described herein comprising synthesizing the peptide on a solid-phase resin using Fmoc amino acids.

[0084] In various embodiments, the method comprises acetylating the peptides by reacting the resin with NMP/Ac₂O/DIEA (10:1:0.1, v/v/v).

[0085] In various embodiments, the method comprises conjugating the peptide to a conjugate moiety, optionally on a lysine residue. In various embodiments, the method

comprises cleaving the protective amino group on lysine, reacting the peptide with 2x Fmoc-amino PEG(2) followed by amino acid, followed by conjugation of the lipid or fatty acid moiety.

[0086] It is understood that each feature or embodiment, or combination, described herein is a non-limiting, illustrative example of any of the aspects of the invention and, as such, is meant to be combinable with any other feature or embodiment, or combination, described herein. For example, where features are described with language such as "one embodiment", "some embodiments", "certain embodiments", "further embodiment", "specific exemplary embodiments", and/or "another embodiment", each of these types of embodiments is a non-limiting example of a feature that is intended to be combined with any other feature, or combination of features, described herein without having to list every possible combination.

[0087] Such features or combinations of features apply to any of the aspects of the invention. Where examples of values falling within ranges are disclosed, any of these examples are contemplated as possible endpoints of a range, any and all numeric values between such endpoints are contemplated, and any and all combinations of upper and lower endpoints are envisioned.

BRIEF DESCRIPTION OF THE DRAWINGS

[0088] Figure 1 illustrates use of a peptoid or electronic linker in a CNP conjugate described herein.

[0089] Figure 2 shows the stability of CNP variants in human plasma over a period of 24 hrs.

[0090] Figure 3 shows the stability of CNP variants under different culture conditions.

[0091] Figure 43 shows the effects of a CNP variant (Pro-Gly-CNP) on cells carrying either NPR2 homozygous or heterozygous mutations, as measured by cGMP stimulation.

[0092] Figure 5 shows the nucleotide and predicted protein sequence of the first exon in NPR2 mutant clones transfected into RCS cells.

[0093] Figure 6 shows exemplary NPR2 mutations analyzed for response to CNP.

[0094] Figure 7 shows exemplary mutations associated with short stature in FGFR3, IGF1R, NPPC, NPR2 and SHOX.

[0095] Figures 8A-8F illustrate the combined effect of PRS and rare coding variants on Height. Fig. 8A. Effects on height as a quantitative trait, samples were divided in five groups based on their PRS, violin-plots with horizontal lines representing the 25%, 50% and 75% percentile of height. Samples were grouped by carrying status of missense, loss of function or None in any of the five core genes. Fig. 8B. Effect reflected on Odds ratios for "Idiopathic Short Stature" or ISS. Odds for ISS using PRS=3 as reference vs the other PRS groups. Fig. 8C. Odds for ISS using PRS = 1 non-carriers as reference vs having missense and/or loss of function variants in core genes. Fig. 8D. Odds for ISS using PRS=2 non-carriers as reference vs having missense and/or loss of function variants in core genes. Fig. 8E. Odds for ISS using PRS =3 non-carriers as reference vs having missense and/or loss of function variants in core genes. Fig. 8F. Odds for ISS using PRS=4 non-carriers as reference vs having missense and/or loss of function variants in core genes.

[0096] Figure 9 illustrates the mechanism of linker cleavage in an embodiment of the disclosure.

[0097] Figure 10 shows the components of a CNP variant, including the CNP, lipid, and linker.

[0098] Figure 11 shows the structure and chemical name of a CNP variant disclosed herein.

[0099] Figure 12 shows a schematic of Phase 1 dosing study of CNP Prodrug in healthy volunteers.

[0100] Figure 13 shows visual appearance of CNP prodrug citrate-based formulations

[0101] Figure 14 shows CNP variant content when stored in different formulation buffers was analyzed over time, at a range of temperatures from -20 to 37° C.

[0102] Figure 15 shows free drug content release from different formulation buffers analyzed over time at a range of temperatures.

[0103] Figure 16 shows linker area of the drug conjugate when stored in different formulation buffers over time at a range of temperatures.

[0104] Figure 17 shows CNP conjugate content when stored in different formulation buffers was analyzed over time at a range of temperatures.

[0105] Figure 18 shows variant concentration as measured by UV when stored in different formulation buffers was analyzed over time.

[0106] Figure 19 shows variant content in formulations with different excipients (histidine, glycine and sorbitol) over time at pH 5.5 or 6.

[0107] Figure 20 shows results of a manufacturing feasibility study for CNP prodrug.

[0108] Figure 21 shows batch analysis of formulated CNP prodrug in histidine or acetate buffer with trehalose and mannitol at different ratios (4:1, or 1:4, respectively).

[0109] Figure 22 shows area under the curve after reverse phase chromatography.

[0110] Figure 23 shows in-use stability and free drug content assessed over time at a range of temperatures and pH 5.2 to 6.0.

[0111] Figure 24 shows visual comparison of CNP prodrug in different formulations.

[0112] Figure 25 shows naso-anal lengths in WT and *Raf1*^{+/-L613V} mice after 6 weeks of treatment.

[0113] Figure 26 shows naso-anal lengths in WT and Rit^{+/-} mice treated for 10 weeks. IP, intraperitoneal; MEKi, mitogen-activated protein kinase kinase inhibitor; N A, naso anal; QAD, every other day; QD, daily; SC, subcutaneous; Veh., vehicle; WT, wild type. # Significantly different from corresponding vehicle-treated group by un-paired t-test. * Significantly different from CNP-treated genotype-match group by un-paired t-test.

[0114] Figure 27 shows whole body μ CT images of WT mice treated with vehicle (left) or CNP Prodrug (right). μ CT, micro computed tomography; WT, wild-type.

[0115] Figure 28 shows plasma pharmacokinetics of CNP prodrug and released vosoritide in mice following a single SC dose.

[0116] Figure 29 shows mean ($\pm$ SD) plasma vosoritide and CNP prodrug concentration-time profiles after a single dose of CNP prodrug.

[0117] Figure 30A and 30B show mean ($\pm$ SD) concentration vs. time curves for CNP prodrug (Fig. 30A) and vosoritide (Fig. 30B) in NHP plasma following IV or SC administration of CNP prodrug.

[0118] Figure 31A and 31B show mean ($\pm$ SD) concentration vs. time curves for CNP prodrug (Fig. 31A) and vosoritide (Fig. 31B) in male and female NHP plasma following IV or SC administration of CNP prodrug.

[0119] Figure 32A shows mean ($\pm$ SD) plasma vosoritide and CNP prodrug concentration-time and dose normalized mean plasma vosoritide and CNP prodrug concentration-time profile. Figure 32B shows mean ($\pm$ SD) plasma vosoritide and CNP prodrug concentration-

time and dose normalized mean plasma vosoritide and CNP prodrug concentration-time profile.

[0120] Figure 33 shows mean ($\pm$ sd) plasma vosoritide and CNP prodrug concentration-time comparing Day 1 vs Day 22.

[0121] Figure 34 shows binding of CNP Prodrug to NPR-B and NPR-C compared to BMN111.

DETAILED DESCRIPTION

[0122] The present disclosure relates to stable CNP variants useful in treating skeletal dysplasias and bone growth disorders.

[0123] As used in the specification and the appended claims, the indefinite articles “a” and “an” and the definite article “the” include plural as well as singular referents unless the context clearly dictates otherwise.

[0124] The term “about” or “approximately” means an acceptable error for a particular value as determined by one of ordinary skill in the art, which depends in part on how the value is measured or determined. In certain embodiments, the term “about” or “approximately” means within 1, 2, 3, or 4 standard deviations. In certain embodiments, the term “about” or “approximately” means within 30%, 25%, 20%, 15%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, 0.5%, or 0.05% of a given value or range. Whenever the term “about” or “approximately” precedes the first numerical value in a series of two or more numerical values, it is understood that the term “about” or “approximately” applies to each one of the numerical values in that series.

[0125] The term “C-type natriuretic peptide” or “CNP” refers to a small, single chain peptide having a 17-amino acid loop structure at the C-terminal end (GenBank Accession No. NP_077720, for the CNP precursor protein, NPPC) and variants thereof. The 17-mer CNP loop structure is also referred to as CNP 17, the CNP ring, or CNP cyclic domain. CNP includes the active 53-amino acid peptide (CNP-53) and the mature 22-amino acid peptide (CNP-22), and peptides of varying lengths between the two peptides.

[0126] In various embodiments, a “CNP variant” is at least about 40%, 50%, 60%, 70%, 75%, 80%, 85%, 90%, or 95% homologous to the wild type NPPC over the same number of amino acid residues. It is further contemplated that a CNP variant peptide may comprise from about 1 to about 53, or 1 to 39, or 1 to 38, or 1 to 37, or 1 to 35, or 1 to 34, or 1 to 31, or 1 to 27, or 1 to 22, or 10 to 35, or about 15 to about 37 residues

of the NPPC polypeptide. In one embodiment, a CNP variant may comprise a sequence of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, or 53 amino acids derived from the NPPC polypeptide.

[0127] The term “conjugate moiety” refers to a moiety that is conjugated to the variant peptide. Conjugate moieties include a lipid, fatty acid, hydrophilic spacer, synthetic polymer, linker, or optionally, combinations thereof.

[0128] The term “effective amount” refers to a dosage sufficient to produce a desired result on a health condition, pathology, or disease of a subject or for a diagnostic purpose. The desired result may comprise a subjective or objective improvement in the recipient of the dosage. “Therapeutically effective amount” refers to that amount of an agent effective to produce the intended beneficial effect on health. An appropriate “effective” amount in any individual case may be determined by one of ordinary skill in the art using routine experimentation. It will be understood that the specific dose level and frequency of dosage for any particular patient may be varied and will depend upon a variety of factors, including the activity of the specific compound employed; the bioavailability, metabolic stability, rate of excretion and length of action of that compound; the mode and time of administration of the compound; the age, body weight, general health, sex, and diet of the patient; and the severity of the particular condition.

[0129] “Substantially pure” or “isolated” means an object species is the predominant species present (i.e., on a molar basis, more abundant than any other individual macromolecular species in the composition), and a substantially purified fraction is a composition wherein the object species comprises at least about 50% (on a molar basis) of all macromolecular species present. In one embodiment, a substantially pure composition means that the species of interest comprises at least about 70%, 75%, 80%, 85%, 90%, 95%, 98% or more of the macromolecular species present in the composition on a molar or weight basis. The object species is purified to essential homogeneity (contaminant species cannot be detected in the composition by conventional detection methods) if the composition consists essentially of a single macromolecular species. Solvent species, small molecules (<500 Daltons), stabilizers (e.g., BSA), and elemental ion species are not considered macromolecular species for purposes of this definition. In an embodiment, the compounds of the disclosure are substantially pure or isolated. In another embodiment, the compounds of the disclosure are substantially pure or isolated with respect to the macromolecular starting materials used in their production. In yet another embodiment, the pharmaceutical compositions of the disclosure comprise a substantially pure or isolated CNP variant admixed with one or more pharmaceutically

acceptable excipients, carriers or diluents, and optionally with another biologically active agent.

[0130] "Treatment" refers to prophylactic treatment or therapeutic treatment or diagnostic treatment. In certain embodiments, "treatment" refers to administration of a compound or composition to a subject for therapeutic, prophylactic or diagnostic purposes.

[0131] A "prophylactic" treatment is a treatment administered to a subject who does not exhibit signs of a disease or exhibits only early signs of the disease, for the purpose of decreasing the risk of developing pathology. The compounds or compositions of the disclosure may be given as a prophylactic treatment to reduce the likelihood of developing a pathology or to minimize the severity of the pathology, if developed.

[0132] A "therapeutic" treatment is a treatment administered to a subject who exhibits signs or symptoms of pathology for the purpose of diminishing or eliminating those signs or symptoms. The signs or symptoms may be biochemical, cellular, histological, functional or physical, subjective or objective. The compounds of the disclosure may also be given as a therapeutic treatment or for diagnosis.

[0133] "Diagnostic" means identifying the presence, extent and/or nature of a pathologic condition. Diagnostic methods differ in their specificity and selectivity. While a particular diagnostic method may not provide a definitive diagnosis of a condition, it suffices if the method provides a positive indication that aids in diagnosis.

[0134] "Bone- or cartilage-associated biomarker" or "bone- or cartilage-associated marker" refers to a growth factor, enzyme, protein, or other detectable biological substance or moiety whose level is increased or decreased in association with, e.g., cartilage turnover, cartilage formation, cartilage growth, bone resorption, bone formation, bone growth, or combinations thereof. Such biomarkers may be measured before, during and/or after administration of a CNP variant as described herein. Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, propeptides of collagen type I and fragments thereof, collagen type I and fragments thereof, osteocalcin, proliferating cell nuclear antigen (PCNA), aggrecan chondroitin sulfate, collagen X, alkaline propeptides of type I procollagen and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, collagen X, CXM (noncollagenous 1 (NC1) domain of type X collagen), NTproCNP, N-terminal collagen type I pro-peptide, bone-specific alkaline phosphatase, amino-terminal propeptide of type I collagen/procollagen type I N-propeptide (PINP), cross-linked C-telopeptide of type I

collagen (CTx), cross-linked N-telopeptide of type I collagen (NTx) tartrate-resistant acid phosphatase 5b (TRAP-5b), transcriptomics readouts, e.g., from PAXgene® RNA, and CNP-variant bioactivity. Cartilage- and bone-associated biomarkers can be measured in any appropriate biological sample, including but not limited to tissues, blood, serum, plasma, cerebrospinal fluid, synovial fluid and urine. In some embodiments, the biomarkers are measured in blood, plasma, urine, or serum from subjects undergoing efficacy/pharmacodynamic *in vivo* studies and/or from the conditioned media of *ex vivo* studies.

[0135] "Pharmaceutical composition" or "formulation" refers to a composition suitable for pharmaceutical use in subject animal, including humans and mammals. A pharmaceutical composition comprises a therapeutically effective amount of CNP variant, optionally another biologically active agent, and optionally a pharmaceutically acceptable excipient, carrier or diluent. In an embodiment, a pharmaceutical composition encompasses a composition comprising the active ingredient(s), and the inert ingredient(s) that make up the carrier, as well as any product that results, directly or indirectly, from combination, complexation or aggregation of any two or more of the ingredients, or from dissociation of one or more of the ingredients, or from other types of reactions or interactions of one or more of the ingredients. Accordingly, the pharmaceutical compositions of the present disclosure encompass any composition made by admixing a compound of the disclosure and a pharmaceutically acceptable excipient, carrier or diluent.

[0136] "Pharmaceutically acceptable carrier" refers to any of the standard pharmaceutical carriers, buffers, and the like, such as a phosphate buffered saline solution, 5% aqueous solution of dextrose, and emulsions (e.g., an oil/water or water/oil emulsion). Non-limiting examples of excipients include adjuvants, binders, fillers, diluents, disintegrants, emulsifying agents, wetting agents, lubricants, glidants, sweetening agents, flavoring agents, and coloring agents. Suitable pharmaceutical carriers, excipients and diluents are described in Remington's Pharmaceutical Sciences, 19th Ed. (Mack Publishing Co., Easton, 1995). Preferred pharmaceutical carriers depend upon the intended mode of administration of the active agent. Typical modes of administration include enteral (e.g., oral) or parenteral (e.g., subcutaneous, intramuscular, intravenous or intraperitoneal injection; or topical, transdermal, or transmucosal administration).

[0137] A "pharmaceutically acceptable salt" is a salt that can be formulated into a compound for pharmaceutical use, including but not limited to metal salts (e.g., sodium, potassium, magnesium, calcium, etc.) and salts of ammonia or organic amines.

[0138] By “pharmaceutically acceptable” or “pharmacologically acceptable” is meant a material that is not biologically or otherwise undesirable, *i.e.*, the material may be administered to an individual without causing any undesirable biological effects or without interacting in a deleterious manner with any of the components of the composition in which it is contained or with any components present on or in the body of the individual.

[0139] “Physiological conditions” refer to conditions in the body of an animal (e.g., a human). Physiological conditions include, but are not limited to, body temperature and an aqueous environment of physiologic ionic strength, pH and enzymes. Physiological conditions also encompass conditions in the body of a particular subject which differ from the “normal” conditions present in the majority of subjects, e.g., which differ from the normal human body temperature of approximately 37 °C or differ from the normal human blood pH of approximately 7.4.

[0140] By “physiological pH” or a “pH in a physiological range” is meant a pH in the range of approximately 7.0 to 8.0 inclusive, more typically in the range of approximately 7.2 to 7.6 inclusive.

[0141] As used herein, the term “subject” encompasses mammals and non-mammals. Examples of mammals include, but are not limited to, any member of the mammalian class: humans, non-human primates such as chimpanzees, and other apes and monkey species; farm animals such as cattle, horses, sheep, goats, swine; domestic animals such as rabbits, dogs, and cats; laboratory animals including rodents, such as rats, mice and guinea pigs, and the like. Examples of non-mammals include, but are not limited to, birds, fish, and the like. The term does not denote a particular age or gender. In various embodiments, the subject is human. In various embodiments the subject is a child or adolescent. In various embodiments, the subject is an infant. In various embodiments, the subject is older than 3, older than 2, older than 1, or older than 6 months in age.

C-type natriuretic peptide variants

[0142] C-type natriuretic peptide (CNP) (Biochem. Biophys. Res. Commun., 168: 863-870 (1990) (GenBank Accession No. NP_077720, for the CNP precursor protein, NPPC) (J. Hypertens., 10: 907-912 (1992)) is a small, single chain peptide in a family of peptides (ANP, BNP, CNP) having a 17-amino acid loop structure (Levin et al., N. Engl. J. Med., 339: 863-870 (1998)) and have important roles in multiple biological processes. CNP interacts with natriuretic peptide receptor-B (NPR-B, GC-B) to stimulate the generation of cyclic- guanosine monophosphate (cGMP) (J. Hypertens., 10: 1111-1 114 (1992)). CNP is expressed more widely, including in the central nervous system,

reproductive tract, bone and endothelium of blood vessels (Hypertension, 49: 419-426 (2007)).

[0143] Natural CNP gene and polypeptide have been previously described. U.S. Patent No. 5,352,770 discloses isolated and purified CNP-22 from porcine brain identical in sequence to human CNP and its use in treating cardiovascular indications. U.S. Patent No. 6,034,231 discloses the human gene and polypeptide of pre-proCNP (126 amino acids) and the human CNP-53 gene and polypeptide. The mature CNP is a 22-amino acid peptide (CNP-22). Certain CNP variants are disclosed in US Patent 8,198,242, incorporated by reference herein.

[0144] In various embodiments, CNP of the disclosure includes truncated CNP ranging from human CNP-17 (hCNP-17) to human CNP-53 (hCNP-53), and having wild-type amino acid sequences derived from hCNP-53. Such truncated CNP peptides include:

DLRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-53) (SEQ ID NO: 56);
LRVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-52) (SEQ ID NO: 15);
RVDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-51) (SEQ ID NO:16);
VDTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-50) (SEQ ID NO:17);
DTKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-49) (SEQ ID NO: 18)
TKSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-48) (SEQ ID NO:19);
KSRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-47) (SEQ ID NO:20);
SRAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-46) (SEQ ID NO:21);
RAAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-45) (SEQ ID NO:22);
AAWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-44) (SEQ ID NO:23); AWARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-43) (SEQ ID NO:24); WARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-42) (SEQ

ID NO:25); ARLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-41) (SEQ ID NO:26); RLLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-40) (SEQ ID NO:27); LLQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-39) (SEQ ID NO:28); LQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-38) (SEQ ID NO:2); QEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-37) (SEQ ID NO:3); EHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-36) (SEQ ID NO:29); HPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-35) (SEQ ID NO:30); PNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-34) (SEQ ID NO:4); NARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-33) (SEQ ID NO:31); ARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-32) (SEQ ID NO:32); RKYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-31) (SEQ ID NO:33); KYKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-30) (SEQ ID NO:34); YKGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-29) (SEQ ID NO:35); KGANKKGLSKGCFGLKLDRI SMSGLGC (CNP-28) (SEQ ID NO:36); GANKKGLSKGCFGLKLDRI SMSGLGC (CNP-27) (SEQ ID NO:37); ANKKGLSKGCFGLKLDRI SMSGLGC (CNP-26) (SEQ ID NO:38); NKKGLSKGCFGLKLDRI SMSGLGC (CNP-25) (SEQ ID NO:39); KKGLSKGCFGLKLDRI SMSGLGC (CNP-24) (SEQ ID NO:40); KGLSKGCFGLKLDRI SMSGLGC (CNP-23) (SEQ ID NO:41); GLSKGCFGLKLDRI SMSGLGC (CNP-22) (SEQ ID NO: 68); LSKGCFGLKLDRI SMSGLGC (CNP-21) (SEQ ID NO:42); SKGCFGLKLDRI SMSGLGC (CNP-20) (SEQ ID NO:43); KGCFGLKLDRI SMSGLGC (CNP-19) (SEQ ID NO:44); GCFGLKLDRI SMSGLGC (CNP-18) (SEQ ID NO:45); and CFGLKLDRI SMSGLGC (CNP-17) (SEQ ID NO: 67).

[0145] In various embodiments, the CNP variant peptides are modified CNP-37 or CNP-38 peptides, optionally having mutation(s)/substitution(s) at the furin cleavage site (underlined), and/or containing glycine or proline-glycine at the N-terminus. Exemplary CNP-37 variants include but are not limited to:

QEHPNARKYKGANKKGLSKGCFGLKLDRI SNSGLGC [CNP-37(M32N); SEQ ID NO: 46]; MQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (Met-CNP-37; SEQ ID NO: 47); PQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (Pro-CNP-37; SEQ ID NO: 48); GQEHPNARKYKGANKKGLSKGCFGLKLDRI SNSGLGC [Gly-CNP-37 (M32N); SEQ ID NO: 49]; PGQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (Pro-Gly-CNP-37; SEQ ID

NO:1); MGQEHNPARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (Met-Gly-CNP-37; SEQ ID NO: 50);

GQEHNPARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (Gly-CNP-37: SEQ ID NO:51)
 GQEHNPARKYKGANPKGLSKGCFGLKLDRI SMSGLGC (SEQ ID NO:52);
 GQEHNPARKYKGANQKGLSKGCFGLKLDRI SMSGLGC (SEQ ID NO:53);
 GQEHNPARKYKGANQQGLSKGCFGLKLDRI SMSGLGC (SEQ ID NO:54); and
 GQEHNPARKYKGANKPGLSKGCFGLKLDRI SMSGLGC (SEQ ID NO:55);

[0146] In various embodiments, CNP variants of the disclosure include PGQEHQPARRYRGAQRRGLSRGCFGLKLDRI SMSGLGC (SEQ ID NO: 5);
 PGQEHNPARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (SEQ ID NO: 1);
 PGQEHNPARRYRGANRRGLSRGCFGLKLDRI SMSGLGC (SEQ ID NO: 6);
 PGQEHQPARRYRGAQRRGLSRGCFGLKLDRI SMSGLGC (SEQ ID NO: 5);
 PGQEHQPARKYKGAQKKGLSKGCFGLKLDRI SMSGLGC (SEQ ID NO: 7);
 PGQEHQPARRYRGAQRRGLSRGCFGLK(AEEA-AEEA-YGIU-C 18DA) LDRI SMSGLGC (SEQ ID NO:5); and
 PGQEHNPARKYKGANKKGLSKGCFGLK(AEEA-AEEA-YGIU-C18DA) LDRI SMSGLGC (SEQ ID NO: 1).

[0147] In various embodiments, the CNP variant further comprises an acetyl group. In various embodiments, the acetyl group is on the N-terminus, C-terminus or attached to an internal amino acid side group. In various embodiments, the acetyl group is on the N-terminus of the peptide.

[0148] In various embodiments, the peptide variant further comprises an OH or an NH₂ group at the C-terminus.

[0149] In various embodiments, the CNP variants are selected from the group consisting of:

Ac-PGQEHQPARRYRGAQRRGLSRGCFGLKLDRI SMSGLGC-OH (SEQ ID NO: 8),
 Ac-PGQEH PNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC-NH₂ (SEQ ID NO: 9),
 Ac-PGQEHNPARRYRGANRRGLSRGCFGLKLDRI SMSGLGC-OH (SEQ ID NO: 10),
 AC-PGQEHNPARRYRGANRRGLSRGCFGLKLDRI SMSGLGC-NH₂ (SEQ ID NO: 11),
 and

AC-PGQEHPQARRYRGAQRRGLSRGCFGLKLDRI SMSGLGC-NH₂ (SEQ ID NO: 12).

[0150] In various embodiments, the CNP variants are selected from the group consisting of: PGQEHPQARRYRGAQRRGLSRGCFGLKLDRI SMSGLGC-OH (SEQ ID NO: 8), PGQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC-NH₂ (SEQ ID NO: 9), PGQEHPNARRYRGANRRGLSRGCFGLKLDRI SMSGLGC-OH (SEQ ID NO: 10), PGQEHPNARRYRGANRRGLSRGCFGLKLDRI SMSGLGC-NH₂ (SEQ ID NO: 11), and PGQEHPQARRYRGAQRRGLSRGCFGLKLDRI SMSGLGC-NH₂ (SEQ ID NO: 12) PGQEHPQARKYKGAQKKGLSKGCFGLKLDRI SMSGLGC-OH (SEQ ID NO: 7).

[0151] In various embodiments, the CNP variants are selected from the group consisting of:

Ac-PGQEHPQARRYRGAQRRGLSRGCFGLKLDRI SMSGLGC-OH (SEQ ID NO: 8),
Ac-PGQEH PNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC-NH₂ (SEQ ID NO: 9),
Ac-PGQEHPNARRYRGANRRGLSRGCFGLKLDRI SMSGLGC-OH (SEQ ID NO: 10),
Ac-PGQEH PNARRYRGANRRGLSRGCFGLKLDRI SMSGLGC-NH₂ (SEQ ID NO: 11) ,
Ac-PGQEH PQARRYRGAQRRGLSRGCFGLKLDRI SMSGLGC-NH₂ (SEQ ID NO: 12);
Ac-PGQEHPQARKYKGAQKKGLSKGCFGLKLDRI SMSGLGC-NH₂ (SEQ ID NO: 13);
and

Ac-PGQEHPQARKYKGAQKKGLSKGCFGLKLDRI SMSGLGC-OH (SEQ ID NO: 14).

[0152] In various embodiments, the CNP variant is selected from the group consisting of Ac-PGQEHPQARRYRGAQRRGLSRGCFGLK(AEEA-AEEA-YGIU-C18DA)LDRIGSMSGLGC-OH (SEQ ID NO:8). In various embodiments, the CNP variant is Ac-PGQEHPNARKYKGANKKGLSKGCFGLK(AEEA-AEEA-YGIU-C18DA)LDRIGSMSGLGC-OH (SEQ ID NO: 1). In various embodiments, the CNP variant is PGQEH PNARKYKGANKKGLSKGCFGLK(AEEA-AEEA-YGIU-C18DA)LDRIGSMSGLGC-OH (SEQ ID NO: 1).

[0153] In additional embodiments, for any of the CNP variants described herein that have asparagine (Asn/N) residue(s) and/or glutamine (Gln/Q) residue(s), whether they have a wild- type sequence or a non-natural amino acid sequence, any Asn residue(s) and/or any Gln residue(s) can independently be substituted with any other natural or unnatural amino acids, including conservative substitutions such as Asn to Gln. Such substitution(s) are designed in part to minimize or avoid any potential deamidation of asparagine and/or glutamine.

[0154] In one embodiment, the CNP variants are cyclized via formation of a disulfide bond between Cys⁶ and Cys²² as designated in the wtCNP22 peptide. Cys⁶ can be a cysteine analog such as, e.g., homocysteine or penicillamine. In a further embodiment, the CNP variants can be cyclized by a covalent bond formed head-to-tail, side chain-to-side chain, side chain-to-head, or side chain-to-tail. In an embodiment, the covalent bond is formed between an amino acid at or toward the N-terminus and an amino acid at or toward the C-terminus of the peptide (referred to as "terminal" amino acids in this context). In another embodiment, the covalent bond is formed between the side chains of the two terminal amino acids. In yet another embodiment, the covalent bond is formed between the side chain of one terminal amino acid and the terminal group of the other terminal amino acid, or between the terminal groups of the two terminal amino acids.

[0155] Head-to-tail cyclization of the terminal amine to the terminal carboxyl group can be carried out using a number of methods, e.g., using p-nitrophenyl ester, 2,4,5-trichlorophenyl ester, pentafluorophenyl ester, the azide method, the mixed anhydride method, HATU, a carbodimide (e.g., DIC, EDC or DCC) with a catalyst such as HOBt, HONSu or HOAt, or on-resin cyclization.

[0156] In addition, the cyclic structure can be formed via a bridging group involving the side chains of amino acid residues of the CNP variant and/or the terminal amino acid residues. A bridging group is a chemical moiety that allows cyclization of two portions of the peptide. No limiting examples of bridging groups include amides, thioethers, thioesters, disulfides, ureas, carbamates, sulfonamides, and the like. A variety of methods are known in the art for incorporation of units having such bridging groups. For example, a lactam bridge (i.e., a cyclic amide) can be formed between the N-terminal amino group or an amino group on a side chain and the C-terminal carboxylic acid or a carboxyl group on a side chain, e.g., the side chain of lysine or ornithine and the side chain of glutamic acid or aspartic acid. A thioester can be formed between the C-terminal carboxyl group or a carboxyl group on a side chain and the thiol group on the side chain of cysteine or a cysteine analog.

[0157] Alternatively, a cross link can be formed by incorporating a lanthionine (thio-dialanine) residue to link alanine residues that are covalently bonded together by a thioether bond. In another method, a cross-linking agent, such as a dicarboxylic acid (e.g., suberic acid (octanedioic acid)), can link the functional groups of amino acid side chains, such as free amino, hydroxyl, and thiol groups.

[0158] Enzyme-catalyzed cyclization can also be used. For example, it has been reported that the thioesterase domain of tyrocidine synthetase can be used to cyclize a thioester precursor, a subtilisin mutant can be utilized to cyclize peptide glycolate phenylalanylamide esters, and the antibody ligase 16G3 can be employed to cyclize a p-nitrophenylester. For a review of peptide cyclization, see Davies, J. *Peptide Sci.*, 9: 471-501 (2003), incorporated herein by reference in its entirety.

[0159] In certain embodiments, the final product has a purity of at least about 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or at least about 99%.

Peptide Conjugates

[0160] Peptide therapeutics are attractive biological therapeutic agents, but are often disadvantaged by low stability and short half-life in solution (Tang et al., *Eur J Pharm Sci.* 102:63-70, 2017). Attempts to improve efficacy of peptide therapeutics, by enhancing stability and/or increasing the half-life, include attempts to encapsulate hydrophilic peptides into biodegradable particles such as liposomes or polymer particles. However, this has been difficult due to the cationic nature of these peptides and their ability to electrostatically interact with liposomes of negatively charged polymers (Griesser et al., *Int J Pharmaceutics* 520:267-274, 2017). Generation of peptide conjugates has been one means used to enable better encapsulation of hydrophilic polymers into microparticles or liposomes (Lu et al., *Mol. Pharmaceutics* 15:216-225, 2018).

[0161] Peptides can be a string of amino acids from 5 to 100 amino acids. The peptide can have positively charged amino acids, negatively charged amino acids, or a mixture of both, such that the peptide is capable of interacting with charged moieties, e.g., a cation, anion or a combination thereof having charged species opposite to those in the peptide.

[0162] It is contemplated that the peptide is complexed to a moiety, e.g., a conjugate moiety, that confers increased stability or half-life. In various embodiments, the conjugate moiety is complexed via a non-covalent bond or is attached by a covalent bond. The moiety may be non-covalently attached with the peptide via electrostatic interactions. Alternatively, the moiety may be covalently associated to the peptide via one or more linker moieties. Linkers can be cleavable and non-cleavable linkers. Cleavable linkers may be cleaved via enzymes, nucleophilic/basic reagents, reducing agents, photo-irradiation, electrophilic/acidic reagents, organometallic and metal reagents, or oxidizing reagents. Linkers may also be self-immolative linkers. Linkers may also be traceless linkers. Exemplary linkers include, but are not limited to, N-succinimidyl-3- (2-pyridyldithiol) propionate (SPDP), iminothiolane (IT),

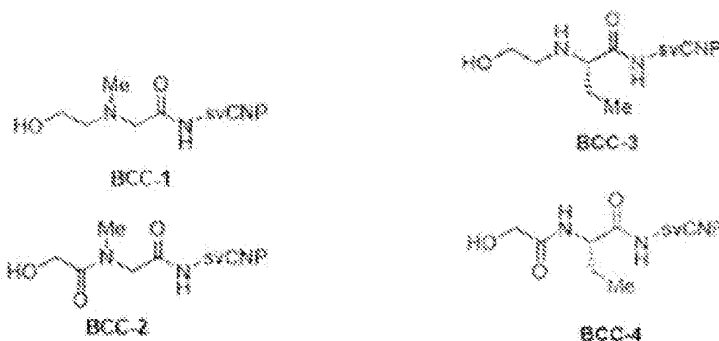
bifunctional derivatives of imidoesters (such as dimethyl adipimidate HCL), active esters (such as disuccinimidyl suberate), aldehydes (such as glutarealdehyde), bis-azido compounds (such as bis (p-azidobenzoyl) hexanediamine), bis- diazonium derivatives (such as bis-(p-diazoniumbenzoyl)-ethylenediamine), diisocyanates (such as tolyene 2,6-diisocyanate), and bis-active fluorine compounds (such as 1,5-difluoro-2,4- dinitrobenzene), beta alanine, 4-aminobutyric acid (GABA), 2-aminoethoxy acid (AEA), aminoethoxy-2-ethoxy acetic acid (AEEA), 5 aminovaleric acid (AVA), 6-aminocaproic acid (Abx), a vicinal diol cleavable linker, Trimethyl Lock Lactonization, p-alkoxyphenyl carbamate, bicin, peptoid or bicin-type linkers, and electronic linkers as described herein.

[0163] In various embodiments, the linker is attached to a residue of the CNP variant within the CNP cyclic domain or at a site other than the CNP cyclic domain. In various embodiments, the linker is attached to a lysine residue. In various embodiments, the linker is attached to a lysine residue in the CNP cyclic domain.

[0164] In various embodiments, the CNP variant is attached to the conjugate moiety via the linker. In various embodiments, the linker is attached to the conjugate moiety via the hydrophilic spacer of the conjugate moiety.

[0165] In various embodiments, the linker is a hydrolysable linker.

[0166] In various embodiments the linker is a peptoid or electronic linker. In various embodiments the linker is a peptoid linker. In various embodiments the linker is an electronic linker. In various embodiments, the linker comprises an SO₂ moiety. Exemplary linkers are illustrated in Figure 1 and below. It is further contemplated that linkers in Figure 1 are modified by substitution on the R groups. For example, bicin-type linkers include the structures as set out below:



[0167] In various embodiments, the moiety conjugated to the peptide is a synthetic polymer such as polyethylene glycol, a linker, a lipid moiety or fatty acid, or a combination thereof. In various embodiments, the CNP variant is conjugated with a fatty acid, an amino acid, a spacer and a linker. In various embodiments, the CNP variant is conjugated with a fatty acid, an amino acid, a polyethylene glycol spacer or a polyethylene glycol derivative spacer, and a linker. In various embodiments, the CNP variant is conjugated with a fatty acid, an amino acid, a spacer, and a linker, wherein the spacer comprises a substituted C-6 to C-20 alkyl chain or any amino acid, or a combination of both, wherein the carbon atoms of the alkyl chain can be replaced by one or more of O, NH, N(C-1 to C-6 alkyl), or carbonyl groups.

[0168] In various embodiments, the CNP variant is conjugated with a fatty acid. It is hypothesized that the lipid technology increases the serum half-life of the CNP variant allowing for less frequent injections and/or improved oral delivery. In various embodiments, the fatty acid is a short chain, medium chain, long chain fatty acid, or a dicarboxylic fatty acid. In various embodiments, the fatty acid is saturated or unsaturated. In various embodiments, the fatty acid is a C-6 to C-20 fatty acid. In various embodiments, the fatty acid is a C-6, C-8, C-10, C-12, C-14, C-16, C-18 or C-20 fatty acid. In various embodiments, the fatty acid is decanoic acid, dodecanoic acid, myristic acid, palmitic acid, stearic acid, arachidic acid, or diacids of the same. In various embodiments, the fatty acid is conjugated to a lysine residue.

[0169] In various embodiments, it is contemplated that the CNP variants described herein comprise a conjugate moiety as described herein. It is contemplated that the conjugate moiety is on a residue of the CNP cyclic domain or at a site other than the CNP cyclic domain. In various embodiments, the conjugate moiety is on a lysine residue. In various embodiments, the conjugate moiety comprises one or more acid moieties. In various embodiments, the acid moiety is a fatty acid.

[0170] In various embodiments, the conjugate moiety comprises an acid moiety linked to a hydrophilic spacer. In various embodiments, the hydrophilic spacer is a substituted C-6 to C-20 alkyl chain or any amino acid, or a combination of both, wherein the carbon atoms of the alkyl chain can be replaced by one or more of O, NH, N(C-1 to C-6 alkyl), or carbonyl groups. In various embodiments, the hydrophilic spacer is any amino acid. In various embodiments, the hydrophilic spacer is gamma glutamic acid (γGlu). In various embodiments, the hydrophilic spacer is a substituted C-6 to C-20 alkyl chain. In

various embodiments, the hydrophilic spacer is a substituted C-6, C-8, C-10, C-12, C-14, C-16, C-18 or C-20 alkyl chain. In various embodiments, the hydrophilic spacer is a substituted C-9 to C-18 alkyl chain. In various embodiments, the hydrophilic spacer is a substituted C-18 alkyl chain. In various embodiments, the hydrophilic spacer is a substituted C-9 alkyl chain. In various embodiments, the hydrophilic spacer is one or more OEG (8-amino-3,6-dioxaoctanoic acid) groups. In various embodiments, the hydrophilic spacer is one or two OEG (8-amino-3,6-dioxaoctanoic acid) groups. In various embodiments, the hydrophilic spacer is OEG (8-amino-3,6-dioxaoctanoic acid). In various embodiments, the spacer is OEG (8-amino-3,6-dioxaoctanoic acid) or yGlu. In various embodiments, the hydrophilic spacer is gamma glutamic acid (yGlu) linked to one or more OEG (8-amino-3,6-dioxaoctanoic acid) groups. In various embodiments, the hydrophilic spacer is gamma glutamic acid (yGlu) linked to one or two OEG (8-amino-3,6-dioxaoctanoic acid) groups (diEG). In various embodiments, the acid moiety and the hydrophilic spacer have the structure AEEA-AEEA-yGlu-d8DA.

[0171] In various embodiments, the CNP variant has the structure:

PGQEHPQARRYRGAQRRGLSRGCFGLK(AEEA-AEEA-yGlu-C18DA)LDRIGSMSGLGC (SEQ

ID NO: 5), or Ac-PGQEHPQARRYRGAQRRGLSRGCFGLK(AEEA-AEEA-yGlu-C18DA)LDRIGSMSGLGC-OH (SEQ ID NO: 8). In various embodiments, the CNP

variant has the structure PGQEHPNARKYKGANKKGLSKGCFGLK(AEEA-AEEA-yGlu-C18DA)LDRIGSMSGLGC, Ac-PGQEHPNARKYKGANKKGLSKGCFGLK(AEEA-AEEA-yGlu-C18DA)LDRIGSMSGLGC-OH (SEQ ID NO: 1), or

PGQEHPNARKYKGANKKGLSKGCFGLK(AEEA-AEEA-yGlu-C18DA)LDRIGSMSGLGC-OH (SEQ ID NO: 1). In various embodiments, the CNP variant comprises Asn to Glu variants of the above peptides.

[0172] In various embodiments, the disclosure contemplates use of hydrophilic or water soluble polymers (e.g., oxygenated alkyl chains, wherein the carbon atoms can be replaced with one or more oxygen atoms, such as polyethylene glycol (PEG) or polyethylene oxide (PEO) and the like). In various embodiments, the water soluble polymers can vary in type (e.g., homopolymer or copolymer; random, alternating or block copolymer; linear or branched; monodispersed or polydispersed), linkage (e.g., hydrolysable or stable linkage such as, e.g., amide, imine, amination, alkylene, or ester bond), conjugation site (e.g., at the N-terminus, internal, and/or C-terminus), and length (e.g., from about 0.2, 0.4 or 0.6 kDa to about 2, 5, 10, 25, 50 or 100 kDa). The

hydrophilic or water-soluble polymer can be conjugated to the CNP variant by means of N-hydroxy succinimide (NHS)- or aldehyde-based chemistry or other chemistry, as is known in the art. In various embodiments, negatively charged PEG-CNP variants can be designed for reduced renal clearance, including but not limited to use of carboxylated, sulfated and phosphorylated compounds (Caliceti, Adv. Drug Deliv. Rev., 55: 1261-77 (2003); Perlman, J. Clin. Endo. Metab., 88: 3227-35 (2003); Pitkin, Antimicrob. Ag. Chemo., 29: 440-444 (1986); Vehaskari, Kidney Int'l, 22: 127-135 (1982)). In one embodiment, the PEG (or PEO) moiety contains carboxyl group(s), sulfate group(s), and/or phosphate group(s).

[0173] In another embodiment, the hydrophilic polymer (e.g., PEG or PEO) moieties conjugated to the N-terminus, C-terminus and/or internal site(s) of CNP variants described herein contain one or more functional groups that are positively charged under physiological conditions. Such moieties are designed, *inter alia*, to improve distribution of such conjugated CNP variants to cartilage tissues. In one embodiment, PEG moieties contain one or more primary, secondary or tertiary amino groups, quaternary ammonium groups, and/or other amine-containing (e.g., urea) groups.

Methods of Making

[0174] Contemplated herein is also a method of making a composition comprising a CNP variant and optionally a conjugate moiety as described herein.

[0175] In various embodiments, the CNP variant is made synthetically using standard protein synthesis chemistry. For example, peptides are synthesized step-wise using a solid-phase resin and standard Fmoc chemistry. Peptides are cleaved from the resin using tri-fluoroacetic acid (TFA) and purified by reverse phase high-performance liquid chromatography (RP-HPLC).

[0176] In various embodiments, the method further comprises, acetylating the peptides by reacting the resin with NMP/Ac₂O/DIEA, optionally at 10:1:0.1, v/v/v.

[0177] Further provided is a method wherein the peptide is conjugated to a conjugate moiety, optionally on a lysine residue. The step comprising cleaving the protective amino group on the lysine, reacting the peptide with 2x Fmoc-amino PEG(2) followed by amino acid, followed by conjugation of the lipid or fatty acid moiety. In various embodiments, the conjugate moiety comprises one or more lipids or fatty acids and a hydrophobic spacer.

[0178] The method further provides a step of cleaving the peptide from the resin by contacting with tri-fluoroacetic acid, and a step of purifying the peptide by reverse phase-HPLC.

[0179] In certain embodiments, the CNP variants described herein are produced by a recombinant process that comprises culturing in a medium a host cell comprising a first polynucleotide encoding a CNP variant polypeptide, optionally linked to a second polynucleotide encoding a cleavable peptide or protein under conditions that result in expression of a fusion polypeptide encoded by the polynucleotides. In some embodiments, the host cell is transformed with an expression vector comprising the polynucleotide encoding the CNP variant polypeptide, optionally linked to the polynucleotide encoding the cleavable peptide or protein. In certain embodiments, the fusion polypeptide is expressed as a soluble protein or as an inclusion body. The expressed fusion polypeptide can be isolated from the host cell or culture medium, and the isolated fusion polypeptide can be contacted with a cleaving agent to release the CNP variant.

[0180] Methods of making CNP variant peptides, including use of host cells, expression vectors, cleavable peptides, and culture parameters, are disclosed in U.S. Patent 8,198,242, hereby incorporated by reference.

Methods of Use

[0181] Achondroplasia is a result of an autosomal dominant mutation in the gene for fibroblast growth factor receptor 3 (FGFR-3), which causes an abnormality of cartilage formation. FGFR-3 normally has a negative regulatory effect on chondrocyte growth, and hence bone growth. In achondroplasia, the mutated form of FGFR-3 is constitutively active, which leads to severely shortened bones. In humans activating mutations of FGFR-3 are the primary cause of genetic dwarfism. Mice having activated FGFR-3 serve as a model of achondroplasia, the most common form of the skeletal dysplasias, and overexpression of CNP rescues these animals from dwarfism. Accordingly, functional variants of CNP are potential therapeutics for treatment of the various skeletal dysplasias

[0182] By stimulating matrix production, proliferation and differentiation of chondrocytes and increasing long bone growth, the CNP variants of the disclosure are useful for treating mammals, including humans, suffering from a bone-related disorder, such as a skeletal dysplasia. Non-limiting examples of CNP-responsive bone-related disorders and skeletal dysplasias include achondroplasia, hypochondroplasia, short stature, dwarfism,

osteochondrodysplasias, thanatophoric dysplasia, osteogenesis congenita, achondrogenesis, chondrodysplasia congenita, homozygous achondroplasia, campomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis congenita, short-rib polydactyly syndromes, rhizomelic type of chondrodysplasia congenita, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenital, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia. Short stature, growth plate disorder, bone-related disorder or skeletal dysplasias contemplated herein include disorders related to NPR2 mutation, SHOX mutation (Turner's syndrome/Leri Weill), and PTPN11 mutations (Noonan's syndrome).

[0183] By stimulating matrix production, proliferation and differentiation of chondrocytes and increasing long bone growth, the CNP variants of the disclosure are useful for treating mammals, including humans, suffering from a bone-related disorder, such as a skeletal dysplasia. Non-limiting examples of CNP-responsive bone-related disorders and skeletal dysplasias include achondroplasia, hypochondroplasia, short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis congenita, achondrogenesis, chondrodysplasia congenita, homozygous achondroplasia, campomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis congenita, short-rib polydactyly syndromes, rhizomelic type of chondrodysplasia congenita, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenital, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia. Short stature, growth plate disorder, bone-related disorder or skeletal dysplasias contemplated herein include disorders related to NPR2 mutation, SHOX mutation (Turner's syndrome/Leri Weill), PTPN11 mutations (Noonan's syndrome) and IGF1R mutation.

[0184] Further provided herein, the CNP variants of the disclosure are useful for treating mammals, including humans, suffering from a bone-related disorder, such as a skeletal dysplasia. Non-limiting examples of CNP-responsive bone-related disorders and skeletal

dysplasias include achondroplasia, hypochondroplasia, short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis congenita, achondrogenesis, chondrodysplasia congenita, homozygous achondroplasia, campomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis congenita, short-rib polydactyly syndromes, rhizomelic type of chondrodysplasia congenita, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenita, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia and osteoporosis.

[0185] Additional short stature and growth plate disorders contemplated by the methods include disorders related to mutations in collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), Indian hedgehog (IHH), PTPN11, NPR2, NPPC, or FGFR3.

[0186] Additional short stature and growth plate disorders contemplated by the methods include disorders related to mutations in collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), Indian hedgehog (IHH), PTPN11, NPR2, NPPC, FGFR3, or IGF1R.

[0187] Also provided herein is treatment of short stature and growth plate disorders including disorders related to mutations in collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), Indian hedgehog (IHH), SHOX, PTPN11, NPR2, NPPC, FGFR3, or IGF1R.

[0188] Further, the CNP variants are useful as an adjunct or alternative to growth hormone for treating idiopathic short stature and other skeletal dysplasias.

[0189] Growth plate disorders include disorders that result in short stature or abnormal bone growth and that may be the result of a genetic mutation in a gene involved in bone growth, including collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), Indian hedgehog (IHH), PTPN11, NPR2, NPPC, or FGFR3. In various embodiments, growth plate disorders include disorders that result in short stature or abnormal bone growth and that may be the result of a genetic mutation in a gene involved in bone growth, including collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), Indian hedgehog (IHH), PTPN11, NPR2, NPPC, FGFR3, or IGF1R. In various embodiments, the growth plate disorder or short stature is associated with one or more mutations in a gene associated with a RASopathy. In various embodiments, the growth

plate disorder or short stature is associated with one or more mutations in the SHOX gene. In various embodiments, a subject with a growth plate disorder is heterozygous for a mutation in a growth plate gene. In various embodiments, the mutation is a loss-of-function mutation. In various embodiments, the mutation is a gain-of-function mutation. Growth plate disorders include, but are not limited to, familial short stature, dominant familial short stature which is also known as dominant inherited short stature, or idiopathic short stature. See, e.g., Plachy et al., J Clin Endocrinol Metab 104: 4273-4281, 2019.

[0190] Mutations in ACAN can give rise to familial osteochondritis dissecans and short stature and eventually osteoarthritis, characterized by areas of bone damage (or lesions) caused by the detachment of cartilage and sometimes bone from the end of the bone at a joint. It has been suggested that the disorganized cartilage network in growing bones impairs their growth, leading to short stature. A mutation associated with ACAN and short stature includes Val2303Met. See Stattin et al., Am J Hum Genet 86(2): 126-37, 2010. It is contemplated that patients with a mutation in ACAN resulting in short stature would benefit from treatment with CNP as administration may be able to increase height in these patients by the known interaction of CNP with FGFR3.

[0191] The natriuretic peptide system, including receptor NPR2, has been shown to be involved in regulation of endochondral bone growth (Vasques et al., Horm Res Pediat 82:222- 229, 2014). Studies have shown that homozygous or compound heterozygous loss-of-function mutations in NPR2 cause acromesomelic dysplasia type Maroteaux (AMDM), which is a skeletal dysplasia having extremely short stature (Vasquez et al., 2014, *supra*). There are reports implicating heterozygous loss-of-function (such as dominant negative) NPR2 mutations as a cause of short stature, whereas gain-of-function NPR2 heterozygous mutations have been found to be responsible for tall stature (Vasquez et al., 2014, *supra*). In view of CNP's interaction with NPR2 to stimulate cGMP generation, increasing cGMP levels is desirable in these conditions and would have therapeutic benefit in the management of the complications from these diseases and conditions.

[0192] Heterozygous mutations of NPR2 are believed to result in idiopathic short stature and other forms of short stature. Mutations in the NPR2 gene are set out below and described in Amano et al., J Clin Endocrinol Metab 99:713-718, 2014, Hisado-Oliva et al., J Clin Endocrinol Metab 100 1133-1 142, 2015 and Vasques et al., J Clin Endocrinol Metab 98: E1636- 1644, 2013, hereby incorporated by reference. It is

contemplated that a subject having short stature to be treated with a CNP variant as described herein has a height SDS of less than -1.0, -1.5, -2.0, -2.5, or -3.0, and has at least one parent with a height SDS of less than -1.0, -1.5, -2.0 or -2.5, optionally wherein the second parent has height within the normal range. In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of between -2.0 to -3.0. In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of between -2.0 to -2.5. However, because de novo mutations in NPR2 can result in short stature as defined by a height SDS of less than -1.5, -2.0, -2.5, or -3.0, treatment of individuals who are heterozygous carriers of a deleterious mutation in NPR2 with neither parent having short stature is also contemplated. Further contemplated is treatment of individuals who are heterozygous for deleterious mutations in other growth plate genes with CNP to improve stature and/or enhance bone growth.

[0193] Exemplary NPR2 mutations in patients that may be treated with a CNP variant include:

Disease	Nucleotide	Mutation	Amino acid change
Short stature	1669C>T	missense	Arg557Cys
Short stature	2794C>T	missense	Arg932Cys
Short stature	2905G>C	missense	Val969Leu
Short stature	3058C>T	missense	Arg1020Trp
Short stature	2972A>G	missense	Glu991Gly
Short stature	1262C>T	missense	Thr421Met
Short stature	766G>T	missense	Asp256Tyr
Short stature	1982C>A	missense	Thr661Lys
Short stature	2449G>A	missense	Glu817Lys
Short stature	1517G>A	missense	Arg506His
Short stature	1802G>C	missense	Arg601Pro
Short stature	1481T>G	missense	Ile494Ser
Short stature	142G>T	missense	Ala48Ser
Short stature	1167G>T	missense	Glu389Asp
Short stature	1249C>G	missense	Gln417Glu
Short stature	328C>T	missense	Arg110Cys
Short stature	2455C>T	missense	Arg819Cys
Short stature	788G>C	missense	Arg263Pro
Short stature	226T>C	missense	Ser76Pro
Short stature	2710A>T	nonsense	Lys904Term

9:35809194:C:G	Leu1009Val
9:35802761:G:C	Leu615Phe
9:35799645:C:T	Pro301Ser
9:35792928:C:T	Arg174Cys
9:35801728:C:G	His508Asp
9:35792713:T:C	Val102Ala
9:35793980:T:A	Tyr250Ter
9:35807085:C:T	Thr861Ile
9:35793906:A:G	Ile226Val
9:35808558:G:A	Arg921Gln
9:35802741:G:A	Glu609Lys
9:35802594:G:A	Arg601His
9:35808663:T:A	Leu956Gln

[0194] NPPC's role in skeletal growth is well documented (Hisado-Oliva et al., Genetics Medicine 20:91-97, 2018). The NPPC knock out mouse showed severe disproportionate form of dwarfism including shortening of limbs and endochondral ossification (Hisado-Oliva et al., 2018, *supra*). Human genome wide studies have shown a link between NPPC and height (Hisado-Oliva et al., 2018, *supra*). Although CNP haploinsufficiency has been believed to be a cause of short stature in humans, a recent study identified heterozygous mutations in families with short stature and hands (Hisado-Oliva et al., 2018, *supra*). These studies observed significant reduction in cGMP production as measured in heterozygous state (Hisado-Oliva et al., 2018, *supra*). Mutations in NPPC include a 355G>T missense mutation causing a Gly1 19Cys change and a 349C>G missense mutation causing a Arg1 17Gly change. A CNP variant rescuing CGMP production may provide therapeutic benefit in the management of a disorder in patients having heterozygous loss-of-function NPPC mutations.

[0195] Isolated SHOX deficiency is one of the more prevalent monogenic causes of short stature (birth prevalence approximately 10 per 100,000 [Marchini et al., Endocr Rev. 37: 417–448, 2016]) (Genoni 2018). The SHOX gene, which is located on both the X and Y chromosomes, encodes for a transcription factor expressed throughout the growth plate with functions including influencing the natriuretic peptide receptor-type B (NPR-B) and fibroblast growth factor receptor 3 (FGFR3) pathways. The SHOX gene has been shown to be a repressor of FGFR3 transcription, and SHOX deficiency leads to increased FGFR3 signaling (Marchini, *supra*). Several pathogenic variants in SHOX are known to give rise to short stature. Individuals with pathogenic variants causing SHOX deficiency present with growth failure in the first year of life, height that often remains below -2.00 SDs compared to CDC

population norms, and no development of a pubertal growth spurt accentuating final statural impairment (Binder 2018; Fukami 2016; Jorge 2010).

[0196] Turner syndrome is a rare chromosomal disorder (birth prevalence 32 per 100,000 females [16 per 100,000 total population] [Martin-Giacalone 2023]) causing short stature and other phenotypic features in girls. It is caused by a structurally abnormal, or a partial or complete absence of 1 X chromosome which can in turn result in a loss of one copy of the SHOX gene (located on the X-chromosome). The absence of 1 copy of the SHOX gene results in disordered growth and skeletal anomalies (Gravholt 2019). Girls with Turner syndrome exhibit growth rate slowing after their first 3 years of life, with adult height deficits further impacted by an absent pubertal growth spurt. Individuals have final heights significantly shorter than average stature women, with a mean height deficit of 20 cm from that predicted by mid-parental height (Karlberg 1991; Rongen-Westerlaken 1997). Given the association of SHOX with FGFR3 and bone growth, it is contemplated that a subject having a homozygous or heterozygous SHOX mutation would benefit from treatment with CNP variants as described herein.

[0197] Leri-Weill dyschondrosteosis (LWD) is a rare genetic disorder characterized by shortening of the forearms and lower legs, abnormal misalignment of the wrist (Madelung deformity of the wrist), and associated short stature. LWD is caused by a heterozygous mutation in the short stature homeobox-containing (SHOX) gene or its regulatory elements located on the pseudoautosomal region 1 (PAR1) of the sex chromosomes. (See the Rare Disease Database and Carmona et al., Hum Mol Genet 20:1547-1559, 2011). The disorder Langer mesomelic dysplasia arises when there are two SHOX mutations, and may result from a mutation on each chromosome, either a homozygous or compound heterozygous mutations. A subset of SHOX mutations give rise to idiopathic short stature. Turner syndrome results due to a deletion on the X chromosome that can include the SHOX gene. SHOX has been identified as involved in the regulation of *FGFR3* transcription and contributes to control of bone growth (Marchini et al., Endocr Rev. 37: 417-448, 2016). SHOX deficiency leads to increased FGFR3 signaling, and there is some evidence to support that SHOX has direct interactions with CNP/NPR2 as well (Marchini, *supra*). Given the association of SHOX with FGFR3 and bone growth, it is contemplated that a subject having a homozygous or heterozygous SHOX mutation would benefit from treatment with CNP variants as described herein.

[0198] RASopathies are a group of rare genetic conditions caused by mutations in genes of the Ras/mitogen-activated protein kinase (MAPK) pathway. RASopathies are a group of disorders characterized by increased signaling through RAS/MAPK pathway.

This pathway leads to downstream activation of the RAF/MEK/ERK pathway. Short stature is a characteristic feature of certain RASopathies. For example, CNP signaling inhibits RAF and leads to decreased MEK and ERK activation.

[0199] Treatment of RASopathies are contemplated herein. RASopathies associated with short stature include Noonan syndrome, Costello syndrome, Cardiofaciocutaneous syndrome, Neurofibromatosis Type 1, and LEOPARD syndrome. Hereditary gingival fibromatosis type 1 is also a RASopathy contemplated herein. RASopathy patients (including Noonan syndrome, Costello syndrome, Cardiofaciocutaneous syndrome, Neurofibromatosis Type 1, LEOPARD syndrome, hereditary gingival fibromatosis type 1) include patients with heterozygous variants in one or more of the following genes: BRAF, CBL, HRAS, KRAS, LZTR1, MAP2K1, MAP2K2, MRAS, NF1, NRAS, PPP1CB, PTPN11, RAF1, RRAS, RIT1, SHOC2, SOS1, or SOS2 (Tajan et al., *Endocr. Rev.* 2018;39(5):676-700).

[0200] CFC is caused by mutations in several genes in the Ras/MAPK signaling pathway, including K-Ras, B-Raf, Mek1 and Mek2. Costello syndrome, also called faciocutaneoskeletal (FCS) syndrome is caused by activating mutations in the H-Ras gene. Hereditary gingival fibromatosis type I (HGF) is caused by dominant mutations in the SOS1 gene (*Son of Sevenless* homolog 1), which encodes a guanine nucleotide exchange factor (SOS) that acts on the Ras subfamily of small GTPases. Neurofibromatosis type I (NF1) is caused by mutations in the neurofibromin 1 gene, which encodes a negative regulator of the Ras/MAPK signaling pathway. Noonan syndrome (NS) is caused by mutations in one of several genes, including PTPN11, which encodes SHP2, and SOS1, as well as K-Ras and Raf-1.

[0201] CNP has been demonstrated to be an effective therapy in RASopathy models. Ono et al. generated mice deficient in *Nf1* in type I collagen producing cells (Ono et al., *Hum. Mol. Genet.* 2013;22(15):3048-62). These mice demonstrated constitutive ERK1/2 activation, and decreased chondrocyte proliferation, and maturation. Daily injections of CNP in these mice led to decreased ERK phosphorylation and corrected the short stature. A mouse model of Cardiofaciocutaneous syndrome using a *Braf* mutation (p.Q241R) (Inoue et al. *Hum. Mol. Genet.* 2019;28(1):74-83). exhibited decreased body length and reduced growth plate width with smaller proliferative and hypertrophic zones compared to wild type, and CNP administration led to increases in body length in these animals.

[0202] Mutations in multiple genes can cause Noonan syndrome, which is characterized by short stature, heart defects, bleeding problems, and skeletal

malformations. Mutations in the PTPN11 gene cause about half of all cases of Noonan's syndrome. SOS1 gene mutations cause an additional 10 to 15 percent, and RAF1 and RIT1 genes each account for about 5 percent of cases. Mutations in other genes each account for a small number of cases. The cause of Noonan syndrome in 15 to 20 percent of people with this disorder is unknown.

[0203] Noonan syndrome (birth prevalence 40 per 100,000 [NORD 2019]) is the most common RASopathy, a clinically defined group of disorders caused by a germline mutation in one of the genes encoding components of the RAS-MAPK pathway, typically resulting in increased signaling through this pathway. The RAS-MAPK pathway leads to downstream activation of RAF/MEK/extracellular-signal-regulated kinase (ERK). C-type natriuretic peptide (CNP) signaling intersects with this pathway by inhibiting RAF, leading to decreased MEK and ERK activation. Noonan syndrome primarily results from gain of function (GoF) mutations in genes encoding components of the RAS-MAPK pathway. Noonan syndrome is characterized by short stature in 50-70% of those affected (Bhambhani 2014), with typical facial features and cardiac defects in more than 80% of patients (Noonan 2005), and multi-system involvement in older children (Allanson 2021; Breilyn 2019). Growth failure occurs in the first year of life and the height of the child often remains below -2.00 SDs until puberty, when the growth is further affected due to an attenuated pubertal growth spurt (Carcavilla 2020). Approximately 50% of adults with Noonan syndrome have significantly reduced height (Noonan 2003).

[0204] The PTPN11, SOS1, RAF1, and RIT1 genes all encode for proteins that are important in the RAS/MAPK cell signaling pathway, which is needed for cell division and growth (proliferation), differentiation, and cell migration. Many of the mutations in the genes associated with Noonan syndrome cause the resulting protein to be turned on (active) and this prolonged activation alters normal RAS/MAPK signaling, which disrupts the regulation of cell growth and division, leading to the characteristic features of Noonan syndrome. See, e.g., Chen et al., *Proc Natl Acad Sci U S A.* 111(31):1 1473-8, 2014, Romano et al., *Pediatrics.* 126(4): 746-59, 2010, and Milosavljevic et al., *Am J Med Genet* 170(7): 1874-80, 2016. It is contemplated that a subject having mutations that activate the MAPK pathway would benefit from treatment with CNP variants as described herein to improve bone growth and short stature. It is also contemplated that a subject having mutations that activate the MAPK pathway would benefit from treatment with CNP variants as described herein to improve other comorbidities associated with an overactive MAPK pathway in other cells throughout the body where the NPR2 receptor is expressed on its surface.

[0205] Mutations in the PTPN11 gene, which encodes the non-receptor protein tyrosine phosphatase SHP-2, lead to disorders characterized by short stature such as Noonan's Syndrome (Musente et al., Eur J Hum Genet 11:201-206 (2003). Musente (*supra*) identifies numerous mutations in the PTPN11 gene that lead to short stature. Gain of function mutations lead to overactive signaling through SHP2 and inhibit Growth Hormone-induced IGF-1 release, thereby contributing to a decrease in bone growth (Rocca Serra-Nedelec, PNAS 109:4257-4262, 2012). It is contemplated that a subject having a homozygous or heterozygous PTPN11 mutation would benefit from treatment with CNP variants as described herein to improve bone growth and short stature.

[0206] Mutations in the Indian hedgehog (IHH) gene, which is related to regulation of endochondral ossification, have also been associated with short stature syndromes (Vasques et al., J Clin Endocrinol Metab. 103:604-614, 2018). Many IHH mutations identified segregate with short stature in a dominant inheritance pattern. Given the association of IHH with bone growth and ossification, it is contemplated that subjects having a homozygous or heterozygous IHH mutation will benefit from treatment with a CNP variant as described herein.

[0207] Mutations in FGFR3, including N540K and K650N, lead to short stature and hypochondroplasia.

[0208] Insulin-like growth factor 1 receptor (IGF1R) is a heterotetrameric ($\alpha 2\beta 2$) transmembrane glycoprotein with an intrinsic kinase activity. IGF1R has been shown to have a role in prenatal and postnatal growth. Heterozygous mutations in IGF1R have been identified in Small for gestational age children (SGA) and individuals with familial short stature (Kawashima et al., Endocrine J. 59:179-185, 2012). Mutations in IGF1R associated with short stature include R108Q/K115N, R59T, R709Q, G1050K, R481Q, V599E, and G1125A (Kawashima, *supra*).

[0209] Height is a highly heritable trait that can be influenced by the combined effect of hundreds or thousands of genes (Wood et al, 2014, Nature Genetics, 46:1173-1189). Short stature in an individual can be the result of the combined effect of these genes, without a single gene being the primary contributor. It is contemplated that such individuals with short stature defined by a height SDS of less than -1.0, -1.5, -2.0, -2.5, or -3.0, can be beneficially treated with a CNP variant given the ability of CNP to increase the length of normal animals, for example, enhance bone growth and length of bones.

[0210] In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of less than -1.0, -1.5, -2.0, -2.5, or -3.0, and having at least one parent with a height SDS of less than -1.0, -1.5, -2.0 or -2.5, optionally wherein the second parent has height within the normal range. In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of between -2.0 to -3.0. In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of between -2.0 to -2.5. In various embodiments, the CNP variants are useful to treat a subject with short stature having a height SDS of -2.00 or less. In various embodiments, the short stature is associated with one or more mutations in a gene associated with short stature, such as, collagen (COL2A1, COL11A1, COL9A2, COL10), aggrecan (ACAN), Indian hedgehog (IHH), PTPN11, NPR2, NPPC, FGFR3, SHOX, or insulin growth factor 1 receptor (IGF1R), or combinations thereof. In various embodiments, mutations in denticleless E3 Ubiquitin Protein Ligase Homolog (DTL), and pregnancy-associated plasma protein A2 (PAPPA2), or combinations thereof are also associated with short stature.

[0211] In various embodiments, the growth plate disorder or short stature is associated with one or more mutations in a gene associated with a RASopathy.

[0212] In various embodiments, the short stature is a result of mutations in multiple genes as determined by polygenic risk score (PRS). Polygenic risk scores (PRS) were calculated for height using the largest published GWAS meta-analysis for height that did not include any samples from the UK Biobank project as described in Example 4. The cohort was divided into five PRS quintiles (PRS 1 being the lowest height, PRS 5 the tallest height). In various embodiments, the subject has a mutation in NPR2 and a low PRS. In various embodiments, the subject has a mutation in FGFR3 and a low PRS. In various embodiments, the subject has a mutation in NPR2 and a low PRS. In various embodiments, the subject has a mutation in IGF1R and a low PRS. In various embodiments, the subject has a mutation in NPPC and a low PRS. In various embodiments, the subject has a mutation in SHOX and a low PRS. In various embodiments, the subject has one or more mutation in one or more of FGFR3, IGF1 R, NPPC, NPR2 and SHOX, and a low PRS. In various embodiments, the subject has one or more mutation in denticleless E3 Ubiquitin Protein Ligase Homolog (DTL), or pregnancy-associated plasma protein A2 (PAPPA2). In various embodiments, the PRS is 1 or 2. In various embodiments, the PRS is 1. In various embodiments, the PRS is 2.

[0213] In addition, the CNP variants are useful for treating other bone-related conditions and disorders, such as rickets, hypophosphatemic rickets [including X-linked hypophosphatemic rickets (also called vitamin D-resistant rickets) and autosomal dominant hypophosphatemic rickets], and osteomalacia [including tumor-induced osteomalacia (also called oncogenic osteomalacia or oncogenic hypophosphatemic osteomalacia)].

[0214] The CNP variants of the disclosure can also be used to treat osteoarthritis. Osteoarthritis is a degenerative disease of the articular cartilage and occurs frequently in the elderly. Osteoarthritis involves destruction of the cartilage and proliferative change in the bone and cartilage resulting from degeneration of articular components, with the change resulting in a secondary arthritis (e.g., synovitis). The extracellular matrix proteins, which are the functional entity of the cartilage, are reduced, and the number of chondrocytes decreases in osteoarthritis (Arth. Rheum. 46(8): 1986-1996 (2002)). By promoting the matrix production, growth and differentiation of chondrocytes, the CNP compositions are useful for countering the undesired effects of FGF-2 and increasing matrix synthesis in subjects suffering from arthritis, including osteoarthritis, thereby treating arthritis, including osteoarthritis.

[0215] In certain embodiments, the CNP variants and compositions and formulations comprising the same of the present disclosure are useful for improving one or more of the symptom(s) or physiological consequences of a skeletal dysplasia, wherein the improvement may be increased absolute growth, increased growth velocity, increased qualitative computed tomography (QCT) bone mineral density, improvement in growth plate morphology, increased long bone growth, improvement in spinal morphology, improved elbow joint range of motion and/or decreased sleep apnea. Additional symptoms that can be improved by CNP therapy include bone tissue mineral density (TMD), bone mineral density (BMD), bone strength, metacarpal length with more cortical area, or combinations thereof. In this regard, it is noted that the terms "improved", "improvement", "increase", "decrease" and grammatical equivalents thereof are all relative terms that when used in relation to a symptom or physiological consequence of a disease state, refer to the state of the symptom or physiological consequence of the disease after treatment with a CNP variant (or composition or formulation comprising the same) of the present invention as compared to the same symptom or physiological consequence of the disease before treatment with a CNP variant (or composition or formulation comprising the same) of the present invention (i.e., as compared to "baseline"). As described above, a "baseline" state can be determined

either through measurement of the state in the subject prior to treatment (which can subsequently be compared to the state in the same subject after treatment), or through measurement of that state in a population of subjects suffering from the same affliction that share the same or similar characteristics (e.g., age, sex and/or disease state or progression).

[0216] Also provided is a method of overcoming cell growth arrest induced by a constitutively active mutant fibroblast growth factor receptor 3 (FGFR-3) comprising contacting a cell expressing the constitutively active FGFR-3 with a CNP variant or a composition as described herein.

[0217] Further provided is a method of stimulating cGMP production in a cell expressing natriuretic peptide receptor B (NPR-B) comprising contacting the cell expressing NPR-B with a CNP variant or a composition as described herein.

[0218] In another aspect, provided herein is a method for increasing facial volume, facial sinus volume, and foramen magnum area in a subject (e.g., 6 months old or less) having a bone-related disorder, skeletal dysplasia or short stature comprising administering CNP variants as described herein. Also provided is a method of decreasing the incidence of sudden infant death, sleep disordered breathing, and necessity for neurosurgical decompression of the foramen magnum in a subject (e.g., 6 months old or less) having a bone-related disorder, skeletal dysplasia or short stature comprising administering CNP variants as described herein.

[0219] In various embodiments, the increase in facial volume, facial sinus volume, and foramen magnum area are measured by magnetic resonance imaging (MRI). In various embodiments, the change in facial volume, facial sinus volume, and foramen magnum area are compared to baseline levels, healthy control subjects or untreated control subjects.

[0220] In yet another embodiment, the disclosure provides CNP variants that *in vitro* or *in vivo* stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 μ M). In a still further embodiment, the CNP variants of the disclosure *in vitro* or *in vivo* stimulate the production of at least about 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140% or 150% of the cGMP level produced under the same concentration of wtCNP22 (e.g., 1 μ M).

[0221] It is contemplated that any of the CNP variants described herein are useful in the methods.

[0222] In various embodiments, the CNP variant is PGQEHPNARKYKGANKKGLSKGCFGLKLDRI GMSGLGC (Pro-Gly-CNP-37) (SEQ ID NO: 1).

[0223] In various embodiments, the peptide further comprises an acetyl group. In various embodiments, the acetyl group is on the N-terminus of the peptide. In various embodiments, the peptide further comprises an OH or an NH₂ group at the C-terminus. In various embodiments, the variant comprises one or more linker groups as described herein. In various embodiments, the linker is a hydrolysable linker.

[0224] Efficacy of treatment is measured by various parameters. In various embodiments, efficacy is assessed as the change in annualized growth velocity from the baseline period to the intervention period. Efficacy will also be assessed as the change in height SDS from baseline to end of treatment as measured using the CDC growth curves, and growth velocity SDS will be based on the Bone Mineral Density in Childhood Study (Kelly et al. , *J. Clin. Endocrinol. Metab.* 2014;99(6):2104-21 12).

[0225] QoLISSY, the Quality of Life in Short Stature Youth, is assessed as directed (*Quality of Life in Short Stature Youth - The QoLISSY Questionnaire User's Manual*. Lengerich: Pabst Science Publishers; 2013). The QoLISSY questionnaire is a disease-specific clinical outcome assessment designed for short stature youth, which has both child self-report and parent-report versions and consists of questions across 8 domains, physical, social, emotional, coping, treatment, belief, future, and effects on parents. Domain total scores are then linearly transformed to standard scores on a scale of 0 to 100: min score = 0 (worst QoL), max score = 100 (best QoL).

[0226] In various embodiments, subjects receiving a CNP Prodrug to treat skeletal dysplasia as described herein have reduced or none of the cardiovascular (CV) side effects such as changes in systemic blood pressures (mean arterial pressure, systolic and diastolic blood pressures, pulse pressure) and heart rate observed with administration of non prodrug CNP variant.

Pharmaceutical Compositions

[0227] The disclosure provides pharmaceutical compositions, including modified release compositions, comprising a CNP variant described herein, and one or more pharmaceutically acceptable excipients, carriers and/or diluents. In certain embodiments,

the compositions further comprise one or more other biologically active agents (*e.g.*, inhibitors of proteases, receptor tyrosine kinases, and/or the clearance receptor NPR-C).

[0228] The disclosure provides for modified release compositions comprising a conjugate moiety as described herein. Modified-release compositions include those that deliver a drug with a delay after its administration (delayed-release dosage) or for a prolonged period of time (extended-release dosage). Various embodiments of a CNP peptide conjugate provided herein include modified-release compositions, such as extended release, sustained or controlled release, and delayed release. The term "extended release composition" refers to a composition formulated in a manner in order to make the active ingredient/drug available over an extended period of time following administration (US Pharmacopeia). Extended-release dosage include sustained-release (SR) or controlled-release (CR) forms in which. Sustained release maintains drug release over a sustained period but not necessarily at a constant rate, while CR maintains drug release over a sustained period at a nearly constant rate (Pharmaceutics: Drug Delivery and Targeting, Yvonne Perrie, Thomas Rades, Pharmaceutical Press, 2009). Delayed-release compositions or products are modified to delay release of the drug substance for some period of time after initial administration.

[0229] In various embodiments, the modified release composition is an extended release composition.

[0230] In various embodiments, for the extended release composition, (i) less than about 20% of peptide is released by day 1; and (ii) about 90% of peptide is released weekly, or about 90% of peptide is released bi-weekly, or about 90% of peptide is released monthly, at pH 7 to 7.6.

[0231] In various embodiments, less than about 20% of peptide is released by day 1 at pH 7 to 7.6. In various embodiments, less than about 10% of peptide is released by day 1 at pH 7 to 7.6. It is further contemplated that (i) less than about 30%, or about 40%, or about 50% of peptide is released by day 1, at pH 7.0 to 7.6; and (ii) about 90% of peptide is released weekly, or about 90% of peptide is released bi-weekly, or about 90% of peptide is released monthly, at pH 7 to 7.6. It is further contemplated that (i) less than about 30%, or about 40%, or about 50%, or about 60% of peptide is released by day 1, at pH 7.0 to 7.6; and (ii) about 70%, about 80%, or about 90% of peptide is released weekly; or about 70%, about 80%, or about 90% of peptide is released bi-weekly; or about 70%, about 80%, or about 90% of peptide is released every three weeks; or about 70%, about 80%, or about 90% of peptide is released monthly, at pH 7

to 7.6. In various embodiments, about 90% of peptide is released weekly, at pH 7 to 7.6. In various embodiments, about 90% of peptide is released biweekly, at pH 7 to 7.6. In various embodiments, about 90% of peptide is released monthly at pH 7 to 7.6. It is further contemplated that the release can be at a pH between pH 7.0 to 7.6, between pH 7.1 to 7.5, between pH 7.2 to 7.4, between pH 7.2 to 7.6, or between pH 7.0 to 7.4.

[0232] In various embodiments, (i) less than about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, or about 75% of peptide is released by day 1, at pH 7.0 to 7.6; and (ii) about 90% of peptide is released weekly, or about 90% of peptide is released bi-weekly, or about 90% of peptide is released monthly, at pH 7 to 7.6. It is further contemplated that (i) less than about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, or about 75% of peptide is released by day 1, at pH 7.0 to 7.6; and (ii) about 70%, about 80%, or about 90% of peptide is released weekly; or about 70%, about 80%, or about 90% of peptide is released bi-weekly; or about 70%, about 80%, or about 90% of peptide is released every three weeks; or about 70%, about 80%, or about 90% of peptide is released monthly, at pH 7 to 7.6; or alternatively ii) about 70%, about 75%, about 80%, about 85%, or about 90% of peptide is released weekly; or about 70%, about 75%, about 80%, about 85%, or about 90% of peptide is released bi-weekly; or about 70%, about 75%, about 80%, about 85%, or about 90% of peptide is released every three weeks; or about 70%, about 75%, about 80%, about 85%, or about 90% of peptide is released monthly, at pH 7 to 7.6.

[0233] In various embodiments, the composition comprises an excipient, diluent or carrier. In various embodiments, the extended release composition comprises an excipient, diluent or carrier. In various embodiments, the excipient, diluent or carrier is a pharmaceutically acceptable excipient, diluent or carrier.

[0234] Non-limiting examples of excipients, carriers and diluents include vehicles, liquids, buffers, isotonicity agents, additives, stabilizers, preservatives, solubilizers, surfactants, emulsifiers, wetting agents, adjuvants, and so on. The compositions can contain liquids (e.g., water, ethanol); diluents of various buffer content (e.g., Tris-HCl, phosphate, acetate buffers, citrate buffers), pH and ionic strength; detergents and solubilizing agents (e.g., Polysorbate 20, Polysorbate 80); anti-oxidants (e.g., methionine, ascorbic acid, sodium metabisulfite); preservatives (e.g., Thimerosal, benzyl alcohol, m-cresol); and bulking substances (e.g., lactose, mannitol, sucrose). The use of excipients, diluents and carriers in the formulation of pharmaceutical

compositions is known in the art; see, e.g., Remington's Pharmaceutical Sciences, 18th Edition, pages 1435-1712, Mack Publishing Co. (Easton, Pennsylvania (1990)), which is incorporated herein by reference in its entirety.

[0235] For example, carriers include without limitation diluents, vehicles and adjuvants, as well as implant carriers, and inert, non-toxic solid or liquid fillers and encapsulating materials that do not react with the active ingredient(s). Non-limiting examples of carriers include phosphate buffered saline, physiological saline, water, and emulsions (e.g., oil/water emulsions). A carrier can be a solvent or dispersing medium containing, e.g., ethanol, a polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol, and the like), a vegetable oil, and mixtures thereof. In some embodiments, the compositions are liquid formulations. In various embodiments, the CNP variant is reconstituted from a lyophilized powder. In certain embodiments, the formulations comprise a CNP variant in a concentration range from about 0.1 mg/ml to about 20 mg/ml, or from about 0.5 mg/ml to about 20 mg/ml, or from about 1 mg/ml to about 20 mg/ml, or from about 0.1 mg/ml to about 10 mg/ml, or from about 0.5 mg/ml to about 10 mg/ml, or from about 0.5 to 5 mg/ml, or from about 0.5 to 3 mg/ml, or from about 1 mg/ml to about 10 mg/ml. In various embodiments, the CNP variant is in a concentration of 0.8 mg/ml to 2 mg/ml. In various embodiments, the CNP variant is at a concentration of 0.8 mg/ml. In various embodiments, the formulation comprises a CNP variant concentration of no less than about 10 mg/ml, no less than about 5 mg/ml, no less than about 1 mg/ml, no less than about 0.5 mg/ml, or no less than about 0.1 mg/ml. In various embodiments, the formulation comprises a CNP variant concentration of no more than about 300 mg/ml, no more than no more than about 250 mg/ml, no more than about 200 mg/ml, no more than about 150 mg/ml, no more than about 100 mg/ml, no more than about 50 mg/ml, or no more than about 40 mg/ml. In certain embodiments, the formulations comprise a CNP variant in a concentration range from about 1 mg/ml to about 300 mg/ml, or from about 5 mg/ml to about 300 mg/ml, or from about 10 mg/ml to about 300 mg/ml, or from about 1 mg/ml to about 150 mg/ml, or from about 1 mg/ml to about 75 mg/ml, or from about 1 to 75 mg/ml, or from about 5 mg/ml to about 50 mg/ml. In various embodiments, the CNP variant is in a concentration of 10 mg/ml to 30 mg/ml. In various embodiments, the CNP variant is at a concentration of 10 mg/ml. In various embodiments, the CNP variant is at a concentration of 30 mg/ml. In other embodiments, the formulations comprise a CNP variant in a concentration range from about 0.1 mg/ml to about 20 mg/ml, or from about 0.5 mg/ml to about 20 mg/ml, or from about 1 mg/ml to about 20 mg/ml, or from about 0.1 mg/ml to about 10 mg/ml, or from about 0.5 mg/ml to about 10 mg/ml, or from about 0.5 to 5 mg/ml,

or from about 0.5 to 3 mg/ml, or from about 1 mg/ml to about 10 mg/ml. In various embodiments, the CNP variant is in a concentration of 0.8 mg/ml to 2 mg/ml. In various embodiments, the CNP variant is at a concentration of 0.8 mg/ml. In various embodiments, the CNP variant is at a concentration of 2.0 mg/ml. In various embodiments, the CNP variant is at a concentration of 10 mg/ml. Exemplary concentrations of the CNP variant in the formulation is 0.1 mg/ml, 0.2 mg/ml, 0.3 mg/ml, 0.4 mg/ml, 0.5 mg/ml, 0.6 mg/ml, 0.7 mg/ml, 0.8 mg/ml, 0.9 mg/ml, 1 mg/ml, 2 mg/ml, 3 mg/ml, 4 mg/ml, 5 mg/ml, 6 mg/ml, 7 mg/ml, 8 mg/ml, 9 mg/ml, 10 mg/ml, 11 mg/ml, 12 mg/ml, 13 mg/ml, 14 mg/ml, 15 mg/ml, 16 mg/ml, 17 mg/ml, 18 mg/ml, 19 mg/ml, 20 mg/ml, 21 mg/ml, 22 mg/ml, 23 mg/ml, 24 mg/ml, 25 mg/ml, 26 mg/ml, 27 mg/ml, 28 mg/ml, 29 mg/ml, 30 mg/ml, 31 mg/ml, 32 mg/ml, 33 mg/ml, 34 mg/ml, 35 mg/ml, 36 mg/ml, 37 mg/ml, 38 mg/ml, 39 mg/ml, 40 mg/ml, 41 mg/ml, 42 mg/ml, 43 mg/ml, 44 mg/ml, 45 mg/ml, 46 mg/ml, 47 mg/ml, 48 mg/ml, 49 mg/ml, 50 mg/ml, 51 mg/ml, 52 mg/ml, 53 mg/ml, 54 mg/ml, 55 mg/ml, 56 mg/ml, 57 mg/ml, 58 mg/ml, 59 mg/ml, 60 mg/ml, 61 mg/ml, 62 mg/ml, 63 mg/ml, 64 mg/ml, 65 mg/ml, 66 mg/ml, 67 mg/ml, 68 mg/ml, 69 mg/ml, 70 mg/ml, 71 mg/ml, 72 mg/ml, 73 mg/ml, 74 mg/ml, 75 mg/ml, 76 mg/ml, 77 mg/ml, 78 mg/ml, 79 mg/ml, 80 mg/ml, 81 mg/ml, 82 mg/ml, 83 mg/ml, 84 mg/ml, 85 mg/ml, 86 mg/ml, 87 mg/ml, 88 mg/ml, 89 mg/ml, 90 mg/ml, 91 mg/ml, 92 mg/ml, 93 mg/ml, 94 mg/ml, 95 mg/ml, 96 mg/ml, 97 mg/ml, 98 mg/ml, 99 mg/ml, 100 mg/ml, 101 mg/ml, 102 mg/ml, 103 mg/ml, 104 mg/ml, 105 mg/ml, 106 mg/ml, 107 mg/ml, 108 mg/ml, 109 mg/ml, 110 mg/ml, 111 mg/ml, 112 mg/ml, 113 mg/ml, 114 mg/ml, 115 mg/ml, 116 mg/ml, 117 mg/ml, 118 mg/ml, 119 mg/ml, 120 mg/ml, 121 mg/ml, 122 mg/ml, 123 mg/ml, 124 mg/ml, 125 mg/ml, 126 mg/ml, 127 mg/ml, 128 mg/ml, 129 mg/ml, 130 mg/ml, 131 mg/ml, 132 mg/ml, 133 mg/ml, 134 mg/ml, 135 mg/ml, 136 mg/ml, 137 mg/ml, 138 mg/ml, 139 mg/ml, 140 mg/ml, 141 mg/ml, 142 mg/ml, 143 mg/ml, 144 mg/ml, 145 mg/ml, 146 mg/ml, 147 mg/ml, 148 mg/ml, 149 mg/ml, 150 mg/ml, 151 mg/ml, 152 mg/ml, 153 mg/ml, 154 mg/ml, 155 mg/ml, 156 mg/ml, 157 mg/ml, 158 mg/ml, 159 mg/ml, 160 mg/ml, 161 mg/ml, 162 mg/ml, 163 mg/ml, 164 mg/ml, 165 mg/ml, 166 mg/ml, 167 mg/ml, 168 mg/ml, 169 mg/ml, 170 mg/ml, 171 mg/ml, 172 mg/ml, 173 mg/ml, 174 mg/ml, 175 mg/ml, 176 mg/ml, 177 mg/ml, 178 mg/ml, 179 mg/ml, 180 mg/ml, 181 mg/ml, 182 mg/ml, 183 mg/ml, 184 mg/ml, 185 mg/ml, 186 mg/ml, 187 mg/ml, 188 mg/ml, 189 mg/ml, 190 mg/ml, 191 mg/ml, 192 mg/ml, 193 mg/ml, 194 mg/ml, 195 mg/ml, 196 mg/ml, 197 mg/ml, 198 mg/ml, 199 mg/ml, 200 mg/ml, 201 mg/ml, 202 mg/ml, 203 mg/ml, 204 mg/ml, 205 mg/ml, 206 mg/ml, 207 mg/ml, 208 mg/ml, 209 mg/ml, 210 mg/ml, 211 mg/ml, 212 mg/ml, 213 mg/ml, 214 mg/ml, 215 mg/ml, 216 mg/ml, 217 mg/ml, 218 mg/ml, 219 mg/ml, 220 mg/ml, 221 mg/ml, 222 mg/ml, 223 mg/ml, 224 mg/ml, 225 mg/ml, 226 mg/ml, 227 mg/ml, 228 mg/ml, 229 mg/ml, 230 mg/ml, 231 mg/ml, 232 mg/ml, 233 mg/ml, 234 mg/ml, 235 mg/ml, 236 mg/ml, 237 mg/ml, 238 mg/ml, 239 mg/ml, 240 mg/ml, 241

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[0236] In further embodiments, the compositions comprise a buffer solution or buffering agent to maintain the pH of a CNP-containing solution or suspension within a desired range. Non-limiting examples of buffer solutions include phosphate buffered saline, Tris buffered saline, and Hanks buffered saline. Buffering agents include without limitation sodium acetate, sodium phosphate, and sodium citrate. Mixtures of buffering agents can also be used. In certain embodiments, the buffering agent is histidine/L-histidine or histidine monohydrochloride monohydrate. In other embodiments, the buffering agent is acetic acid/acetate or citric acid/citrate. The amount of buffering agent suitable in a composition depends in part on the particular buffer used and the desired pH of the solution or suspension. In some embodiments, the buffering agent has a concentration of about $10 \text{ mM} \pm 5 \text{ mM}$. In certain embodiments, the pH of a composition is from about pH 3 to about pH 9, or from about pH 3 to about pH 7.5, or from about pH 3.5 to about pH 7, or from about pH 3.5 to about pH 6.5, or from about pH 4 to about pH 6, or from about pH 4 to about pH 5, or is at about pH 5.0 ± 1.0 . In various embodiments, the pH is about 5.0 to about 6.0 (e.g., 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9 or 6.0). In various embodiments, the pH is 5.5.

[0237] In other embodiments, the compositions contain an isotonicity-adjusting agent to render the solution or suspension isotonic and more compatible for administration. Non-limiting examples of isotonicity agents include NaCl, dextrose, glucose, glycerin, sorbitol, xylitol, and ethanol. In certain embodiments, the isotonicity agent is NaCl. In certain embodiments, NaCl is in a concentration of about $160 \pm 20 \text{ mM}$, or about $140 \text{ mM} \pm 20 \text{ mM}$, or about $120 \pm 20 \text{ mM}$, or about $100 \text{ mM} \pm 20 \text{ mM}$, or about $80 \text{ mM} \pm 20 \text{ mM}$, or about $60 \text{ mM} \pm 20 \text{ mM}$.

[0238] In yet other embodiments, the compositions comprise a preservative. Preservatives include, but are not limited to, m-cresol and benzyl alcohol. In certain embodiments, the preservative is in a concentration of about $0.4\% \pm 0.2\%$, or about $1\% \pm 0.5\%$, or about $1.5\% \pm 0.5\%$, or about $2.0\% \pm 0.5\%$.

[0239] In still other embodiments, the compositions contain an anti-adsorbent (e.g., to mitigate adsorption of a CNP variant to glass or plastic). Anti-adsorbents include without limitation benzyl alcohol, Polysorbate 20, and Polysorbate 80. In certain embodiments, the anti adsorbent is in a concentration from about 0.001% to about 0.5%, or from about 0.01% to about 0.5%, or from about 0.1% to about 1%, or from about 0.5% to about 1%, or from about 0.5% to about 1.5%, or from about 0.5% to about 2%, or from about 1% to about 2%.

[0240] In additional embodiments, the compositions comprise a stabilizer. Non-limiting examples of stabilizers include glycerin, glycerol, thioglycerol, methionine ((L-methionine), and ascorbic acid and salts thereof. In some embodiments, when the stabilizer is thioglycerol or ascorbic acid or a salt thereof, the stabilizer is in a concentration from about 0.1% to about 1%. In other embodiments, when the stabilizer is methionine, the stabilizer is in a concentration from about 0.01% to about 0.5%, or from about 0.01% to about 0.2%. In still other embodiments, when the stabilizer is glycerin, the stabilizer is in a concentration from about 5% to about 100% (neat).

[0241] In further embodiments, the compositions contain an antioxidant. Exemplary antioxidants include without limitation methionine and ascorbic acid. In certain embodiments, the molar ratio of antioxidant to CNP is from about 0.1:1 to about 15:1, or from about 1:1 to about 15:1, or from about 0.5:1 to about 10:1, or from about 1:1 to about 10:1 or from about 3:1 to about 10:1.

[0242] Pharmaceutically acceptable salts can be used in the compositions, including without limitation mineral acid salts (e.g., hydrochloride, hydrobromide, phosphate, sulfate), salts of organic acids (e.g., acetate, propionate, malonate, benzoate, mesylate, tosylate), and salts of amines (e.g., isopropylamine, trimethylamine, dicyclohexylamine, diethanolamine). A thorough discussion of pharmaceutically acceptable salts is found in *Remingtons Pharmaceutical Sciences*, 18th Edition, Mack Publishing Company, (Easton, Pennsylvania (1990)).

[0243] The pharmaceutical compositions can be administered in various forms, such as tablets, capsules, granules, powders, solutions, suspensions, emulsions, ointments, and transdermal patches. The dosage forms of the compositions can be tailored to the desired mode of administration of the compositions. For oral administration, the compositions can take the form of, e.g., a tablet or capsule (including softgel capsule),

or can be, e.g., an aqueous or nonaqueous solution, suspension or syrup. Tablets and capsules for oral administration can include one or more commonly used excipients, diluents and carriers, such as mannitol, lactose, glucose, sucrose, starch, corn starch, sodium saccharin, talc, cellulose, magnesium carbonate, and lubricating agents (e.g., magnesium stearate, sodium stearyl fumarate). If desired, flavoring, coloring and/or sweetening agents can be added to the solid and liquid formulations.

[0244] Other optional ingredients for oral formulations include without limitation preservatives, suspending agents, and thickening agents. Oral formulations can also have an enteric coating to protect the CNP variant from the acidic environment of the stomach. Methods of preparing solid and liquid dosage forms are known, or will be apparent, to those skilled in this art (see, e.g., Remington's Pharmaceutical Sciences, referenced above).

[0245] Formulations for parenteral administration can be prepared, e.g., as liquid solutions or suspensions, as solid forms suitable for solubilization or suspension in a liquid medium prior to injection, or as emulsions. For example, sterile injectable solutions and suspensions can be formulated according to techniques known in the art using suitable diluents, carriers, solvents (e.g., buffered aqueous solution, Ringer's solution, isotonic sodium chloride solution), dispersing agents, wetting agents, emulsifying agents, suspending agents, and the like. In addition, sterile fixed oils, fatty esters, polyols and/or other inactive ingredients can be used. As further examples, formulations for parenteral administration include aqueous sterile injectable solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which can contain suspending agents and thickening agents.

[0246] Compositions comprising a CNP variant can also be lyophilized formulations. In certain embodiments, the lyophilized formulations comprise a buffer and bulking agent, and optionally an antioxidant. Exemplary buffers include without limitation acetate buffers, citrate buffers, and histidine buffers. Exemplary bulking agents include without limitation mannitol (e.g., D-mannitol), sucrose, dextran, lactose, trehalose (e.g., trehalose dihydrate), and povidone (PVP K24). In certain embodiments, mannitol is in an amount from about 3% to about 10%, or from about 4% to about 8%, or from about 4% to about 6%. In certain embodiments, sucrose is in an amount from about 6% to about 20%, or from about 6% to about 15%, or from about 8% to about 12%. Exemplary anti-oxidants include, but are not limited to, methionine and ascorbic acid. In various embodiments, the

lyophilized formulation comprises no less than about 10 mg of the CNP variant, no less than about 5 mg of the CNP variant, no less than about 3 mg of the CNP variant, no less than about 1 mg of the CNP variant, no less than about 0.5 mg of the CNP variant, or no less than about 0.1 mg. In various embodiments, the lyophilized formulation comprises no more than about 300 mg of the CNP variant, no more than about 250 mg of the CNP variant, no more than about 200 mg of the CNP variant, no more than about 150 mg of the CNP variant, no more than about 100 mg of the CNP variant, no more than about 50 mg of the CNP variant, or no more than about 40 mg of the CNP variant. In various embodiments, the lyophilized formulation comprises about 1 mg to about 300 mg of the CNP variant, or from about 5 mg to about 300 mg of the CNP variant, or from about 10 mg to about 300 mg of the CNP variant, or from about 1 mg to about 150 mg of the CNP variant, or from about 5 mg to about 150 mg of the CNP variant, or from about 5 to 150 mg of the CNP variant, or from about 5 to 75 mg of the CNP variant, or from about 10 mg to about 50 mg of the CNP variant. The lyophilized formulation comprises 13 mg to 39 mg of the CNP variant. In various embodiments, the lyophilized formulation comprises 13 mg of the CNP variant. In various embodiments, the lyophilized formulation comprises 39 mg of the CNP variant. In other embodiments, the lyophilized formulation comprises about 0.1 mg to about 20 mg of the CNP variant, or from about 0.4 mg to about 20 mg of the CNP variant, or from about 1 mg to about 20 mg of the CNP variant, or from about 0.1 mg to about 10 mg of the CNP variant, or from about 0.1 mg to about 10 mg of the CNP variant, or from about 0.1 to 5 mg of the CNP variant. In various embodiments, the lyophilized formulation comprises 0.4 mg to 3.5 mg of the CNP variant. In various embodiments, the lyophilized formulation comprises 0.4 mg of the CNP variant. In various embodiments, the lyophilized formulation comprises 0.56 mg of the CNP variant. In various embodiments, the lyophilized formulation comprises 1.2 mg of the CNP variant. In various embodiments, the lyophilized formulation comprises 3.5 mg of the CNP variant. Exemplary concentrations of the CNP variant in the formulation is 0.1 mg, 0.11 mg, 0.12 mg, 0.13 mg, 0.14 mg, 0.15 mg, 0.16 mg, 0.17 mg, 0.18 mg, 0.19 mg, 0.2 mg, 0.21 mg, 0.22 mg, 0.23 mg, 0.24 mg, 0.25 mg, 0.26 mg, 0.27 mg, 0.28 mg, 0.29 mg, 0.3 mg, 0.31 mg, 0.32 mg, 0.33 mg, 0.34 mg, 0.35 mg, 0.36 mg, 0.37 mg, 0.38 mg, 0.39 mg, 0.4 mg, 0.41 mg, 0.42 mg, 0.43 mg, 0.44 mg, 0.45 mg, 0.46 mg, 0.47 mg, 0.48 mg, 0.49 mg, 0.5 mg, 0.51 mg, 0.52 mg, 0.53 mg, 0.54 mg, 0.55 mg, 0.56 mg, 0.57 mg, 0.58 mg, 0.59 mg, 0.6 mg, 0.61 mg, 0.62 mg, 0.63 mg, 0.64 mg, 0.65 mg, 0.66 mg, 0.67 mg, 0.68 mg, 0.69 mg, 0.7 mg, 0.71 mg, 0.72 mg, 0.73 mg, 0.74 mg, 0.75 mg, 0.76 mg, 0.77 mg, 0.78 mg, 0.79 mg, 0.8 mg, 0.81 mg, 0.82 mg, 0.83 mg, 0.84 mg, 0.85 mg, 0.86 mg, 0.87 mg, 0.88 mg, 0.89 mg, 0.9 mg, 0.91 mg, 0.92 mg, 0.93 mg,

0.94 mg, 0.95 mg, 0.96 mg, 0.97 mg, 0.98 mg, 0.99 mg, 1 mg, 1.5 mg, 2 mg, 2.5 mg, 3 mg, 3.5 mg, 4 mg, 4.5 mg, 5 mg, 5.5 mg, 6 mg, 6.5 mg, 7 mg, 7.5 mg, 8 mg, 8.5 mg, 9 mg, 9.5 mg, 10 mg, 10.5 mg, 11 mg, 11.5 mg, 12 mg, 12.5 mg, 13 mg, 13.5 mg, 14 mg, 14.5 mg, 15 mg, 15.5 mg, 16 mg, 16.5 mg, 17 mg, 17.5 mg, 18 mg, 18.5 mg, 19 mg, 19.5 mg, 20 mg, 20.5 mg, 21 mg, 21.5 mg, 22 mg, 22.5 mg, 23 mg, 23.5 mg, 24 mg, 24.5 mg, 25 mg, 25.5 mg, 26 mg, 26.5 mg, 27 mg, 27.5 mg, 28 mg, 28.5 mg, 29 mg, 29.5 mg, 30 mg, 30.5 mg, 31 mg, 31.5 mg, 32 mg, 32.5 mg, 33 mg, 33.5 mg, 34 mg, 34.5 mg, 35 mg, 35.5 mg, 36 mg, 36.5 mg, 37 mg, 37.5 mg, 38 mg, 38.5 mg, 39 mg, 39.5 mg, 40 mg, 40.5 mg, 41 mg, 41.5 mg, 42 mg, 42.5 mg, 43 mg, 43.5 mg, 44 mg, 44.5 mg, 45 mg, 45.5 mg, 46 mg, 46.5 mg, 47 mg, 47.5 mg, 48 mg, 48.5 mg, 49 mg, 49.5 mg, 50 mg, 50.5 mg, 51 mg, 51.5 mg, 52 mg, 52.5 mg, 53 mg, 53.5 mg, 54 mg, 54.5 mg, 55 mg, 55.5 mg, 56 mg, 56.5 mg, 57 mg, 57.5 mg, 58 mg, 58.5 mg, 59 mg, 59.5 mg, 60 mg, 60.5 mg, 61 mg, 61.5 mg, 62 mg, 62.5 mg, 63 mg, 63.5 mg, 64 mg, 64.5 mg, 65 mg, 65.5 mg, 66 mg, 66.5 mg, 67 mg, 67.5 mg, 68 mg, 68.5 mg, 69 mg, 69.5 mg, 70 mg, 70.5 mg, 71 mg, 71.5 mg, 72 mg, 72.5 mg, 73 mg, 73.5 mg, 74 mg, 74.5 mg, 75 mg, 75.5 mg, 76 mg, 76.5 mg, 77 mg, 77.5 mg, 78 mg, 78.5 mg, 79 mg, 79.5 mg, 80 mg, 80.5 mg, 81 mg, 81.5 mg, 82 mg, 82.5 mg, 83 mg, 83.5 mg, 84 mg, 84.5 mg, 85 mg, 85.5 mg, 86 mg, 86.5 mg, 87 mg, 87.5 mg, 88 mg, 88.5 mg, 89 mg, 89.5 mg, 90 mg, 90.5 mg, 91 mg, 91.5 mg, 92 mg, 92.5 mg, 93 mg, 93.5 mg, 94 mg, 94.5 mg, 95 mg, 95.5 mg, 96 mg, 96.5 mg, 97 mg, 97.5 mg, 98 mg, 98.5 mg, 99 mg, 99.5 mg, 100 mg, 100.5 mg, 101 mg, 101.5 mg, 102 mg, 102.5 mg, 103 mg, 103.5 mg, 104 mg, 104.5 mg, 105 mg, 105.5 mg, 106 mg, 106.5 mg, 107 mg, 107.5 mg, 108 mg, 108.5 mg, 109 mg, 109.5 mg, 110 mg, 110.5 mg, 111 mg, 111.5 mg, 112 mg, 112.5 mg, 113 mg, 113.5 mg, 114 mg, 114.5 mg, 115 mg, 115.5 mg, 116 mg, 116.5 mg, 117 mg, 117.5 mg, 118 mg, 118.5 mg, 119 mg, 119.5 mg, 120 mg, 120.5 mg, 121 mg, 121.5 mg, 122 mg, 122.5 mg, 123 mg, 123.5 mg, 124 mg, 124.5 mg, 125 mg, 125.5 mg, 126 mg, 126.5 mg, 127 mg, 127.5 mg, 128 mg, 128.5 mg, 129 mg, 129.5 mg, 130 mg, 130.5 mg, 131 mg, 131.5 mg, 132 mg, 132.5 mg, 133 mg, 133.5 mg, 134 mg, 134.5 mg, 135 mg, 135.5 mg, 136 mg, 136.5 mg, 137 mg, 137.5 mg, 138 mg, 138.5 mg, 139 mg, 139.5 mg, 140 mg, 140.5 mg, 141 mg, 141.5 mg, 142 mg, 142.5 mg, 143 mg, 143.5 mg, 144 mg, 144.5 mg, 145 mg, 145.5 mg, 146 mg, 146.5 mg, 147 mg, 147.5 mg, 148 mg, 148.5 mg, 149 mg, 149.5 mg, 150 mg, 150.5 mg, 151 mg, 151.5 mg, 152 mg, 152.5 mg, 153 mg, 153.5 mg, 154 mg, 154.5 mg, 155 mg, 155.5 mg, 156 mg, 156.5 mg, 157 mg, 157.5 mg, 158 mg, 158.5 mg, 159 mg, 159.5 mg, 160 mg, 160.5 mg, 161 mg, 161.5 mg, 162 mg, 162.5 mg, 163 mg, 163.5 mg, 164 mg, 164.5 mg, 165 mg, 165.5 mg, 166 mg, 166.5 mg, 167 mg, 167.5 mg, 168 mg, 168.5 mg, 169 mg, 169.5 mg, 170 mg, 170.5 mg, 171 mg, 171.5 mg, 172 mg, 172.5 mg, 173 mg, 173.5 mg, 174 mg, 174.5 mg, 175 mg, 175.5 mg, 176 mg,

176.5 mg, 177 mg, 177.5 mg, 178 mg, 178.5 mg, 179 mg, 179.5 mg, 180 mg, 180.5 mg, 181 mg, 181.5 mg, 182 mg, 182.5 mg, 183 mg, 183.5 mg, 184 mg, 184.5 mg, 185 mg, 185.5 mg, 186 mg, 186.5 mg, 187 mg, 187.5 mg, 188 mg, 188.5 mg, 189 mg, 189.5 mg, 190 mg, 190.5 mg, 191 mg, 191.5 mg, 192 mg, 192.5 mg, 193 mg, 193.5 mg, 194 mg, 194.5 mg, 195 mg, 195.5 mg, 196 mg, 196.5 mg, 197 mg, 197.5 mg, 198 mg, 198.5 mg, 199 mg, 199.5 mg, 200 mg, 200.5 mg, 201 mg, 201.5 mg, 202 mg, 202.5 mg, 203 mg, 203.5 mg, 204 mg, 204.5 mg, 205 mg, 205.5 mg, 206 mg, 206.5 mg, 207 mg, 207.5 mg, 208 mg, 208.5 mg, 209 mg, 209.5 mg, 210 mg, 210.5 mg, 211 mg, 211.5 mg, 212 mg, 212.5 mg, 213 mg, 213.5 mg, 214 mg, 214.5 mg, 215 mg, 215.5 mg, 216 mg, 216.5 mg, 217 mg, 217.5 mg, 218 mg, 218.5 mg, 219 mg, 219.5 mg, 220 mg, 220.5 mg, 221 mg, 221.5 mg, 222 mg, 222.5 mg, 223 mg, 223.5 mg, 224 mg, 224.5 mg, 225 mg, 225.5 mg, 226 mg, 226.5 mg, 227 mg, 227.5 mg, 228 mg, 228.5 mg, 229 mg, 229.5 mg, 230 mg, 230.5 mg, 231 mg, 231.5 mg, 232 mg, 232.5 mg, 233 mg, 233.5 mg, 234 mg, 234.5 mg, 235 mg, 235.5 mg, 236 mg, 236.5 mg, 237 mg, 237.5 mg, 238 mg, 238.5 mg, 239 mg, 239.5 mg, 240 mg, 240.5 mg, 241 mg, 241.5 mg, 242 mg, 242.5 mg, 243 mg, 243.5 mg, 244 mg, 244.5 mg, 245 mg, 245.5 mg, 246 mg, 246.5 mg, 247 mg, 247.5 mg, 248 mg, 248.5 mg, 249 mg, 249.5 mg, 250 mg, 250.5 mg, 251 mg, 251.5 mg, 252 mg, 252.5 mg, 253 mg, 253.5 mg, 254 mg, 254.5 mg, 255 mg, 255.5 mg, 256 mg, 256.5 mg, 257 mg, 257.5 mg, 258 mg, 258.5 mg, 259 mg, 259.5 mg, 260 mg, 260.5 mg, 261 mg, 261.5 mg, 262 mg, 262.5 mg, 263 mg, 263.5 mg, 264 mg, 264.5 mg, 265 mg, 265.5 mg, 266 mg, 266.5 mg, 267 mg, 267.5 mg, 268 mg, 268.5 mg, 269 mg, 269.5 mg, 270 mg, 270.5 mg, 271 mg, 271.5 mg, 272 mg, 272.5 mg, 273 mg, 273.5 mg, 274 mg, 274.5 mg, 275 mg, 275.5 mg, 276 mg, 276.5 mg, 277 mg, 277.5 mg, 278 mg, 278.5 mg, 279 mg, 279.5 mg, 280 mg, 280.5 mg, 281 mg, 281.5 mg, 282 mg, 282.5 mg, 283 mg, 283.5 mg, 284 mg, 284.5 mg, 285 mg, 285.5 mg, 286 mg, 286.5 mg, 287 mg, 287.5 mg, 288 mg, 288.5 mg, 289 mg, 289.5 mg, 290 mg, 290.5 mg, 291 mg, 291.5 mg, 292 mg, 292.5 mg, 293 mg, 293.5 mg, 294 mg, 294.5 mg, 295 mg, 295.5 mg, 296 mg, 296.5 mg, 297 mg, 297.5 mg, 298 mg, 298.5 mg, 299 mg, 299.5 mg, or 300 mg.

[0247] In various embodiments, the formulation comprises L-histidine, histidine monohydrochloride monohydrate, trehalose, mannitol, methionine, polysorbate 80, and optionally sterile water for injection (WFI). In various embodiments, the formulation comprises citric acid, sodium citrate, trehalose, mannitol, methionine, polysorbate 80, and optionally sterile water for injection (WFI).

[0248] The disclosure also provides kits containing, e.g., bottles, vials, ampoules, tubes, cartridges and/or syringes that comprise a liquid (e.g., sterile injectable)

formulation or a solid (e.g., lyophilized) formulation. The kits can also contain pharmaceutically acceptable vehicles or carriers (e.g., solvents, solutions and/or buffers) for reconstituting a solid (e.g., lyophilized) formulation into a solution or suspension for administration (e.g., by injection), including without limitation reconstituting a lyophilized formulation in a syringe for injection or for diluting concentrate to a lower concentration. Furthermore, extemporaneous injection solutions and suspensions can be prepared from, e.g., sterile powder, granules, or tablets comprising a CNP-containing composition. The kits can also include dispensing devices, such as aerosol or injection dispensing devices, pen injectors, autoinjectors, needleless injectors, syringes, and/or needles.

[0249] As a non-limiting example, a kit can include syringes having a single chamber or dual chambers. For single-chamber syringes, the single chamber can contain a liquid CNP formulation ready for injection, or a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) that can be reconstituted into a solution or suspension for injection. For dual-chamber syringes, one chamber can contain a pharmaceutically acceptable vehicle or carrier (e.g., solvent system, solution or buffer), and the other chamber can contain a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) which can be reconstituted into a solution or suspension, using the vehicle or carrier from the first chamber, for injection.

[0250] As a further example, a kit can include one or more pen injector or autoinjector devices, and dual-chamber cartridges. One chamber of a cartridge can contain a pharmaceutically acceptable vehicle or carrier (e.g., solvent system, solution or buffer), and the other chamber can contain a solid (e.g., lyophilized) CNP formulation or a liquid formulation of a CNP variant in a relatively small amount of a suitable solvent system (e.g., glycerin) which can be reconstituted into a solution or suspension, using the vehicle or carrier from the first chamber, for injection. A cartridge can comprise an amount of the CNP variant that is sufficient for dosing over a desired time period (e.g., 2 days, 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, etc.). The pen injector or autoinjector can be adjusted to administer a desired amount of the CNP formulation from a cartridge.

Administration and Dosing

[0251] The CNP variants, or pharmaceutical compositions or formulations comprising them, can be administered to subjects in various ways such as, e.g., subcutaneously, intraarticularly, intraperitoneally, intramuscularly, intradermally or orally. In one

embodiment, the CNP variant composition is administered once daily, once weekly, once every two weeks, once every three weeks, once every 4 weeks, once every 6 weeks, once every two months, once every three months or once every six months.

[0252] The CNP variants or compositions thereof can also be administered by implantation of a depot at the target site of action (e.g., an abnormal or degenerated joint or cartilage area).

[0253] Alternatively, the CNP variant can be administered sublingually under the tongue (e.g., sublingual tablet) by transdermal delivery (e.g., by means of a patch on the skin) or orally in the form of microspheres, microcapsules, liposomes (uncharged or charged (e.g., cationic)), polymeric microparticles (e.g., polyamides, polylactide, polyglycolide, poly(lactide-glycolide)), microemulsions, and the like.

[0254] The CNP variant compositions described herein can be administered to patients in need thereof at therapeutically effective doses to treat, ameliorate or prevent bone-related disorders (e.g., skeletal dysplasias, including achondroplasia). The CNP variant compositions described herein can be administered to patients in need thereof at therapeutically effective doses to treat, ameliorate or prevent bone-related disorders or short stature disorders (e.g., skeletal dysplasias, including achondroplasia, hypochondroplasia, or idiopathic short stature). The safety and therapeutic efficacy of the CNP variant can be determined by standard pharmacological procedures in cell cultures or experimental animals, such as, for example, by determining the LD₅₀ (the dose lethal to 50% of the population) and the ED₅₀ (the dose therapeutically effective in 50% of the population). The dose ratio between toxic and therapeutic effects is the therapeutic index and it can be expressed as the ratio LD₅₀/ED₅₀. Active agents exhibiting a large therapeutic index are normally preferred.

[0255] In certain embodiments, the CNP variant compositions described herein are administered at a dose in the range from about 3, 4, 5, 6, 7, 8, 9 or 10 nmol/kg to about 300 nmol/kg, or from about 20 nmol/kg to about 200 nmol/kg. In some embodiments, the CNP compositions are administered at a dose of about 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, 120, 125, 130, 140, 150, 160, 170, 175, 180, 190, 200, 210, 220, 230, 240, 250, 260, 270, 280, 290, 300, 350, 400, 450, 500, 750, 1000, 1250, 1500, 1750 or 2000 nmol/kg or other dose deemed appropriate by the treating physician. In other embodiments, the CNP variant compositions are administered at a dose of about 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, 200, 250, 300, 350, 400, 450, 500, 550, 600, 650, 700,

750, 800, 850, 900, 950 or 1000 pg/kg, or about 0.5, 0.8, 1.0, 1.25, 1.5, 2, 2.5, 3, 3.5, 4, 4.5, 5, 5.5, 6, 6.5, 7, 7.5, 8, 8.5, 9, 9.5 or 10 mg/kg, or other dose deemed appropriate by the treating physician. In various embodiments, the CNP Prodrug is administered at a dose from about 5 µg/kg to 500 µg/kg, from about 15 µg/kg to 350 µg/kg, from about 25 µg/kg to 300 µg/kg, from about 50 µg/kg to 250 µg/kg or from about 75 µg/kg to 200 µg/kg. In various embodiments, the CNP variant is administered at a dose of about 15 µg/kg, 20 µg/kg, 25 µg/kg, 30 µg/kg, 35 µg/kg, 40 µg/kg, 45 µg/kg, 50 µg/kg, 60 µg/kg, 70 µg/kg, 75 µg/kg, 80 µg/kg, 90 µg/kg, 100 µg/kg, 125 µg/kg, 150 µg/kg, 175 µg/kg, 200 µg/kg, 225 µg/kg, 250 µg/kg, 275 µg/kg, 300 µg/kg, 325 µg/kg, 350 µg/kg, 400 µg/kg, 450 µg/kg, or 500 µg/kg. The doses of CNP or CNP variant described herein can be administered according to the dosing frequency/frequency of administration described herein, including without limitation daily, 2 or 3 times per week, weekly, every 2 weeks, every 3 weeks, monthly, etc. In various embodiments, the CNP or CNP variant is administered daily subcutaneously. In various embodiments, the CNP or CNP variant is administered weekly subcutaneously. In various embodiments, the CNP variant is administered at a dose of 2.5 pg/kg/day to 60 pg/kg/day, 10pg/kg/day to 45 pg/kg/day, or 15pg/kg/day to 30 pg/kg/day. In various embodiments, the CNP variant is administered at a dose of 15 pg/kg/day. In various embodiments, the CNP variant is administered at a dose of 30 pg/kg/day.

[0256] In various embodiments, the pharmaceutical composition is a sustained release composition. In various embodiments, the pharmaceutical composition comprising the CNP prodrug has a longer half-life, improved C_{max} and improved AUC compared to a non-sustained release composition comprising the same active CNP compound, i.e., free drug.

[0257] The frequency of dosing/administration of a CNP variant for a particular subject may vary depending upon various factors, including the disorder being treated and the condition and response of the subject to the therapy. The CNP variant can be administered in a single dose or in multiple doses per dosing. In certain embodiments, the CNP variant composition is administered, in a single dose or in multiple doses, once daily, once weekly, once every two weeks, once every three weeks, once every 4 weeks, once every 6 weeks, once every two months, once every three months or once every six months, or as deemed appropriate by the treating physician. In various embodiments, the CNP variant is administered for 3 months, 6 months, 12 months or more.

[0258] In some embodiments, a CNP variant composition is administered so as to allow for periods of growth (e.g., chondrogenesis), followed by a recovery period (e.g., osteogenesis). For example, the CNP composition may be administered subcutaneously

or by another mode daily or multiple times per week for a period of time, followed by a period of no treatment, then the cycle is repeated. In some embodiments, the initial period of treatment (e.g., administration of the CNP variant composition daily or multiple times per week) is for 3 days, 1 week, 2 weeks, 3 weeks, 4 weeks, 5 weeks, 6 weeks, 7 weeks, 8 weeks, 9 weeks, 10 weeks, 11 weeks or 12 weeks. In a related embodiment, the period of no treatment lasts for 3 days, 1 week, 2 weeks, 3 weeks or 4 weeks. In certain embodiments, the dosing regimen of the CNP variant compositions is daily for 3 days followed by 3 days off; or daily or multiple times per week for 1 week followed by 3 days or 1 week off; or daily or multiple times per week for 2 weeks followed by 1 or 2 weeks off; or daily or multiple times per week for 3 weeks followed by 1, 2 or 3 weeks off; or daily or multiple times per week for 4, 5, 6, 7, 8, 9, 10, 11 or 12 weeks followed by 1, 2, 3 or 4 weeks off.

Biomarkers

[0259] For treatment of bone-related disorders, indicators of growth can be measured, such as long bone growth measurements *in utero* and neonatal and measurements of bone growth biomarkers such as CNP, cGMP, Collagen II, Collagen X, osteocalcin, and Proliferating Cell Nuclear Antigen (PCNA).

[0260] One CNP signaling marker is cGMP (guanosine 3', 5' cyclic monophosphate). The level of this intracellular signaling molecule increases after CNP binds to and activates its cognate receptor NPR-B. Elevated levels of cGMP can be measured from cell culture extracts (*in vitro*) after CNP exposure, conditioned media from bone ex-plant studies (*ex vivo*) after CNP exposure, and in the plasma (*in vivo*) within minutes of CNP administration subcutaneously, intravenously, or via other routes of administration known in the art.

[0261] Cartilage and bone-specific analytes (or cartilage- and bone-associated markers) can also be measured to assess CNP efficacy. For example, fragments of cleaved collagen type II are a cartilage-specific marker for cartilage turnover. Type II collagen is the major organic constituent of cartilage and fragments of type II collagen (cleaved collagen) are released into circulation, and subsequently secreted into the urine, following cartilage turnover. Cartilage turnover precedes new bone formation.

[0262] A bone-specific biomarker for bone formation which can be measured is N-terminal propeptides of type I procollagen (PINP). The synthesis of type I collagen is an important step in bone formation, as type I collagen is the major organic component in

bone matrix. During collagen synthesis, propeptides are released from the procollagen molecule and can be detected in serum. In addition, fragments of collagen type I can be measured as a marker for bone resorption.

[0263] Other potential biomarkers for cartilage and bone formation and growth include aggrecan chondroitin sulfate (cartilage-specific marker for cartilage turnover), propeptides of type II collagen (cartilage-specific marker for cartilage formation), collagen type I C-telopeptide (CTX), alkaline phosphatase (bone-specific) and osteocalcin (bone-specific marker for bone formation). Biomarkers also include proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, collagen X, CXM (noncollagenous 1 (NC1) domain of type X collagen), NTproCNP, and alkaline phosphatase, N-terminal collagen type I pro-peptide, bone-specific alkaline phosphatase, amino-terminal propeptide of type I collagen/procollagen type I N-propeptide (PINP), cross-linked C-telopeptide of type I collagen (CTX), cross-linked N-telopeptide of type I collagen (NTx) tartrate-resistant acid phosphatase 5b (TRAP-5b), transcriptomics readouts, e.g., from PAXgene® RNA, and CNP-variant bioactivity. Cartilage- and bone-associated biomarkers can be measured, e.g., in serum from efficacy/pharmacodynamic *in vivo* studies and from the conditioned media of *ex vivo* studies, using commercially available kits.

[0264] In one embodiment, the level of at least one bone- or cartilage-associated biomarker is assayed or measured in a subject that has been administered a CNP variant or composition described herein in order to monitor the effects of the CNP composition on bone and cartilage formation and growth *in vivo*. For example, an increase in the level of at least one bone- or cartilage-associated biomarker may indicate that administration of a CNP variant or composition has a positive effect on bone growth and is a useful treatment for skeletal dysplasias and other bone- or cartilage-related diseases or disorders associated with decreased CNP activity.

[0265] Exemplary bone- or cartilage-associated biomarkers include, but are not limited to, CNP (e.g., endogenous levels of CNP), cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, collagen type I C-telopeptide (CTX), osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, collagen X, aggrecan chondroitin sulfate, and alkaline phosphatase

[0266] In various embodiments, the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP, cGMP, propeptides of collagen type II and

fragments thereof, collagen type II and fragments thereof, Collagen Type I C-Telopeptide (CTx), osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, collagen X, CXM (noncollagenous 1 (NC1) domain of type X collagen), NTproCNP, and alkaline phosphatase, N-terminal collagen type I pro-peptide, bone-specific alkaline phosphatase, amino-terminal propeptide of type I collagen/procollagen type I N-propeptide (PINP), cross-linked C-telopeptide of type I collagen (CTx), cross-linked N-telopeptide of type I collagen (NTx) tartrate-resistant acid phosphatase 5b (TRAP-5b), transcriptomics readouts, e.g., from PAXgene® RNA, and CNP-variant bioactivity. NTproCNP is an amino-terminal propeptide (NTproCNP) of CNP that is released from cells at an equimolar ratio with CNP. The biologically active forms of CNP are found in plasma in low concentrations due to the quick clearance rate of the peptide. NTproCNP is not cleared via the same mechanism and it is found in the circulation at 20- to 50-fold higher concentration (Olney et al., Clin Endocrinol (Oxf). 2012, 77:416–422).

[0267] Collagen type X biomarker (CXM) is a degradation fragment of collagen type X, comprising intact trimeric noncollagenous 1 (NC1) domain of type X collagen. CXM is released by active growth plates and decreases in samples as subjects age. CXM levels have been correlated with growth velocity in children (Coghlan et al., Sci Transl Med 2017, 9(419):eaan4669).

[0268] Bone-specific alkaline phosphatase (BSAP or BAP) is a bone growth biomarker produced by osteoblasts and osteoclasts in growth plates and mineralized bone. Changes in BSAP may reflect growth plate activity, bone growth, and / or bone remodeling activity.

[0269] A bone-specific biomarker for bone formation which can be measured is N-terminal propeptides of type I procollagen (PINP). The synthesis of type I collagen is an important step in bone formation, as type I collagen is the major organic component in bone matrix. During collagen synthesis, propeptides are released from the procollagen molecule and can be detected in serum. In addition, fragments of collagen type I can be measured as a marker for bone resorption.

[0270] Other potential biomarkers for cartilage and bone formation and growth include aggrecan chondroitin sulfate (cartilage-specific marker for cartilage turnover), propeptides of type II collagen (cartilage-specific marker for cartilage formation), collagen type I C-telopeptide (CTx), alkaline phosphatase (bone-specific) and osteocalcin (bone-specific marker for bone formation). Cartilage- and bone-associated biomarkers can be measured, e.g., in serum from efficacy/pharmacodynamic *in vivo* studies and from the conditioned media of *ex vivo* studies, using commercially available kits.

[0271] In various embodiments, biomarkers are measured by obtaining a biological sample from a subject who will be administered, is being administered or has been administered a CNP variant. Biomarkers can be measured using techniques known in the art, including, but not limited to, Western Blot, enzyme linked immunosorbant assay (ELISA), and enzymatic activity assay. The biological sample can be blood, serum, urine, or other biological fluids.

Formulations

[0272] In additional embodiments, the disclosure contemplates use of pharmaceutical compositions and formulations comprising a CNP variant peptide, and one or more pharmaceutically acceptable excipients, carriers and/or diluents. In certain embodiments, the compositions further comprise one or more other biologically active agents (*e.g.*, inhibitors of proteases, receptor tyrosine kinases, and/or the clearance receptor NPR-C).

[0273] Some of the possible formulations are disclosed in the examples section. In any formulation, the concentration of the CNP Variant in units of mg/mL is typically 0.5 or greater, 1 or greater, 5 or greater, 10 or greater, 15 or greater, 20 or greater, 25 or greater, or even 30 or greater. In any formulation, the concentration of the CNP Variant in units of mg/mL is typically 40 or less, 35 or less, 30 or less, 25 or less, 20 or less, 15 or less, 10 or less, or even 5 or less.

[0274] Non-limiting examples of excipients, carriers and diluents include vehicles, liquids, buffers, isotonicity agents, additives, stabilizers, preservatives, solubilizers, surfactants, emulsifiers, wetting agents, adjuvants, and so on. The compositions can contain liquids (*e.g.*, water, ethanol); diluents of various buffer content (*e.g.*, Tris-HCl, phosphate, acetate buffers, citrate buffers), pH and ionic strength; detergents and solubilizing agents (*e.g.*, Polysorbate 20, Polysorbate 80 (sometimes called PS 80)); anti-oxidants (*e.g.*, methionine, ascorbic acid, sodium metabisulfite); preservatives (*e.g.*, Thimerosal, benzyl alcohol, m-cresol); and bulking substances (*e.g.*, lactose, mannitol, sucrose). The use of excipients, diluents and carriers in the formulation of pharmaceutical compositions is known in the art; see, *e.g.*, Remington's Pharmaceutical Sciences, 18th Edition, pages 1435-1712, Mack Publishing Co. (Easton, Pennsylvania (1990)), which is incorporated herein by reference in its entirety.

[0275] For example, carriers include without limitation diluents, vehicles and adjuvants, as well as implant carriers, and inert, non-toxic solid or liquid fillers and encapsulating materials that do not react with the active ingredient(s). Non-limiting examples of carriers

include phosphate buffered saline, physiological saline, water, and emulsions (e.g., oil/water emulsions). A carrier can be a solvent or dispersing medium containing, e.g., ethanol, a polyol (e.g., glycerol, propylene glycol, liquid polyethylene glycol, and the like), a vegetable oil, and mixtures thereof.

[0276] In some embodiments, the compositions are liquid formulations. In certain embodiments, the formulations comprise a CNP variant peptide in a concentration range from about 0.1 mg/ml to about 20 mg/ml, or from about 0.5 mg/ml to about 20 mg/ml, or from about 1 mg/ml to about 20 mg/ml, or from about 0.1 mg/ml to about 10 mg/ml, or from about 0.5 mg/ml to about 10 mg/ml, or from about 1 mg/ml to about 10 mg/ml, or from about 2 mg/ml to about 10 mg/ml, or about 2 mg/ml or about 10 mg/ml. In other embodiments, the formulation may be a lyophilized formulation or may be a liquid formulation that was previously reconstituted from a lyophilized formulation.

[0277] In further embodiments, the compositions comprise a buffer solution or buffering agent to maintain the pH of a CNP-containing solution or suspension within a desired range. Non-limiting examples of buffer solutions include phosphate buffered saline, Tris buffered saline, and Hank's buffered saline. Buffering agents include without limitation sodium acetate, sodium phosphate, citric acid monohydrate and sodium citrate dihydrate. Mixtures of buffering agents can also be used. In certain embodiments, the buffering agent is acetic acid/acetate or citric acid/citrate. The amount of buffering agent suitable in a composition depends in part on the particular buffer used and the desired pH of the solution or suspension. For example, acetate is a more efficient pH buffer at pH 5 than pH 6, so less acetate may be used in a solution at pH 5 than at pH 6. In some embodiments, the buffering agent has a concentration of about 5-15 mM (e.g., 10 mM + 5 mM). In certain embodiments, the pH of a composition is from about pH 3 to about pH 7.5, or from about pH 3.5 to about pH 7, or from about pH 3.5 to about pH 6.5, or from about pH 4 to about pH 6, or from about pH 4 to about pH 5, or is at about pH 5.0 + 1.0, or is at about pH 5.5 + 1.0.

[0278] In other embodiments, the compositions contain an isotonicity agent to render the solution or suspension isotonic and more compatible for injection. Non-limiting examples of isotonicity agents include NaCl, trehalose, mannitol, dextrose, glucose, glycerin, sorbitol, xylitol, and ethanol. In certain embodiments, the isotonicity agent is trehalose or mannitol, which can be employed individually or in combination. In certain embodiments, trehalose or mannitol is in a concentration of about 160 + 20 mM, or about 140 mM + 20 mM, or about 120 + 20 mM, or about 100 mM + 20 mM, or about 80 mM + 20 mM, or about 60

mM + 20 mM. The ratio of trehalose to mannitol can be about 4:1, such as about 3:1 to about 5:1.

[0279] In various embodiments, the compositions may comprise a preservative. Preservatives include, but are not limited to, m-cresol and benzyl alcohol. In certain embodiments, the preservative is in a concentration of about 0.4% + 0.2%, or about 1% + 0.5%, or about 1.5% + 0.5%, or about 2.0% + 0.5%. In certain embodiments of the invention, the composition or formulation does not contain a preservative.

[0280] In various embodiments, the compositions contain an anti-adsorbent agent (e.g., to mitigate adsorption of a CNP variant to glass or plastic). Anti-adsorbent agents include without limitation benzyl alcohol, polysorbate 20, and polysorbate 80. In certain embodiments, the anti-adsorbent is in a concentration from about 0.001% to about 0.5%, or from about 0.01% to about 0.5%, or from about 0.1% to about 1%, or from about 0.5% to about 1%, or from about 0.5% to about 1.5%, or from about 0.5% to about 2%, or from about 1% to about 2%.

[0281] In various embodiments, the compositions comprise a stabilizer. Non-limiting examples of stabilizers include glycerin, glycerol, thioglycerol, methionine, and ascorbic acid and salts thereof. In some embodiments, when the stabilizer is thioglycerol or ascorbic acid or a salt thereof, the stabilizer is in a concentration from about 0.1% to about 1%.

[0282] In various embodiments, the compositions contain an antioxidant. An exemplary anti-oxidant is without limitation ascorbic acid. In certain embodiments, the molar ratio of antioxidant to CNP variant peptide is from about 0.1:1 to about 15:1, or from about 1:1 to about 15:1, or from about 0.5:1 to about 10:1, or from about 1:1 to about 10:1 or from about 3:1 to about 10:1.

[0283] Pharmaceutically acceptable salts can be used in the compositions, including without limitation mineral acid salts (e.g., hydrochloride, hydrobromide, phosphate, sulfate), salts of organic acids (e.g., acetate, propionate, malonate, benzoate, mesylate, tosylate), and salts of amines (e.g., isopropylamine, trimethylamine, dicyclohexylamine, diethanolamine). A thorough discussion of pharmaceutically acceptable salts is found in *Remington's Pharmaceutical Sciences*, 18th Edition, Mack Publishing Company, (Easton, Pennsylvania (1990)).

[0284] Formulations for parenteral administration can be prepared, e.g., as liquid solutions or suspensions, as solid forms suitable for solubilization or suspension in a liquid

medium prior to injection, or as emulsions. For example, sterile injectable solutions and suspensions can be formulated according to techniques known in the art using suitable diluents, carriers, solvents (e.g., buffered aqueous solution, Ringer's solution, isotonic sodium chloride solution), dispersing agents, wetting agents, emulsifying agents, suspending agents, and the like. In addition, sterile fixed oils, fatty esters, polyols and/or other inactive ingredients can be used. As further examples, formulations for parenteral administration include aqueous sterile injectable solutions, which can contain antioxidants, buffers, bacteriostats, and solutes that render the formulation isotonic with the blood of the intended recipient; and aqueous and nonaqueous sterile suspensions, which can contain suspending agents and thickening agents.

[0285] Exemplary CNP peptide-containing formulations are described in U.S. Patents 8,198,242 and 8,598,121. Use of CNP formulations having a pH in the range from about 4 to about 6 is contemplated.

[0286] In various embodiments, CNP variant peptides can be formulated in pharmaceutical carriers for administration to subjects affected by skeletal dysplasia. In some embodiments, liquid formulations of CNP variant peptides are formulated according to any combinations of the ingredients and their amounts or concentrations are described below:

[0287] Compositions comprising a CNP variant peptide can also be lyophilized formulations. In certain embodiments, the lyophilized formulations comprise a buffer and bulking agent, and optionally an antioxidant. Exemplary buffers include without limitation acetate buffers and citrate buffers. Exemplary bulking agents include without limitation mannitol, sucrose, dextran, lactose, trehalose, and povidone (PVP K24). In certain embodiments, mannitol and/or trehalose is in an amount from about 3% to about 10%, or from about 4% to about 8%, or from about 4% to about 6%. In certain embodiments, sucrose is in an amount from about 6% to about 20%, or from about 6% to about 15%, or from about 8% to about 12%.

[0288] In various embodiments, lyophilized formulations of CNP variant peptides are prepared from formulations formulated according to any combinations of the ingredients and their amounts or concentrations described below:

[0289] In various embodiments, a formulation comprising a CNP variant peptide has a pH of about 3-7, or about 3-6, or about 3.5-6.5, or about 4-6, or about 4-5, or about 4.5-5.5. In some embodiments, for pH 4-5.5 a suitable buffering agent is acetic acid/acetate (e.g.,

sodium acetate), and for pH 5.5-6 a suitable buffering agent is citric acid/citrate. Citric acid/citrate (e.g., sodium citrate) is also a suitable buffering agent in the range of pH 3-6 or pH 4-6. In certain embodiments, the buffering agent has a concentration in the formulation of about 2-50nM, or about 2-40 nM, or about 2-30 nM, or about 5-30 nM, or about 2-20 nM, or about 5-20 nM, or about 5-15 nM.

[0290] Also to minimize or avoid deamidation of a CNP variant peptide, water can be removed from the formulation by lyophilization. In some embodiments, lyophilized formulations contain any combinations of the following components: buffer: sodium acetate and acetic acid, or sodium citrate and citric acid; isotonicity/bulking agent: mannitol (e.g., 3- 10%, 2-8% or 4-6%); sucrose (e.g., 6-20%, 5-15% or 8-12%); antioxidants: methionine and/or ascorbic acid with molal ratio of each antioxidant to CNP variant peptide from about 0.1:1 to about 1:1, or from about 0.5:1 to about 5:1, or from about 1:1 to about 15:1, or from about 1:1 to about 10:1, or from about 3:1 to about 10:1.

[0291] Deamidation can also be minimized or avoided by storing a CNP composition (e.g., a liquid formulation or a lyophilized formulation) at lower temperature, such as at about 5 °C, 0 °C, -10 °C, -20 °C, -30 °C, -40 °C, -50 °C, -60 °C, -70 °C, -80 °C, -90 °C, or -100 °C.

[0292] To minimize or avoid oxidation of oxidizable residues (e.g., methionine) in a CNP variant peptide, the variant can be formulated with one or more antioxidants. Exemplary antioxidants include, but are not limited to, methionine, ascorbic acid, and thioglycerol. Oxidation of, e.g., methionine residues can also be minimized or prevented by purging oxygen from a liquid medium (if a liquid formulation) with nitrogen or argon, and/or by purging oxygen from a container or packaging with nitrogen or argon.

[0293] In some embodiments, to minimize or prevent adsorption (e.g., adsorption of a CNP variant peptide to plastic or glass), polysorbate 20, polysorbate 80 or benzyl alcohol, or a combination thereof, is added to a CNP formulation. In certain embodiments, each of the anti-adsorbent(s) is in a concentration from about 0.001% to about 0.5%, or from about 0.01% to about 0.5%, or from about 0.1% to about 1%, or from about 0.5% to about 1%, or from about 0.5% to about 1.5%, or from about 0.5% to about 2%, or from about 1% to about 2%. Exemplary range(s) of anti-adsorbent(s) in the formulation include without limitation from about 0.001% to about 0.5% of Polysorbate 20, from about 0.001% to about 0.5% of Polysorbate 80, and/or from about 0.5% to about 1.5% of benzyl alcohol.

[02994] The disclosure also provides kits containing, e.g., bottles, vials, ampoules, tubes, cartridges and/or syringes that comprise a liquid (e.g., sterile injectable) formulation or a solid (e.g., lyophilized) formulation. The kits can also contain pharmaceutically acceptable vehicles or carriers (e.g., solvents, solutions and/or buffers) for reconstituting a solid (e.g., lyophilized) formulation into a solution or suspension for administration (e.g., by injection), including without limitation reconstituting a lyophilized formulation in a syringe for injection or for diluting concentrate to a lower concentration. Furthermore, extemporaneous injection solutions and suspensions can be prepared from, e.g., sterile powder, granules, or tablets comprising a CNP-containing composition. The kits can also include dispensing devices, such as aerosol or injection dispensing devices, pen injectors, autoinjectors, needleless injectors, syringes, and/or needles.

[0295] Additional aspects and details of the disclosure will be apparent from the following examples, which are intended to be illustrative rather than limiting.

EMBODIMENTS

1. A pharmaceutical composition comprising a variant of C-type natriuretic peptide (CNP) PGQEHPNARKYKGANKKGLSKGCFGLKLDRIGSMSGLGC (SEQ ID NO: 1), a pharmaceutically acceptable excipient, and a carrier or a diluent, wherein the CNP variant comprises an acid moiety, a spacer, a hydrolysable linker, and is characterized by a structure as shown in Figure 11.

2. A pharmaceutical composition comprising

(4R,10S,16S,19S,22S,28S,31S,34S,37S,40S,43S,49S,52R)-52-(2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-1-(L-prolylglycyl-L-glutaminyll-L-glutamyll-L-histidyl)pyrrolidine-2-carboxamido)-4-amino-4-oxobutanamido)propanamido)-5-guanidinopentanamido)-6-aminohexanamido)-3-(4-hydroxyphenyl)propanamido)-6-aminohexanamido)acetamido)propanamido)-4-amino-4-oxobutanamido)-6-aminohexanamido)-6-aminohexanamido)acetamido)-4-methylpentanamido)-3-hydroxypropanamido)-6-aminohexanamido)acetamido)-49-benzyl-28-((S)-sec-butyl)-34-(carboxymethyl)-40-((S)-33,51-dicarboxy-8-(2-hydroxyethyl)-6,12,21,30,35-pentaoxo-14,17,23,26-tetraoxa-5,8,11,20,29,34-hexaazahenpentacontyl)-31-(3-guanidinopropyl)-16,22-bis(hydroxymethyl)-10,37,43-triisobutyl-19-(2-(methylthio)ethyl)-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51-hexadeca-1,2-dithia-5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50-hexadecaazacyclotripentacontane-4-carboxylic acid, a pharmaceutically acceptable excipient, and a carrier or a diluent.

3. The pharmaceutical composition of embodiment 1 or 2, wherein the pharmaceutical composition is lyophilized.
4. The pharmaceutical composition of any one of embodiments 1-3, wherein the carrier or diluent comprises a buffer.
5. The pharmaceutical composition of embodiment 4, wherein the buffer comprises a buffer selected from citrate, acetate, phosphate, TRIS, and a combination thereof.
6. The pharmaceutical composition of embodiment 5, wherein the buffer further comprises histidine, a salt thereof, a solvate thereof, or a solvate of a salt thereof.
7. The pharmaceutical composition of any one of embodiments 4-6, wherein the buffer is present in a concentration of 5-15 mM.
8. The pharmaceutical composition of any one of embodiments 4-7, having a pH of 3-9.
9. The pharmaceutical composition of embodiment 8, having a pH of 4-6.
10. The pharmaceutical composition of embodiment 9, having a pH of 5-6.
11. The pharmaceutical composition of embodiment 10, having a pH of 5.2 or 5.5.
12. The pharmaceutical composition of any one of embodiments 1-11, wherein the pharmaceutically acceptable excipient is selected from a bulking agent, a tonicity agent, an antioxidant, a surfactant, a solubilizing agent, a stabilizing agent, and a combination thereof.
13. The pharmaceutical composition of embodiment 12, wherein the bulking agent is selected from mannitol, sucrose, dextran, lactose, trehalose, and povidone (PVP K24), and a combination thereof.
14. The pharmaceutical composition of embodiment 12 or 13, wherein the bulking agent comprises trehalose or a solvate thereof, mannitol, or a combination thereof.
15. The pharmaceutical composition of embodiment 14, wherein the bulking agent comprises trehalose and mannitol in a weight ratio of 3:1 to 1:1.
16. The pharmaceutical composition of embodiment 15, wherein the weight ratio of trehalose to mannitol is 3.9:1.
17. The pharmaceutical composition of any one of embodiments 13-16, wherein trehalose is present in an amount of 3-6 wt% of the composition.

18. The pharmaceutical composition of embodiment 17, wherein trehalose is present in an amount of 3.5-5.8 wt% of the composition.

19. The pharmaceutical composition of embodiment 18, wherein trehalose is present in an amount of 3.8-4.8 wt% of the composition.

20. The pharmaceutical composition of any one of embodiments 12-19, comprising a tonicity agent selected from sodium chloride, dextrose, glucose, glycerin, sorbitol, xylitol, ethanol, and a combination thereof.

21. The pharmaceutical composition of any one of embodiments 12-20, comprising an antioxidant selected from methionine, ascorbic acid, salt forms of ascorbic acid, thioglycerol, and combinations thereof.

22. The pharmaceutical composition of embodiment 21, wherein the antioxidant is methionine.

23. The pharmaceutical composition of any one of embodiments 1-22, comprising a stabilizing agent or surfactant selected from glycine, sorbitol, polysorbate, and a combination thereof.

24. The pharmaceutical composition of embodiment 23, comprising polysorbate.

25. The pharmaceutical composition of any one of embodiments 1-24, wherein the pharmaceutical composition comprises L-histidine, histidine monohydrochloride monohydrate, trehalose dihydrate, D-mannitol, L-methionine, and polysorbate.

26. The pharmaceutical composition of any one of embodiments 1-24, wherein the pharmaceutical composition comprises citrate buffer, trehalose dihydrate, D-mannitol, L-methionine, and polysorbate.

27. A pharmaceutical composition comprising
 (4R,10S,16S,19S,22S,28S,31S,34S,37S,40S,43S,49S,52R)-52-(2-((S)-2-((S)-2-((S)-2-
 ((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-1-(L-prolylglycyl-L-glutaminyll-L-glutamyl-L-histidyl)pyrrolidine-2-carboxamido)-4-amino-4-oxobutanamido)propanamido)-5-guanidinopentanamido)-6-aminoxohexanamido)-3-(4-hydroxyphenyl)propanamido)-6-aminoxohexanamido)acetamido)propanamido)-4-amino-4-oxobutanamido)-6-aminoxohexanamido)-6-aminoxohexanamido)acetamido)-4-methylpentanamido)-3-hydroxypropanamido)-6-aminoxohexanamido)acetamido)-49-benzyl-28-((S)-sec-butyl)-34-(carboxymethyl)-40-((S)-33,51-dicarboxy-8-(2-hydroxyethyl)-6,12,21,30,35-pentaaxo-14,17,23,26-tetraoxa-5,8,11,20,29,34-hexazahenpentacontyl)-31-(3-guanidinopropyl)-16,22-bis(hydroxymethyl)-10,37,43-trisobutyl-19-(2-(methylthio)ethyl)-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51-hexadecaaxo-1,2-dithia-

5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50-hexadecaazacyclotripentacontane-4-carboxylic acid, a buffer comprising histidine or a salt thereof, and one or more pharmaceutically acceptable excipients.

28. The pharmaceutical composition of embodiment 27, wherein the buffer comprises L-histidine hydrochloride monohydrate.

29. The pharmaceutical composition of embodiment 27 or 28, comprising a pharmaceutically acceptable excipient selected from a bulking agent, a stabilizer, an anti-adsorbent, a diluent, and a combination thereof.

30. The pharmaceutical composition of any one of embodiments 27-29, wherein the pharmaceutically acceptable excipient comprises a bulking agent comprising trehalose or a solvate thereof.

31. The pharmaceutical composition of embodiment 30, wherein the bulking agent comprises trehalose dihydrate.

32. The pharmaceutical composition of any one of embodiments 27-31, wherein pharmaceutically acceptable excipient comprises a bulking agent comprising mannitol.

33. The pharmaceutical composition of embodiment 32, wherein the mannitol is D-mannitol.

34. The pharmaceutical composition of any one of embodiments 27-33, wherein the pharmaceutically acceptable excipient comprises a stabilizer comprising methionine.

35. The pharmaceutical composition of embodiment 34, wherein the methionine is L-methionine.

36. The pharmaceutical composition of any one of embodiments 27-35, wherein the pharmaceutically acceptable excipient comprises an anti-adsorbent comprising polysorbate.

37. The pharmaceutical composition of embodiment 36, wherein the polysorbate is polysorbate 80.

38. The pharmaceutical composition of any one of embodiments 27-37, wherein the pharmaceutical composition is substantially free of a citrate buffer.

39. A pharmaceutical composition comprising a C-type natriuretic peptide (CNP) variant that is (4R,10S,16S,19S,22S,28S,31S,34S,37S,40S,43S,49S,52R)-52-(2-((S)-2-((S)-2-((S)-2-(2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-1-(L-prolylglycyl- L-glutaminy- L-glutamyl- L-histidyl)pyrrolidine-2-carboxamido)-4-amino-4-oxobutanamido)propanamido)-5-guanidinopentanamido)-6-aminohexanamido)-3-(4-

hydroxyphenyl)propanamido)-6-aminohexanamido)acetamido)propanamido)-4-amino-4-oxobutanamido)-6-aminohexanamido)-6-aminohexanamido)acetamido)-4-methylpentanamido)-3-hydroxypropanamido)-6-aminohexanamido)acetamido)-49-benzyl-28-((S)-sec-butyl)-34-(carboxymethyl)-40-((S)-33,51-dicarboxy-8-(2-hydroxyethyl)-6,12,21,30,35-penta-14,17,23,26-tetra-5,8,11,20,29,34-hexaazapentacetyl)-31-(3-guanidinopropyl)-16,22-bis(hydroxymethyl)-10,37,43-trisobutyl-19-(2-(methylthio)ethyl)-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51-hexadeca-1,2-dithia-5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50-hexadecaazacyclotripentacontane-4-carboxylic acid, a histidine buffer, trehalose dihydrate, D-mannitol, L-methionine, polysorbate 80, and water.

40. The pharmaceutical composition of embodiment 39, wherein the CNP variant is present at a concentration of 10 mg/mL.

41. The pharmaceutical composition of embodiment 39, wherein the CNP variant is present at a concentration of 30 mg/mL.

42. The pharmaceutical composition of any one of embodiments 39-41, wherein the histidine buffer comprises L-histidine and histidine monohydrochloride monohydrate.

43. The pharmaceutical composition of embodiment 42, wherein L-histidine is present at a concentration of 0.35 mg/mL.

44. The pharmaceutical composition of embodiment 42, wherein L-histidine is present at a concentration of 2.2 mM.

45. The pharmaceutical composition of any one of embodiments 42-44, wherein L-histidine monohydrochloride monohydrate is present at a concentration of 1.6 mg/mL.

46. The pharmaceutical composition of any one of embodiments 42-44, wherein L-histidine monohydrochloride monohydrate is present at a concentration of 7.8 mM.

47. The pharmaceutical composition of any one of embodiments 39-46, wherein the trehalose dihydrate is present at a concentration of 58 mg/mL.

48. The pharmaceutical composition of any one of embodiments 39-46, wherein the trehalose dihydrate is present at a concentration of about 127 mM to about 153 mM.

49. The pharmaceutical composition of embodiment 48, wherein the trehalose dihydrate is present at a concentration of 127 mM.

50. The pharmaceutical composition of embodiment 48, wherein the trehalose dihydrate is present at a concentration of 153.3 mM.

51. The pharmaceutical composition of any one of embodiments 39-50, wherein the D-mannitol is present at a concentration of 15 mg/mL.
52. The pharmaceutical composition of any one of embodiments 39-50, wherein the D-mannitol is present at a concentration of about 68 mM to about 82 mM.
53. The pharmaceutical composition of embodiment 52, wherein the D-mannitol is present at a concentration of 68.1 mM.
54. The pharmaceutical composition of embodiment 52, wherein the D-mannitol is present at a concentration of 82.3 mM.
55. The pharmaceutical composition of any one of embodiments 39-54, wherein the L-methionine is present at a concentration of 0.7 mg/mL.
56. The pharmaceutical composition of any one of embodiments 39-54, wherein the L-methionine is present at a concentration of 4.9 mM.
57. The pharmaceutical composition of any one of embodiments 39-56, wherein the polysorbate 80 is present at a concentration of 0.05 mg/mL.
58. The pharmaceutical composition of any one of embodiments 39-56, wherein the polysorbate 80 is present at a concentration of 0.005% (v/v).
59. The pharmaceutical composition of any one of embodiments 1-58, wherein the composition exhibits lower C_{max} and higher AUC compared to free drug, e.g., an equivalent composition lacking an acid moiety, a spacer, and a hydrolysable linker.
60. A pharmaceutical kit comprising the pharmaceutical composition of any one of embodiments 1-58.
61. A pharmaceutical kit comprising the pharmaceutical composition of any one of embodiments 39-58, wherein the CNP variant is present in amount of 13 mg.
62. A pharmaceutical kit comprising the pharmaceutical composition of any one of embodiments 39-58, wherein the CNP variant is present in amount of 39 mg.
63. A method of treating a bone-related disorder or skeletal dysplasia in a subject in need thereof comprising administering to the subject a composition according to any one of embodiments 1-59.
64. The method of embodiment 63, wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of osteoarthritis, hypophosphatemic rickets, achondroplasia, hypochondroplasia, short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis imperfecta, achondrogenesis, chondrodysplasia punctata, homozygous achondroplasia, campomelic dysplasia, congenital lethal

hypophosphatasia, perinatal lethal type of osteogenesis imperfecta, short-rib polydactyly syndromes, rhizomelic type of chondrodysplasia punctata, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenita, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia, NPR2 mutation, SHOX mutation (Turner's syndrome/Leri Weill), PTPN11 mutations (Noonan's syndrome), insulin growth factor 1 receptor (IGF1R) mutation, idiopathic short stature and osteoporosis.

65. A method of elongating a bone or increasing long bone growth in a subject in need thereof, comprising administering to the subject a composition according to any one of embodiments 1 to 59, and wherein the administering elongates a bone or increases long bone growth.

66. The method of any one of embodiments 63-64, wherein the composition is administered subcutaneously, intradermally, intraarticularly, orally, or intramuscularly.

67. The method of any one of embodiments 63-66 wherein the composition provides an extended release composition.

68. The method of any one of embodiments 63-66, wherein the composition is administered, once every 5 days, once a week, once every two weeks, once every three weeks, once every 4 weeks, once every 6 weeks, once every two months, once every three months or once every six months.

69. A method of treating a CNP-responsive condition or disorder, comprising
-administering to a subject a composition according to any one of
embodiments 1 to 58, and

-monitoring the level of at least one bone- or cartilage-associated biomarker
in the subject,

wherein an increase in the level of the at least one bone- or cartilage-
associated biomarker indicates a therapeutic effect of the CNP variant on the subject or the
condition or disorder.

70. The method of embodiment 69, further comprising adjusting the amount or
frequency of administration of the composition, wherein

i) the amount or frequency of administration of the composition is increased if the level of the at least one bone- or cartilage-associated biomarker is below a target level; or

ii) the amount or frequency of administration of the composition is decreased if the level of the at least one bone- or cartilage-associated biomarker is above a target level.

71. The method of embodiment 69 or 70, wherein the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type II and fragments thereof, collagen type I C-telopeptide (CTx), osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, collagen X, alkaline phosphatase, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, collagen X, CXM (noncollagenous 1 (NC1) domain of type X collagen), NTproCNP, N-terminal collagen type I pro-peptide, bone-specific alkaline phosphatase, amino-terminal propeptide of type I collagen/procollagen type I N-propeptide (PINP), cross-linked C-telopeptide of type I collagen (CTx), cross-linked N-telopeptide of type I collagen (NTx) tartrate-resistant acid phosphatase 5b (TRAP-5b), transcriptomics readouts, and CNP-variant bioactivity.

72. The method of any one of embodiments to 63-70, wherein the administration increases the annualized growth velocity (AGV) in the subject at 12 months, optionally compared to baseline or to a normal control.

73. The method of embodiment 72, wherein the AGV in the subject increases over 1 year or over 2 years, or more.

74. The method of any one of embodiment 63-72, wherein the administration improves the height Z score at 12 months, optionally compared to baseline or to a normal control.

75. The method of any one of embodiments to 63-73, wherein the subject is greater than 3 years old.

76. The method of any one of embodiments to 63-74, wherein the subject is between 3 and 17 years old.

77. The method of any one of embodiments 63-75, wherein the subject has open epiphyses.

78. The method of any one of embodiments 63-77, wherein the composition is administered at a dose from about 5 µg/kg to 500 µg/kg or from about 15 µg/kg to 350 µg/kg.

79. The method of any one of embodiments 63-77, wherein the administration does not result in cardiovascular (CV) side effects.

80. The method of embodiment 79, wherein the CV side effect is change in systemic blood pressure, mean arterial pressure, systolic and/or diastolic blood pressures, pulse pressure or heart rate.

EXAMPLES

[0296] In these examples and the related schematics, tables, graphs, charts, and the like, "Free Drug" is sometimes used to refer to CNP that is not bound or conjugated to a linker and/or polymer.

Example 1: Synthesis of CNP Variants

[0297] CNP variant peptides were synthesized on solid-phase using a resin that would leave a C-terminal COOH on a Symphony/Prelude (Protein Technologies Inc., USA), Voyager (CEM GmbH, Germany), or Syroll (MultiSyntech, Germany) synthesizer.

[0298] All Fmoc-amino acids were purchased from Biosolve (Netherlands) or Bachem GmbH (Germany) with side-chain functional groups protected with N-f-Boc (KW), O-f-Bu (DESTY), N-Trt (HNQ), S-Trt (C), or N-Pbf (R) groups. A 5-fold excess of HBTU/HOBt/amino acid/DIPEA (1:1:1:2) in NMP with a 20 minute activation time using double couplings was employed for every amino acid coupling step.

[0299] Acetylation (Ac) of the peptide was performed by reacting the resin with NMP/Ac₂O/DIEA (10:1:0.1, v/v/v) for 30 min at room temperature.

[0300] For conjugation of a moiety, the protective amino group on lysine was cleaved to create a reactive group. Standard Fmoc synthesis was used to react 2x Fmoc-amino PEG(2) followed by glutamic acid, followed by C18-diacid.

[0301] The completed peptide was cleaved from the resin by reaction with a TFA (40 mL/mmol resin) for 2 hours at room temperature. Crude peptide was filtered, precipitated with ice-cold Et₂O followed by lyophilization and ultimately purification by preparative reverse phase-high-performance liquid chromatography (RP-HPLC). Final products and purity were confirmed by mass spectrometry.

[0302] CNP variants were generated based on the sequence of Pro-Gly CNP-37 (PGQEHPNARKYKGANKKGLSKGCGFLKLDRIGSMGLGC (SEQ ID NO: 1)), optionally

having changes in different amino acid residues, and/or acetylation at the N-terminus, and/or other modifications, and include (amino acid changes underlined):

CNP-8: Ac-PGQEHPQARRYRGAQRRGLSRGCFGLKLDRI~~GS~~MSGLGC-OH (SEQ ID NO: 8), CNP-5: Ac-PGQEHPNARKYKGANKKGLSKGCFGLKLDRI~~GS~~MSGLGC-NH₂ (SEQ ID NO: 9), CNP-6: Ac-PGQEHPNARRYRGANRRGLSRGCFGLKLDRI~~GS~~MSGLGC-OH (SEQ ID NO: 10),

CNP-7: Ac-PGQEHPNARRYRGANRRGLSRGCFGLKLDRI~~GS~~MSGLGC-NH₂ (SEQ ID NO: 11), CNP-9: Ac-PGQEHPQARRYRGAQRRGLSRGCFGLKLDRI~~GS~~MSGLGC-NH₂ (SEQ ID NO: 12),

AC-PGQEHPQARRYRGAQRRGLSRGCFGLK(AEEA-AEEA-YGIU-C

18DA)LDRIGSMSGL

GC-OH (SEQ ID NO: 8), and

PGQEHPQARKYKGAQKKGLSKGCFGLKLDRI~~GS~~MSGLGC-OH (SEQ ID NO: 7).

[0303] Fully reduced peptides were dissolved in 0.1 M Tris-buffer (pH 8.0) with or without guanidine HCl, containing 1 mM cysteine (SS-form) and 8 mM cysteine (SH-form) in a final concentration of 0.1 mg/ml and stirred at room temperature. Disulfide formation was monitored by HPLC-analysis until no further peak changes were observed. Mixtures were loaded on preparative RP-HPLC for purification.

Example 2: Characterization of Synthesized CNP Variants

[0304] CNP variants synthesized as in Example 1 were analyzed by mass spectrometry and UV spectroscopy to determine purity and stability after 10 days.

[0305] Stability was measured using RP-HPLC analysis of purity over time T0 to T10. Briefly, the CNP variants were diluted 1:5 in buffer: 5mM citrate, 6% sucrose, 1.5% mannitol, 0.7mg/ml methionine and 0.005% tween80, pH 5.6. An injection volume of 10 µL was injected onto a Phenomenex Aeris XB-C18 RP column (2 µm, 100Å, 2.1 x 250 mm; p/n 00G-4505-AN) HPLC conditions were: Mobile Phase A: H₂O / 0.05% TFA, pH 3 w/NH₄OH (~3mM final), Mobile Phase B: 70% CH₃CN in H₂O / 0.05% TFA, pH 3 w/NH₄OH (~3mM final) and a flow rate of 0.25mL/min. Measurement was carried out with a column temperature of 55° C and UV: 214nm (bw 4nm); ref 360nm (bw 20nm), UV: 280nm (bw 4nm); ref 360nm (bw 20nm). CNP variants were held 10 days in PBS, pH 7.4, at 37° C and stability measurements obtained.

[0306] Results of stability measurements are shown in Table 1 below as % variant detected at T0 (0 days) or T10 (10 days).

Table 1

Peptide	T0, %	T10, %	EC50, T0, nm
Pro-Gly CNP37	76.7	39.5	1.7
CNP-5	83	47	3
CNP-6	90	58	0.4
CNP-7	77	53	2.1
CNP-8	91	88	0.2
CNP-9	92	77	0.9

[0307] CNP variants were tested for activity by a cGMP stimulation assay using a CatchPoint Cyclic-GMP Fluorescent Assay Bulk kit (Molecular Devices, R8075). Briefly, NIH3T3 cells (ATCC, CRL-1658) and HEK293 cells were seeded at 60,000 cells/well in a 96-well plate (96-well black imaging plates, Grenier, #655090). Culture media was as follows: NIH3T3 culture media: DMEM high glucose, pyruvate (Thermo, 11995-073) + 10% FBS + 1x Pen Strep (abbrev P/S, Thermo, cat# 15140122). NIH3T3 was the control system for the cGMP assay HEK293 culture media: EMEM + 10% FBS + 1x P/S + 1x GMAX. Serum free NIH3T3 media: DMEM + 1x P/S for treatment of cells with IBMX (CAS 28822-58-4); Serum free NIH3T3 media with BSA: DMEM + 1x P/S + 0.5 mg/mL BSA (Thermo, A9418-100G) for treatment of cells with CNP.

[0308] Cells were incubated for 24 hours at 37° C, 5% CO₂. For cells to be treated with CNP variants, plates were pre-treated with IBMX (Enzo life sciences, 89161-340, 1g) 15 minutes prior to use. IBMX is a potent, non-specific inhibitor of phosphodiesterases. An 800 mM stock solution of IBMX is diluted in IBMX dilution media (serum-free media (DMEM + 1x PBS mixed 1:1 with 1x PBS) to a 0.75 mM working stock.

[0309] CNP variants were prepared as follows: Dilute 10 mg/ml CNP solution 1:1000 in CNP dilution media (DMEM + 1x P/S + 0.5 mg/mL BSA). This solution is further diluted to obtain a CNP starting solution plated at 100 nM CNP/well. This 100 nM CNP solution is further serially diluted 1:5 for six dilutions to obtain a lower end concentration of 0.0064 nM CNP /well. This provides a 7-point dose curve for analysis.

[0310] For cell treatment, cells were removed from the incubator, growth media was removed from cells and cells treated with IBMX. 80 µL 0.75 mM IBMX was added to each well and cells returned to 37° C incubator for 15 minutes. After 15 minutes CNP (40 µL/well) was added to each test well and cells returned to 37° C incubator for 15 minutes. The plate

was mixed by gentle tapping. The plate was imaged on a Solentim cell metric to visualize cells and determine if there is any cell lifting and then placed back into 37° C incubator.

[0311] The reaction was stopped and cells lysed by adding 40 µL lysis buffer (from cGMP kit). Plate was placed on a shaker for 5 minutes to complete lysis. The cell lysate was used in the cGMP assay.

[0312] The cGMP assay was carried out using a cGMP calibrator, rabbit anti-cGMP antibody and HRP-cGMP prepared as according to manufacturer's protocol. 40 µL of calibrator was added to wells of an anti-cGMP antibody coated plate, and 40 µL of lysate to be analyzed added to the appropriate wells. 40 µL of reconstituted rabbit anti-cGMP antibody was added to all wells and plates placed on shaker five minutes for mixing. 40 µL of reconstituted HRP-cGMP was added to each well and incubated for 2 hours at room temperature. Plates were manually aspirated and washed 4x with 300 µL wash buffer. 100 µL of stoplight red substrate was added to each well, the plate covered and left at room temperature for at least 10 mins, protected from light. The plate was read for fluorescence intensity on a Spectramax M, or similar instrument, at excitation 530 nm and emission at 590 nm.

[0313] Table 1 shows that the CNP variants stimulate cGMP production, suggesting that the stable variants described herein are useful as therapeutics to treat bone-related disorders.

Example 2A: Stability of CNP Variants or Conjugates in Plasma

[0314] Stability of different CNP variants or CNP conjugates were tested for stability in human plasma over a 24 hour period. Briefly, a C18 fatty acid was fused to gamma glutamic acid using 2 OEG spacers (see e.g., Lau et al., J. Med. Chem 58:7370-7380, 2015). For the chromatography, Waters UPLC H-Class connected with BEH C18 1.7 µm, 2.1 x 150 mm; Mobile Phase A: 1% DMSO 0.1% Formic Acid in Water; Mobile Phase B: 1% DMSO 0.1% Formic Acid in CAN. Mass Spectrometry was carried out using an AB Sciex QTRAP. Each CNP variant was prepared at 200nM in Human Plasma Li Heparin and samples Incubated at 37° C 5% CO₂. The reaction was quenched at 0, 1, 2, 4, 8, 24 hours with 0.5M Sodium Citrate pH 4. Plasma proteins were precipitated with 0.2% Formic Acid in MeOH and samples prepared using WCX 96-well uElution plate and analyzed by LC-MS/MS 6500.

[0315] Variants CNP-R refers to CNP37 variants in which the K residues not in the ring portion are changed to R residues, and CNP Q/R refers to CNP37 variants in which N residues are changed to Q and K residues not in the ring portion are changed to R.

[0316] Figure 1 illustrates CNP conjugates having different linker structures. Figure 2 shows that lipidated conjugate has improved stability in plasma compared to PEGylated or non-conjugated peptides in plasma.

[0317] Stability of different conjugates was also analyzed at varying conditions. Briefly, the same protocol as above was used at 37° C (control), 37° C+ 0.5M NaCl, 37°C + protease inhibitors or 4° C. Figure 3 shows that PG-CNP37 and variants are prone to proteolysis in human plasma and PG-CNP37 shows greater stability compared to other variants.

Example 3: Heterozygous NPR2 Mutations Are Responsive to CNP Treatment

[0318] To determine the effects of CNP on subjects with short stature resulting from NPR2 mutations, cellular models of NPR2 mutations were developed. Exemplary NPR2 mutations analyzed are set out in Figure 6. Rat chondrosarcoma (RCS) cells having either a knockout of, or heterozygous loss of function mutations in, the NPR2 gene were made by RNP transfection into RCS cells using 125ng NPR2 variants or wild-type NPR2 plasmid DNA transfected into RCS or HEK293 cells. Single cell clones were seeded and genotyped by Sanger sequencing. Cell models are able to reproduce published cGMP phenotypes of the different mutations.

[0319] The NPR2 clones were created by creating insertions and deletions in the first exon of NPR2 in RCS cells. The sequence of the first exon in NPR2 was confirmed by next-generation sequencing and is set out in Figure 5. NPR2 mutant cells were tested for activity in response to CNP administration by a cGMP stimulation assay using a CatchPoint Cyclic-GMP Fluorescent Assay after treatment with 6nM of Pro-Gly CNP37. RCS (rat chondrosarcoma) cells were seeded at 40,000 cells/well in RCS culture media: DMEM + 10% FBS + 1x Pen Strep. Figure 4 shows that adding exogenous Pro-Gly-CNP37 variant rescues cGMP readout in a NPR2 +/- rat chondrosarcoma cell model.

[0320] Previous activation data reports cGMP EC50 in the range of 40 to 360nM for activation of PRKG2 (Campbell et al., ACS Chem Biol 12, 2388-2398, 2017); Vaandrager et al., J Biol Chem 272, 11816-23, 1997); Pohler et al., FEBS Lett 374, 419-25, 1995). In the heterozygous NPR2 knockout cells, a CNP dose >0.163nM is able to achieve an intracellular concentration exceeding the EC50 range for PRKG2 activation cGMP (Figure 4). Whereas, in wild-type cells a CNP dose of 0.040nM is able to achieve the same cGMP concentration. These results demonstrate that CNP supplementation can achieve the cGMP levels necessary for PRKG2 activation and growth in cells with loss-of-function mutations of NPR2.

[0321] These results also suggest that administration of CNP variants is useful to restore bone growth in subjects with short stature that have reduced activity of NPR2. It is further contemplated that treatment with CNP variants will be beneficial in subjects having mutations in other growth plate genes in which cGMP signaling may be impaired.

Example 4: Identification of Mutations Associated With Short Stature

[0322] It is hypothesized that genes showing clear evidence of genetically-driven bi-directional effects are more likely to represent therapeutic targets that can be effectively modulated in a broad patient population. To identify genes that are core regulators of growth, the intersections of five gene lists were analyzed including, the list of genes from genome-wide association study (GWAS). Core growth regulators would be the most likely to contain rare coding mutations with bidirectional effects (i.e. short stature or skeletal dysplasia AND tall stature or overgrowth).

[0323] Databases queried include: GWAS 2,067 non-repeating closest genes for each of the 3,290 independent genetic variants reported by a large GWAS meta-analysis of height using ~700,000 individuals were extracted; HGMD The "allmut" table from HGMD version v2019_2 was queried looking for all pathogenic variants labelled as "DM" having either "short stature" and "tall stature or overgrowth" in the same genes; OMIM The list of OMIM genes related to growth disorders was previously described and was created using the keywords: short stature, overgrowth, skeletal dysplasia, brachydactyly.

[0324] First, the Human Gene Mutation Database (HGMD version v2019_2) was queried for genes associated with short or tall stature (Stenson et al., Hum Genet 136:665-677, 2017). There were 47 genes annotated with at least one pathogenic variant reported in the literature to cause "short stature". Only 20 genes were annotated as tall stature or overgrowth genes.

[0325] Secondly, a manually curated list of 258 OMIM genes was used (248 short, 20 tall) which was created using the keywords: short stature, overgrowth, skeletal dysplasia, brachydactyly (Wood et al., Nat Genet 46:1173-86, 2014). Third, the intersection of these lists was compared with the list of genes from GWAS. At the intersection of these list there were three genes known to be associated with height (IGF1R, NPPC, NPR2) and two additional genes were identified (FGFR3, SHOX).

[0326] Additional analysis led to generation of a new group of five Core genes that showed significantly decreased height ($\beta=-0.20$, 95%CI [-0.26 to -0.14], $p=4.04 \times 10^{-11}$) and significantly increased risk for Idiopathic Short Stature (ISS) (OR=2.75, 95%CI [1.92 - 3.96]).

Each of the core five genes (FGFR3, IGF1R, NPPC, NPR2 and SHOX) were associated with height when considered individually, and also were associated with short stature when taken in combination with other mutations. Exemplary mutations in FGFR3, IGF1R, NPPC, NPR2 and SHOX are set out in Figure 7.

[0327] Combined LoF (loss of function) and Missense variants in NPR2 and IGF1R were also associated with increased risk for ISS (OR=3.31, P= 0.001, OR= 2.85, P=0.002, respectively). Entire gene deletions and/or mutations causing loss of protein function in SHOX, IGF1R, NPPC, NPR2 have been reported in familial short stature with various degrees of severity. Mutations in DTL, and pregnancy-associated plasma protein A2 (PAPPA2), or combinations thereof also contribute to skeletal dysplasia.

[0328] Analysis shows that carriers of variants in any of the five core genes are at approximately a 3-fold increased risk for ISS and account for 6.7% of the total ISS population. Furthermore, a dose-dependent rescue of NPR2 signaling in a cell model of NPR2 haploinsufficiency after adding exogenous CNP was demonstrated.

[0329] According to the omnigenic model (Liu, et al., Cell 177:1022-1034 e6 (2019); Boyle et al., Cell 169:1177-1186 (2017)) if these genes are core human growth genes, then their effects should be modulated by multiple weaker common genetic variants driving regulatory networks. To indirectly test this hypothesis, polygenic risk scores (PRS) were calculated for height using the largest published GWAS meta-analysis for height that did not include any samples from the UK Biobank project. The cohort was divided into five equally-sized (n=6,824) PRS quintiles (PRS 1 being the lowest height, PRS 5 the tallest height). There was a dose-dependent relationship between increasing PRS score and mean height ($\beta=0.30$ per each PRS quintile increase) (Fig. 8A). Carriers of LoF variants in the five core genes were consistently shorter than non-carriers across the five different PRS backgrounds. See Figure 8. The data suggest that the combined effect of PRS and rare protein variants is consistent with an additive model: polygenic effects modulated height in both carriers and non-carriers.

[0330] The risk for ISS across PRS groups was calculated using PRS=3 as a reference. The lowest PRS group was associated with increased risk for ISS and the highest PRS group with a decreased risk (OR=5.43, P=8.58x10⁻³⁴; OR=0.22, P=4.49x10⁻⁷ for PRS 1 and PRS 5 respectively). The effect of rare coding variants of the five core genes was evaluated for ISS stratified by PRS group. Carriers of any of the five core genes were at increased risk for ISS in the first three quintiles (OR=2.64, P=3.09x10⁻⁵; OR=2.17, P=0.04;

OR=5.29, $P=1.58 \times 10^{-5}$; OR=2.72, $P=0.09$ Figs. 8C-F). Consistent directions of effect were observed for carriers of each individual core gene on ISS risk stratified by PRS (Figs. 8C-F).

[0331] Further, additive effects of PRS, mostly coming from multiple common genetic variation with individual small effects, predicted 20.1% of the variance in height in the dataset. These additive effects of PRS appeared to have similar magnitude on carriers of rare coding variation of core genes as well as in non-carriers. This observation indicates that PRS may be a strong contributor in the differences in penetrance of rare pathogenic variants (especially in models of haploinsufficiency such as the ones described here). Supporting this idea, it was observed that two of eight NPR2 variant carriers with low NPR2 activity had a low-normal height. This data suggests that most ISS individuals possessing mutations in NPR2 may also have a polygenic background that made them more susceptible to the pathogenic effect of losing NPR2 activity.

[0332] These results support the idea that CNP-based treatments could be effective in NPR2 haploinsufficient patient populations. Further, results showing a significant bi-directional (LoF and GoF) correlation of cGMP levels and height in NPR2 carriers of the general population suggest that targeting this receptor with CNP analogs could be an effective therapy for all ISS individuals. Methods of identifying a variant gene associated with short stature that is a GoF or LoF variant using a reporter construct/barcode assay is described in PCT/US 24/32942, incorporated by reference herein.

Example 5: CNP Formulations

[0333] Different buffering agents for CNP variant formulations were evaluated for particulates. Particularly, lyophilized CNP formulations reconstituted with water were analyzed by visual and UV concentration measurements to identify particulates. As shown in Figure 13, visual appearance shows that visible particles are formed in citrate-based formulations. Histidine buffer exhibited the best appearance out of all the different buffers including citrate.

[0334] As shown below, the UV analysis highlights the drop in the concentration after precipitation.

Sample	Test	Initial (T=0)	25 °C		5 °C		
			2W	1M	2W	1M	3M
pH 5.5C	UV concentration	0.74	0.68	0.53	0.73	0.74	0.57
pH 5.5FB	UV concentration	0.77	0.72	0.53	0.75	0.73	0.42
pH 6H	UV concentration	0.76	0.74	0.75	0.75	0.75	0.75

Example 6

[0335] Variant content when stored in different formulation buffers was analyzed over time, up to 13 months, at a range of temperatures from -20 to 37° C. Results are shown in Figure 14.

Example 7

[0336] Free drug content release from different formulation buffers was analyzed over time, up to 13 months, at a range of temperatures from -20 to 37° C. Results are shown in Figure 15.

Example 8

[0337] Linker area of the drug conjugate when stored in different formulation buffers was analyzed over time, up to 13 months, at a range of temperatures from -20 to 37° C. Results are shown in Figure 16.

Example 9

[0338] CNP conjugate content when stored in different formulation buffers was analyzed over time, up to 13 months, at a range of temperatures from -20 to 37° C. Results are shown in Figure 17.

Example 10

[0339] Variant concentration as measured by UV when stored in different formulation buffers was analyzed over time, up to 3 months. Results are shown in Figure 18.

Example 11

[0340] Variant content in formulations with different excipients (histidine, glycine and sorbitol) was analyzed over time, up to 13 months, pH 5.5 or 6 was assessed. Results are shown in Figure 19. No precipitation was observed, and excipients did not reduce drug cleavage. There was no significant difference in the histidine formulation at pH 5.5 and 6.

Example 12

[0341] A manufacturing feasibility study was carried out for CNP prodrug. Results are shown in Figure 20. Free drug cleavage was observed up to 0.5% at 5° C and 2.5% up to 25° C. There was no significant difference between pH 5.5 and 6.

Example 13

[0342] Batches of formulated CNP prodrug were analyzed for product degradation over 1 month in histidine or acetate buffer with trehalose and mannitol at different ratios (4:1, or 1:4, respectively). Results are shown in Figure 21. Free drug increases as a function of

increasing time, temperature and pH. Dextran containing formulations at pH 4 had lower free drug cleavage that is likely due to the lower pH.

Example 14

[0343] Different formulations of CNP prodrug were tested with buffers, bulking agents and other excipients.

Formulation	
1.	10 mM Histidine pH 6 with Trehalose, Mannitol, Methionine, PS80
2.	10 mM Histidine pH 5.5 with Trehalose, Mannitol, Methionine, PS80
3.	10 mM Histidine pH 5.2 with Trehalose, Mannitol, Methionine, PS80
4.	Acetate pH 4 with Trehalose, Methionine, PS80
5.	10 mM Citrate pH 3 with Trehalose, Methionine, PS80
6.	5 mM Citrate pH 5.5 with Trehalose, Mannitol, Methionine, PS80 (Control)

Tonicity: concentration 5 mM Osmo- 5, 10, 20 mg/mL

10 mM Acetate	5.5	279
10 mM Acetate	4	273
10 mM Citrate	3	273
Free drug (5 mM Citrate)	5.5	280

10 mM Histidine FB pH 5.5 with prodrug

10 mM Histidine FB pH 5.5 with CNP Variant HCl	pH	Average n=3 Osmolality (mOsm/kg)	UV concentration (mg/mL)
Buffer only	5.58	269	0
5 mg/mL	5.04	277	4.1
10 mg/mL	4.69	286	8.3
20 mg/mL	4.44	295	15.0
40 mg/mL	4.39	317	29.3

Example 17

[0346] Area under the curve of drug product after reverse phase chromatography was assessed. Results are shown in Figure 22.

Example 18

[0347] Drug product stability was assessed under the following conditions:

Buffer	10 mM Histidine 4.8% Trehalose Formulation Buffer
Buffer pH	5.2, 5.5, 6.0
Drug Substance	CNP Variant
Sample Concentration	50, 150, 500, 1667 µg/mL

10 mM Histidine 4.8% Trehalose Formulation Buffer			
Component/Target Amount (g/L)	pH 5.2	pH 5.5	pH 6.0
L-Histidine	0.196	0.347	0.740
Histidine Monohydrochloride Monohydrate	1.832	1.627	1.096
Trehalose Dihydrate		48.0	
D-Mannitol		12.4	
L-methionine		0.73	
Polysorbate 80, 1% (w/w)		5.0	
Water		QS	

Storage and Testing Requirements				
Storage Condition / Timepoint	0	6-8 h	3 d	2 W
Initial	Full testing	-	-	-
5 °C	-	-	Full testing	Full testing
25 °C	-	Full testing	Full testing	-
3x Freeze/Thaw (<-60 °C and 25 °C/60% RH)	Full testing	-	-	-

Full testing: appearance, osmolality, pH, UV concentration, RP-Perchlorate UPLC

Example 19

[0348] In use stability and free drug content was assessed over time, up to 13 months, at a range of temperatures from 5 to 25° C and pH 5.2 to 6.0. Results are shown in Figure 23.

Example 20

[0349] Buffering formulations for CNP prodrug are described.

Component	Grade	Function	Strength (mM,%)
L-Histidine	Ph. Eur., ChP, JP, USP	Buffering species	2.2mM
Histidine Monohydrochloride Monohydrate	Ph. Eur., BP, ChP, JP	Buffering species	7.8 mM
Trehalose Dihydrate	Ph. Eur., ChP, JP, NF	Bulking/tonicity agent	127 mM
D-Mannitol	Ph. Eur., BP, ChP, JP, USP	Bulking/tonicity agent	68.1 mM
L-Methionine	Ph. Eur., BP, JP, USP	Antioxidant	4.9 mM

Polysorbate 80	Ph. Eur., JP, NF	Surfactant/anti-absorbent	0.005%
QS to final volume with WFI			

Buffer range: 5-15 mN concentration

Trehalose : mannitol ratio of about 4:1 (e.g, 3:1 to 5:5)

[0350] Antioxidant in the buffer is not necessary, but an antioxidant is added in some case. When used, L-methionine is particularly employed as an antioxidant.

[0351] PS 80 (polysorbate 80) is the preferred surfactant, because it minimizes loss of the CNP Variant because of the CNP Variant's absorption on the materials (e.g., vessel in which the formulation is prepared).

Example 21 – Visual comparison of different amounts of trehalose

[0352] Visual comparison of CNP prodrug in different formulations is shown in Figure 24. Trehalose at 3.5-5.5%, such as 3.8-4.8% trehalose, may be acceptable. 4.8% trehalose was most preferred, because of favorable properties by visual inspection (among other reasons).

Example 22 (Prophetic) – Analytical techniques to analyze binding in rat plasma and/or and linker cleavage and release of CNP from the conjugate.

Preparation of materials to be used in the analysis

[0353] For the purposes of this example, CNP means a CNP Variant; it is particularly envisaged that the analysis will be undertaken with the CNP variant shown in Figure 10, although it is also envisaged that other CNP variants as described herein could be analyzed by the same or analogous methods.

0.1M Methionine

Weigh 60 mg methionine. Add 4.0 mL water in a 5 mL tube. Mix well, tightly capped and store on wet-ice and discard after use.

0.5 M Sodium Citrate (pH 4.0)

Weigh 36.8 g sodium citrate dihydrate. Dissolve in approximately 220mL of water. Adjust pH to 4.0 using 1.0 M citric acid while stirring. Transfer the solution to a 250-mL volumetric flask and keep adding water until the meniscus of the solution reaches the graduation line. Put on the volumetric flask stopper and invert the flask to thoroughly mix the solution.

Protease inhibitor cocktail

- 1) Dissolve protease inhibitor cocktail (Sigma P2714) in 10 mL Milli-Q water to get inhibitor solutions A.
- 2) Dissolve 250 mg 3-isobutyl-1-methylxanthine (phosphodiesterase inhibitor) (SigmaI5879) in 11.25 mL methanol to get inhibitor solution B.
- 3) Combine 0.83 mL of inhibitor solution B with 9.17 mL of inhibitor solution A to get 10.0 mL protease inhibitor cocktail. Keep the remaining inhibitor solution B at -20°C for up to 2 months.

Treated rat plasma K2EDTA matrix

- 1) Chill rat K2EDTA whole blood on ice and aliquot 10 mL into a 15-mL tube.
- 2) Add 2.0 mL of 0.5 M sodium citrate (pH 4.0) and 60 μ L of protease inhibitor cocktail into 10 mL ice-chilled rat K2EDTA whole blood.
- 3) Gently mix and then centrifuge the 15-mL tube at 1,500 $\times$ g for approximately 15 minutes at room temperature.
- 4) Take the supernatant as the treated rat plasma K2EDTA matrix.

CNP Stock Solution

[0354] 10 mg of dry or lyophilized CNP is reconstituted in 1 mL of formulation buffer to provide 10 mg/mL stock solution. The concentration is then corrected to approximately 2.3 mM based on absorbance readings at 275 nm.

CNPox Stock Solution

[0355] 10 mg CNP111ox was reconstituted in 1 mL of formulation buffer to get 10 mg/mL

Lip-BC-CNPox stock solution. After A275 nm reading, concentration is corrected to 8.64 mg/mL (2.10 mM).

Lip-BC-CNP Stock Solution

[0356] 10 mg Lipid-BC-CNP (MW: 4962.11) was reconstituted in 1 mL of formulation buffer to get 10 mg/mL Lip-BC-CNP stock solution. After A275 nm reading, concentration is corrected to 6.88 mg/mL (1.38 mM).

Lip-BC-CNPox Stock Solution

[0357] 10 mg Lipid-BC-CNPox was reconstituted in 1 mL of formulation buffer to get 10 mg/mL Lip-BC-CNPox stock solution. After A275 nm reading, concentration is corrected to 6.75 mg/mL (1.36 mM).

Mix ISTD Stock Solution

[0358] Prepare all ISWS on ice. Add 10 μ L of 5 μ M CNP (old) and 10 μ L of 5 μ M CNPox (old) into 5 mL of 0.1% FA, 20% ACN in water. Vortex thoroughly prior to aliquoting. Keep on ice till use. Add 10 mL of 5 mM Lip-BC-CNP[^] (new) and 10 mL of 5 mM Lip-BC-CNPox[^] (new) into 5 mL of formulation buffer. Vortex it thoroughly prior to aliquoting. Keep on ice till use.

Standard Solutions

[0359] Prepare standard working solutions as follows using 0.1% FA, 20% ACN in water as the diluent:

Working Solution ID	Source Solution ID	Source Solution Concentration (pM)	Volume Source Solution (μ L)	Volume Diluent (μ L)	Final Volume (mL)	Final Concentration (pM)
CNP-D01	CNP Stock	2,310,000,000	20.0	1873	1.893	24,400,000
CNP-D02	D01	24,400,000	20.0	1980	2.00	244,000

CNPox-D03	CNPox stock	2,100,000,000	20.0	1701	1.721	24,400,000
CNPox-D04	D03	24,400,000	20.0	1980	2.00	244,000
Lipid-BC-CNP D05	Lipid-BC-CNP Stock	1,380,000,000	20.0	1,111	1.131	24,400,000
Lipid-BC-CNPox D06	Lipid-BC-CNPox Stock	1,360,000,000	20.0	1,095	1.115	24,400,000

[0360] Prepare CNP and CNPox standards using treated rat plasma K2EDTA in 1.5-mL protein low-binding tubes;

Standard ID	Source Solution ID	Source Solution Concentration (pM)	Volume Source Solution (μL)	Volume Diluent (μL)	Final Volume (mL)	Final Concentration (pM)
STD-8	D02; D04	244,000	50;50	2340	2.440	5,000
STD-7	STD-8	5,000	720	480	1.200	3,000
STD-6	STD-7	3,000	600	1200	1.800	1,000
STD-5	STD-6	1,000	960	240	1.200	800
STD-4	STD-5	800	750	450	1.200	500
STD-3	STD-4	500	480	720	1.200	200
STD-2	STD-3	200	300	900	1.200	50.0
STD-1	STD-2	50.0	600	600	1.200	25.0

[0361] Prepare Lip-BC-CNP and Lip-BC-CNPox standards using treated rat plasma K2EDTA in 1.5-mL protein low-binding tubes;

Standard ID	Source Solution ID	Source Solution Concentration (pM)	Volume Source Solution (μL)	Volume Diluent (μL)	Final Volume (mL)	Final Concentration (pM)
STD-8	D05; D06	24,400,000	50;50	2340	2.440	500,000
STD-7	STD-8	500,000	480	720	1.200	200,000
STD-6	STD-7	200,000	450	750	1.200	75,000
STD-5	STD-6	75,000	400	800	1.200	25,000
STD-4	STD-5	25,000	300	700	1.000	7,500
STD-3	STD-4	7,500	400	800	1.200	2,500
STD-2	STD-3	2,500	400	600	1.000	1,000
STD-1	STD-2	1,000	500	500	1.000	500

QC Solutions

[0362] Prepare Free Peptides CNP QCs as follows using citrate and inhibitors treated rat plasma K2EDTA as the diluent:

QC ID	Source Solution ID	Source Solution Concentration (pM)	Volume Source Solution (μL)	Volume Diluent (μL)	Final Volume (mL)	Final Concentration (pM)
HQC	D02; D04	244,000	20.0; 20.0	1,261	1.301	3,750
MQC	HQC	3,750	320	880	1.200	1,000
LQC	MQC	1,000	180	1,020	1.200	150

[0363] Prepare Lip-BC-CNP and Lip-BC-CNPox QCs as follows using citrate and inhibitors treated rat plasma K2EDTA as the diluent:

QC ID	Source Solution ID	Source Solution Concentration (pM)	Volume Source Solution (μL)	Volume Diluent (μL)	Final Volume (mL)	Final Concentration (pM)
HQC	D05; D06	24,400,000	15.0; 15.0	946	0.976	375,000
MQC	HQC	375,000	52	1148	1.200	16,250
LQC	MQC	16,250	120	1180	1.300	1,500

LC-MS ANALYTICAL CONDITIONS

Chromatographic Conditions

Column	Waters ACQUITY UPLC Peptide BEH C18, 130 Å, 1.7μm, 2.1 x 50 mm		
Pre-filter	Waters, 2 μm (or other equivalent pre-filters)		
Column Temp	50°C		
Mobile Phase	A: 0.1% FA, 1% DMSO in water B: 0.1% FA, 1% DMSO in acetonitrile		
LC Gradient	Time (min)	Events	%B
	0.00	B.conc.	10.0
	3.00	B.conc.	40.0
	3.05	B.conc.	95.0
	4.50	B.conc.	95.0
	4.55	B.conc.	10.0
	5.00	B.conc.	10.0
	6.00	System controller	Stop
Flow Rate	0.5 mL/min		
Equilibration back Pressure	380 bar		
Sample Tray Temp	6°C		
Injection Volume	20 μL (Typical)		
Wash Solution (S2)	0.1% FA in water		
Needle Wash – Multi-Wash	S1: Seat back flush/needle wash, 5 sec		
Acquisition Time	5.00 min		

Mass Spectrometer Parameters

Mass Spectrometer		Sciex Triple Quad 6500+					
Ionization/Mode		Positive ion, MRM					
Curtain Gas (CUR)		35.0					
Collision Gas (CAD)		10					
IonSpray Voltage (V)		5500					
Capillary Temp (°C)		550					
Ion Source Gas 1 (GS1)		70.0					
Ion Source Gas 2 (GS2)		70.0					
Compound Name	Q1 (m/z)	Q3 (m/z)	Schedule MRM Cycle (sec)	CE	DP	CXP	EP
CNP	513.900	574.600	0.4	21.0	80.0	18.0	10.0
CNP^	516.000	577.000	0.4	21.0	80.0	18.0	10.0
CNPox	515.900	568.600	0.4	21.0	80.0	18.0	10.0
CNPox^	518.000	570.900	0.4	21.0	80.0	18.0	10.0
Lip-BC-CNP	710.000	658.700	0.4	21.0	80.0	18.0	10.0
Lip-BC-CNP	711.600	660.100	0.4	21.0	80.0	18.0	10.0
Lip-BC-CNPox	712.300	660.900	0.4	21.0	80.0	18.0	10.0
Lip-BC-CNPox^	713.700	662.400	0.4	21.0	80.0	18.0	10.0

Example 22

[0364] Buffering Formulation for CNP Variant are described.

Component	Grade	Function	Strength (mM,%)
L-Histidine	Ph. Eur., ChP, JP, USP	Buffering species	2.2mM
Histidine Monohydrochloride Monohydrate	Ph. Eur., BP, ChP, JP	Buffering species	7.8 mM
Trehalose Dihydrate	Ph. Eur., ChP, JP, NF	Bulking/tonicity agent	153.3 mM
D-Mannitol	Ph. Eur., BP, ChP, JP, USP	Bulking/tonicity agent	82.3 mM
L-Methionine	Ph. Eur., BP, JP, USP	Antioxidant	4.9 mM
Polysorbate 80	Ph. Eur., JP, NF	Surfactant/anti-absorbent	0.005%
QS to final volume with WFI			

Example 23 – CNP Prodrug Drug Substance and Drug Product

The following abbreviations are used herein:

μ CT	micro computed tomography
ACH	achondroplasia
ADA	antidrug antibody
AE	adverse event
AGV	annualized growth velocity
ANP	atrial natriuretic peptide
AUC	area under the plasma concentration-time curve
$AUC_{0-\infty}$	area under the plasma concentration-time curve from time 0 to infinity
AUC_{0-t}	area under the plasma concentration-time curve from time 0 to the last measurable concentration
BAP	bone-specific alkaline phosphatase
BBB	blood brain barrier
BCRP	breast cancer resistance protein
BLQ	below the limit of quantification
cGMP	cyclic guanosine monophosphate
C_{max}	maximum observed plasma concentration
CMC	chemistry, manufacturing, and control
CNP	C-type natriuretic peptide
CNS	central nervous system
C_{trough}	trough plasma concentration

CTx-Ia	telo peptide of type I collagen
CTx-II	telo peptide of type II collagen
CV	cardiovascular
CYP	cytochrome P450
DLT	dose-limiting toxicity
DP	drug product
DS	drug substance
DXA	dual X-ray absorptiometry
ECG	electrocardiogram
EOI	event of interest
ERK	extracellular signal-regulated kinase
EU	European Union
FDA	Food and Drug Administration
FGF	fibroblast growth factor
FGFR3	fibroblast growth factor receptor-3
<i>FGFR3</i>	fibroblast growth factor receptor-3 gene (human)
<i>Fgfr3</i>	fibroblast growth factor receptor-3 gene (mouse)
FIH	first-in-human
Fmoc	<i>N</i> -a-9 fluorenylmethoxycarbonyl
GD	gestational day
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
HCH	hypochondroplasia
HCM	hypertrophic cardiomyopathy
IB	Investigator's Brochure
ICH	International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use
IND	Investigational New Drug
IP	intraperitoneal
IV	intravenous
LD	lactation day
MAPK	mitogen-activated protein kinase
MATE	multidrug and toxin extrusion protein
MEKi	mitogen-activated protein kinase kinase inhibitor
NA	not applicable
NCE	New Chemical Entity
NDA	New Drug Application
NHP	non-human primate
NOAEL	no-observed-adverse-effect level
NOEL	no-observed-effect level

NPR	natriuretic peptide receptor
NPR-B	natriuretic peptide receptor B
NS	Noonan Syndrome
OAT	organic anion transporter
OATP	organic anion transporting polypeptides
OCT	organic cationic transporter
OECD	Organisation for Economic Co-operation and Development
OLE	open-label extension
P-gp	permeability glycoprotein
Ph. Eur.	European Pharmacopeia
PK	pharmacokinetic(s)
PND	postnatal day
PPL	Polypeptide Laboratories
pQCT	peripheral quantitative computed tomography
PREA	Pediatric Research Equity Act
Pro C1	fragment of N-terminal type I collagen
Pro C2	fragment of N-terminal type IIB procollagen
Pro-C10 HP	fragment of the NC1 domain of Collagen X
QAD	once every other day
QSAR	Quantitative Structure-activity Relationship
QTc	corrected QT interval
QTcF	heart rate corrected QT interval using Fridericia's formula
QW	once weekly
RAF-1	rapidly accelerating fibrosarcoma 1
RP-UPLC	reversed phase ultra performance liquid chromatography
SC	subcutaneous
SOC	system organ class
SPPS	Solid-Phase Peptide Synthesis
SRC	Safety Review Committee
$t_{1/2}$	terminal half-life
T2CM	type II collagen neoepitope
TD	thanatophoric dysplasia
TEAE	treatment-emergent adverse event
TK	toxicokinetic(s)
T_{max}	time to peak concentration
US	United States
USP	United States Pharmacopeia
WFI	water for injection
WT	wild-type

Drug Substance (DS)

[0365] CNP Prodrug is comprised of CNP, a pH responsive self-immolative linker, spacer, and C-18 fatty acid albumin binding domain (attached to the Lys²⁷ side chain).

[0366] The sequence of the CNP Prodrug is as follows: Pro¹-Gly-Gln-Glu-His⁵-Pro-Asn-Ala-Arg-Lys¹⁰-Tyr-Lys-Gly-Ala-Asn¹⁵-Lys-Lys-Gly-Leu-Ser²⁰-Lys-Gly-Cys-Phe-Gly²⁵-Leu-Lys²⁷(X)-Leu-Asp-Arg³⁰-Ile-Gly-Ser-Met-Ser³⁵-Gly-Leu-Gly-Cys-OH

(X) = OH-1,18-octadecandioyl-Glu(NH-PEG₂-carboxy)₂-NH-CH₂CH₂-Gly(N-CH₂CH₂OH)-OH

[0367] The drug substance is isolated as a hydrochloride salt. The empirical molecular formula is C₂₁₇H₃₆₃N₆₁O₆₅S₃ (as free base). The average molecular mass is 4962.77 m.u.

Physico-chemical Properties

[0368] The available relevant physico-chemical properties of the DS are provided in Table .

Table 3: Physico-chemical Properties of CNP Prodrug

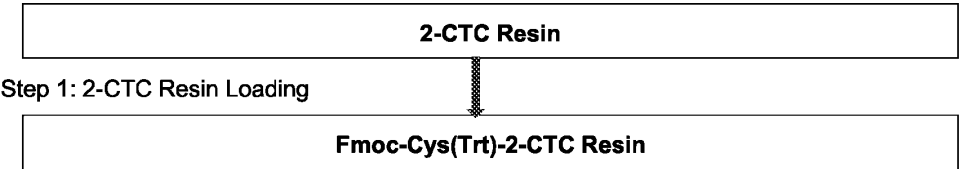
Physico-Chemical Property	Details
Appearance of lyophilized powder.	White to off-white powder
Hygroscopicity	Very hygroscopic

Manufacture

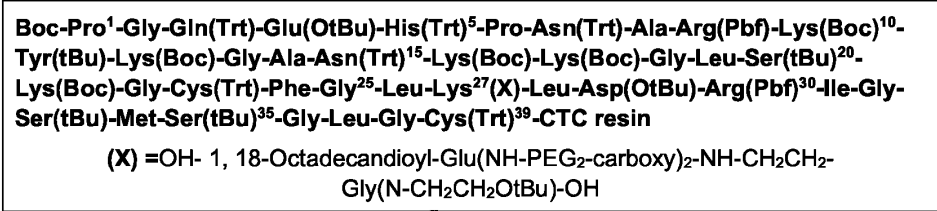
[0369] The synthesis of the CNP Prodrug DS applies SPPS technology, utilizing Fmoc amino acids derivatives as building blocks to assemble the peptide onto a resin (solid support) and Fmoc protected building blocks to assemble the side chain (including the pH responsive self-immolative linker, spacer, and C-18 fatty acid albumin binding domain) out of Lys²⁷. Post-synthesis, the peptide is cleaved from the resin, cyclized, purified and isolated, as a lyophilized powder. A description of the synthetic process is provided in Table 4 and 5.

[0370] The release of all starting materials is done in accordance with ICH Q7. These procedures are part of the standard quality program that ensures the high quality of the peptide and batch-to-batch consistency.

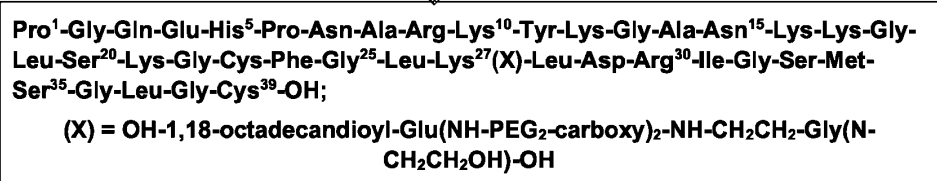
Table 4: Manufacturing Flowchart for CNP Prodrug Drug Substance



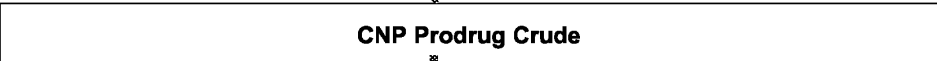
Step 2: SPPS



Step 3: TFA Cleavage

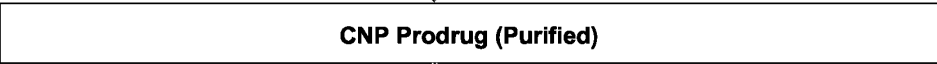


Step 4: Cyclization



Step 5: Purification (TFA system)

Step 6: Salt exchange purification



Step 7: Lyophilization



2-CTC, 2-chlorotrityl chloride; SPPS, Solid-Phase Peptide Synthesis; TFA, trifluoroacetic acid.

Table 5: Description of Manufacturing Process

Step	Process	Description
1	2-Chlorotrityl Chloride Resin Loading	The 2-Chlorotrityl Chloride Resin was swelled (or swollen) in dry DMF and was loaded with Fmoc-Cys(Trt)-OH, in the presence of DIPEA, followed by washing with DMF.

Step	Process	Description
2	SPPS	The peptide chain is built up from the C-terminus to N-terminus by repeated cycles until the resin carries the complete amino acid sequence. • N_α-deprotection with piperidine to enable the coupling reaction • Coupling of protected building block • Acetylation (capping) of remaining free amino groups • Attachment of C18 diacid construct at Lys²⁷ • Dry under reduced pressure and elevated temperature Each cycle consists of the following operations: • Addition of solvent / reagents to the resin • Stirring of the reaction mixture • Removal of solvents/ reagents by filtration and washing
3	TFA Cleavage	Cleavage of the peptide from the resin and simultaneous cleavage of the side-chain protecting groups is accomplished by treatment of the peptide resin with TFA in the presence of suitable scavenger. The product is precipitated in Heptane/MTBE filtered and dried
4	Cyclization	Acyclic crude peptide is cyclized to form disulfide bridge between Cys ²³ and Cys ³⁹ with iodine and then quenched with ascorbic acid
5	Purification (TFA system)	The crude cyclized product is dissolved in a diluent containing TEAP, ACN and Purified water and is purified by HPLC, on a reversed-phase column, with ACN gradient elution and UV detection. The collected fractions are analyzed by HPLC and pooled accordingly
6	Salt exchange purification	A HCl, ACN and purified water system is used for the salt exchange by HPLC
7	Lyophilization	The solution from step 6 is filtered through a 0.2 μm filter and lyophilized, to yield to the product

ACN, acetonitrile; DIPEA, diisopropylethylamine; DMF, dimethylformamide; Fmoc, *N*-α-9-fluorenylmethoxycarbonyl; HPLC, high-performance liquid chromatography; ; MTBE, methyl tert-butyl ether; SPPS, Solid-Phase Peptide Synthesis; TEAP, triethylammonium phosphate; TFA, trifluoroacetic acid; UV, ultraviolet.

Characterization

[0371] The structure of CNP Prodrug has been elucidated by various analytical techniques (Table).

Table 6: Structure Elucidation of CNP Prodrug

Analytical Technique	Structure characteristics
Chemical Structure by Nuclear magnetic resonance	Consistent with assigned structure and functional groups
Chiral analysis of Amino Acids by GC-Mass spectrometry	The results confirmed the intended identity and optical purity of the amino acids.
Molecular mass and CNP Prodrug sequence (MALDI-TOF MS-MS)	The results are consistent with the intended monoisotopic mass and sequence of CNP Prodrug. This includes the peptide sequence of the CNP active moiety.
Identity and amount of the counter-ion by IC	The identity of chloride was confirmed.

GC, gas chromatography; IC, ion chromatography; MALDI, matrix-assisted laser desorption/ionization; MS, mass spectrometry; TOF, time of flight.

Biological Activity

[0372] CNP Prodrug is inactive as a prodrug of CNP. Hydrolysis of the linker forms the active parent, CNP, and inactive metabolites consisting of the lipid and hydrolyzed linker. Pharmacodynamics of CNP Prodrug is being evaluated in mice and NHPs to demonstrate the dose response effect of CNP is retained when active CNP is released continuously from its albumin bound inactive prodrug form into the circulation compared to daily pulsatile delivery of CNP. Completed studies demonstrated the expected dose responsive endochondral skeletal growth effect of CNP is retained with CNP Prodrug treatment, as shown by increases in cellularity and height of the hypertrophic zone of the growth plate and/or increased length of long bones.

CNP Prodrug Drug Substance Preliminary Specification and Batch Analysis Results

[0373] The controls on residual solvents and elemental impurities will be based on ICH Q3C and ICH Q3D. Obligatory tests will be based on ICH Q6A. For the clinical batch analysis, a reference material was prepared and characterized to be used for reversed phase ion-pair high performance liquid chromatography mass spectrometry purity and impurity testing. Preliminary batch release data are provided herein.

CNP Prodrug Drug Substance Stability Protocol

[0374] The stability of non-GMP (nonclinical DS batches) and GMP (clinical DS batches) are obtained as per ICH Q1A conditions. The purity method used in analysis of stability samples will be confirmed as stability indicating by a forced degradation study performed as per ICH Q1A. The stability data will be evaluated as per ICH Q1E for setting of a retest date at the long-term storage condition which is $-20 \pm 5^{\circ}\text{C}$. Additional stability data will be

obtained at intermediate condition of $5 \pm 3^{\circ}\text{C}$ and accelerated condition of $25 \pm 2^{\circ}\text{C}/60 \pm 5\%$ relative humidity.

[0375] The stability protocol for CNP Prodrug DS is provided in Tables 7-9.

Stability Data Summary

[0376] Up to 9 months representative stability data are currently available for non-GMP batch. The results show the DS is stable at both $-20 \pm 5^{\circ}\text{C}$ and $5 \pm 3^{\circ}\text{C}$ for 9 months. Stability studies at $25 \pm 2^{\circ}\text{C}/60\% \pm 5\%$ RH conditions show elevated impurities and an increase in moisture content over 6 months. However, based on these results it is expected that the DS will be stable for at least 12 months.

Table 7: CNP Prodrug Drug Substance Stability Protocol – Long Term (-20 ± 5°C)

Test Parameter	Method	Testing ^a	Time Points									
			Months									
			0	1	3	6	9	12	18	24	36	48
Appearance	Visual	White to off-white powder	x	x	x	x	x	x	x	x	x	x
Purity	RP-UPLC	Purity: ≥ 95% by Area	x	x	x	x	x	x	x	x	x	x
		CNP Content: TBD	x	x	x	x	x	x	x	x	x	x
		Total Impurities: ≤ 5%	x	x	x	x	x	x	x	x	x	x
		Report impurities ≥ 0.10% by RRT	x	x	x	x	x	x	x	x	x	x
Water Content	Karl Fischer	TBD	x	x		x		x		x	x	x

CNP, C-type natriuretic peptide; GMP, Good Manufacturing Practices; RP-UPLC, reversed phase ultra performance liquid chromatography; TBD, to be determined.
^a Testing applies to GMP Batches only.

Table 8: Drug Substance Stability Protocol for Accelerated Storage Condition (5 ± 3 °C)

Test Parameter	Method	Testing ^a	Time Points							
			Months							
			0	1	3	6	9	12	18	24
Appearance	Visual	White to off-white powder	x	x	x	x	x	x	x	x
Purity	RP-UPLC	Purity: ≥ 95% by Area	x	x	x	x	x	x	x	x
		CNP Content: TBD	x	x	x	x	x	x	x	x
		Total Impurities: ≤5%	x	x	x	x	x	x	x	x
		Report impurities ≥ 0.10% by RRT	x	x	x	x	x	x	x	x
Water Content	Karl Fischer	TBD	x	x		x		x		x

CNP, C-type natriuretic peptide; GMP, Good Manufacturing Practices; RP-UPLC, reversed phase ultra performance liquid chromatography; TBD, to be determined.

^a Testing apply to GMP Batches only.

Table 9: Drug Substance Stability Protocol for Accelerated Storage Condition (25 ± 2 °C/60% ± 5% RH)

Test Parameter	Method	Testing ^a	Time Points			
			Months			
			0	1	3	6
Appearance	Visual	White to off-white powder	x	x	x	x
Purity	RP-UPLC	Purity: ≥ 95% by Area	x	x	x	x
		CNP Content: TBD	x	x	x	x
		Total Impurities: ≤5%	x	x	x	x
		Report impurities ≥ 0.10% by RRT	x	x	x	x
Water Content	Karl Fischer	TBD	x	x	x	x

CNP, C-type natriuretic peptide; GMP, Good Manufacturing Practices; RP-UPLC, reversed phase ultra performance liquid chromatography; TBD, to be determined.

^a Testing apply to GMP Batches only.

CNP Prodrug Drug Product**CNP Prodrug Compounding****Table 10-1: Drug Substance Characteristics**

Name:	CNP Prodrug HCl Salt
Appearance:	White to off-white powder
Purity Factor P (%):	81.3%
CNP equivalence Factor B 1	0.827
Hygroscopicity:	Very hygroscopic
Storage and handling requirements:	-20°C or lower Protect from light and moisture
OHC:	Category 3a (Occupational Exposure Limit: 1-10µg/m ³)

¹ CNP:CNP Prodrug used to determine the amount of c-type natriuretic peptide (CNP) equivalents in the CNP Prodrug DS

Table 10-2: Drug Product Inactive Ingredients

Component	CAS No.	Grade	Molar Mass (g/mol)
L-Histidine	71-00-1	Ph. Eur., ChP, JP, USP	155.16
Histidine Monohydrochloride Monohydrate	5934-29-2	Ph. Eur., BP, ChP, JP	209.63
Trehalose Dihydrate	6138-23-4	Ph. Eur., ChP, JP, NF	378.33
D-Mannitol	69-65-8	Ph. Eur., BP, ChP, JP, USP	182.17
L-Methionine	63-68-3	Ph. Eur., BP, JP, USP	149.21
Polysorbate 80	9005-65-6	Ph. Eur., JP, NF	1310
Hydrochloric Acid	7647-01-0	Ph. Eur., BP, JP, NF, ChP	36.46
Sodium hydroxide	1310-73-2	Ph. Eur., BP, JP, NF	40.00

1% (w/v) Polysorbate 80

Preparation for 1% (w/v) Polysorbate 80 is described below in Table 11.

Table 11: 1% (w/v) Polysorbate 80 Preparation Instructions

Name:	1% (w/v) Polysorbate 80	
Composition:		
	Component	Strength (mg/mL)
	Polysorbate 80	10.0
	WFI for Injection	q.s.ad final volume
Procedure:	1. Add Polysorbate 80 to an appropriately sized plastic volumetric flask. 2. Add 90% of the required WFI, then mix gently until a homogenous solution is achieved. 3. q.s.ad to final volume with WFI, then mix gently until a homogenous solution is achieved. 4. Filter using a Polyether Sulfone (PES) membrane with a pore size of 0.2µm or less.	
Appearance:	Clear colorless liquid	
Storage requirements and stability:	2°C to 8°C no more than 2 months	

Table 12: CNP Prodrug Formulation Buffer Preparation Instructions

Name:	CNP Prodrug formulation buffer		
Composition:			
	Component	Strength	
		(mg/mL)	(mM)
	L-Histidine	0.347	2.2
	Histidine Monohydrochloride Monohydrate	1.627	7.8
	Trehalose Dihydrate	58.00	153.3
	D-Mannitol	15.00	82.3
	L-Methionine	0.730	4.9
	Polysorbate 80	0.050	0.04
	WFI for Injection	q.s.ad final volume	q.s.ad final volume

Procedure:	1. Combine all components in a volumetric flask with 90% of the required WFI. 2. Mix until all powders are completely dissolved, and a homogenous solution is achieved. 3. q.s.ad to final volume with WFI, then mix gently until a homogenous solution is achieved. 4. Verify that the pH is 5.5 ± 0.1. Record the pH. 5. Filter using a PVDF membrane with a pore size of $0.22\mu\text{m}$. Discard the flush volume determined based on the filtration study.
Appearance:	Clear colorless liquid
Storage requirements and stability:	2°C to 8°C no more than 2 months

Drug Substance Target Calculation

[0377] Based on the required DP concentration and volume, the DS target amount to weigh is determined using the following equation:

Drug Substance Target Calculation

$$\text{DS Target (mg)} = \frac{\text{DP Target Volume (mL)} \times \text{DP Target Concentration (mg/mL)} \times 100}{\text{Purity Factor P (\%)} \times \text{CNP eq. Factor B}}$$

Note:

- CNP Variant HCl Salt, Purity Factor P = 81.3%
- CNP equivalence Factor B = 0.827

Figure 4.1.2 is an example determining the mass of DS in milligrams needed to prepare 100 mL of DP Solution at a 0.500 mg/mL concentration.

Drug Substance Target Calculation

$$\text{DS Target (mg)} = \frac{100 \text{ (mL)} \times 0.500 \text{ (mg/mL)} \times 100}{81.3 \text{ (\%)} \times 0.827} = 74.4 \text{ mg}$$

Drug Product Solution Preparation Instructions

Component	Strength (mg/mL)
CNP Prodrug HCl salt	Target concentration
Sodium hydroxide	As needed to pH 5.5 ± 0.1
CNP Prodrug Formulation Buffer	q.s.ad final volume

Procedure:

1. Determine DS target amount using the calculation in Figure 4.1.1.
2. Weigh the DS amount determined in Step 1. Record weight.

3. Calculate the FB volume needed to achieve the required DP concentration using the actual DS amount weighted in Step 2 and the following equation:

$$\text{FB Volume (mL)} = \frac{\text{DS Weighed (mg)} \times \text{Purity Factor P (\%)} \times \text{CNP eq. Factor B}}{\text{DP Target Concentration (mg/mL)} \times 100}$$

4. Add 98% to 99% of the calculated CNP Prodrug FB volume from Step 3 to the previously weighed DS in Step 2 while withholding 1-2% of the FB volume to account for pH adjustments in next steps.
Note: The FB volume to be withheld is based on scale; the larger the scale the more volume is withheld.
5. Mix until the DS is completely dissolved, and a homogeneous solution is achieved.
6. Test and record the initial pH.
7. Adjust the pH to 5.5 ± 0.1 using the 1N sodium hydroxide. Record the volume used.
8. Q.s.ad to final volume with CNP Prodrug FB, then mix gently until a homogenous solution is achieved.
9. Verify that the pH is 5.5 ± 0.1 . Record the final pH.

Appearance: clear colorless liquid

Storage requirements/stability: In-use: 25 °C, no more than 6 hours;
Short-term: 2-8 °C, no more than 3 days; and
Long term: -60 °C or less, no more than 1 month.

Description and Composition of the Drug Product

[0378] CNP Prodrug DP is supplied as lyophilized, preservative-free, white to yellow powder for reconstitution with sterile water for injection (WFI). The reconstituted solution is colorless to yellow. The drug product contains 15.7 mg/vial or 47.2 mg/vial of CNP prodrug substance which is equivalent to 13 mg/vial or 39 mg/vial of CNP, respectively. The reconstituted solution contains 10 mg/mL or 30 mg/mL of CNP equivalent with a target pH of 5.5. Sterile WFI will also be supplied for reconstitution. The clinical DP will be supplied in sterile, Type I, single-dose, borosilicate glass vials with coated stopper and flip-off aluminum cap. The formulation buffer contains 10 mM histidine buffer, 58.00 mg/mL trehalose dihydrate, 15.00 mg/mL D-mannitol, 0.73 mg/mL L-methionine and 0.05 mg/mL polysorbate 80. It will be used as placebo for clinical study.

Table 13: Composition of CNP Prodrug Drug Product

Component	Compendial grade	Quantity per mL ^a (mg)	Quantity per vial (mg) ^c		Function
			13 mg/vial ^b	39 mg/vial	
CNP Prodrug Drug Substance (dosage strength is equivalent to CNP)	In-house	10, 30 ^c	15.7 mg	47.2 mg	Active Ingredient
L-Histidine	USP, Ph. Eur., JP	0.35	0.46 mg	0.46 mg	Buffering Agent
Histidine Monohydrochloride Monohydrate	Ph. Eur., JP	1.63	2.12 mg	2.12 mg	Buffering Agent
Trehalose, Dihydrate	USP, Ph. Eur., JP	58.00	75.40 mg	75.40 mg	Bulking/Isotonicity Agent
D-Mannitol	USP, Ph. Eur., JP	15.00	19.50 mg	19.50 mg	Bulking/Isotonicity Agent
L-Methionine	USP, Ph. Eur., JP	0.73	0.95 mg	0.95 mg	Stabilizer
Polysorbate 80	USP, Ph. Eur., JP	0.05	0.065 mg	0.065 mg	Anti-Adsorbent
Water for injection (q.s) ^c	USP, Ph. Eur., JP	1.0	1.3	1.3	Diluent

JP: Japanese Pharmacopoeia; Ph. Eur.: European Pharmacopoeia; q.s., as much as needed; USP: United States Pharmacopeia.

^a Nominal concentration per mL after reconstitution. Each vial is filled to a target of 1.3 mL prior to lyophilization.

^b Amount of CNP Equivalent (Molar ratio of CNP to CNP prodrug is 0.827)

^c Water removed during lyophilization

[0379] The container closure system for the CNP Prodrug DP is compliant with USP <660> and USP <381> (Table 14).

Table 14: CNP Prodrug Drug Product Container Closure System

Component	Description	Quality Standard
Vial	10R Type I borosilicate glass (Topline)	USP, Ph. Eur., JP
Stopper	20 mm ETFE laminated Chlorobutyl Rubber Lyophilization Stopper	USP, Ph. Eur., JP
Flip cap	20 mm Aluminum-plastics Combination Caps	In-house WEST Standard

ERFE, ethylene tetrafluoroethylene; JP, Japanese Pharmacopoeia; Ph. Eur., European Pharmacopeia; USP, United States Pharmacopeia.

Pharmaceutical Development

[0380] The DP being proposed for use in clinical studies is representative to the DP used in the animal toxicology studies that support the established no observed adverse effect level (NOAEL) as described herein. A description of chemistry and manufacturing

differences between the DP proposed for clinical use and the DP used in the animal toxicology studies to establish the NOAEL is provided below.

Table 15: Summary of Drug Product Samples for Nonclinical Studies and Clinical

Buffer Formulation	Manufacturing Process
Pre-clinical formulation was composed of 10 mM histidine, pH 5.5, 48.00 mg/mL Trehalose, 12.40 mg/mL D-Mannitol, 0.73 mg/mL L-methionine, 0.05 mg/mL polysorbate 80	NA – Non-clinical studies utilized DS prepared manually with buffer formulation.
Formulation is described in Tables 12 and 13.	

DS, drug substance; NA, not applicable; TBD, to be determined.

[0381] The formulation development began with a series screening studies using different buffering species and pH levels ranging from pH 3 to pH 8. Based on the outcome of these studies, histidine buffer pH 5.5 was selected. The pH ranging studies indicated impurities are generated in liquid phase and a lyophilization process was selected as stabilization strategy. A series of studies was conducted with varying bulking agents and their concentrations to develop an isotonic and lyophilizable formulation for CNP Prodrug. Trehalose and mannitol with above mentioned amount and ratio (Table) provided pharmaceutically elegant cake structure and isotonic formulation. L-methionine is added to CNP Prodrug formulation to derisk the potential for oxidation while polysorbate-80 is included to minimize adsorption of CNP Prodrug to product contacting material.

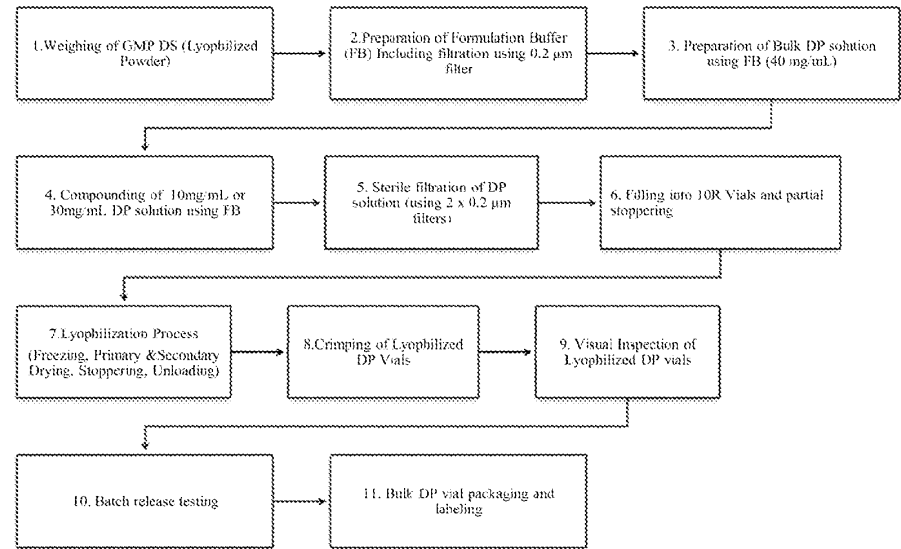
[0382] A preliminary in-use study was conducted, and no risk was detected. Compatibility studies with the manufacturing process components were conducted and no risk was observed. Compatibility studies for the administration components and container closure system are planned and will be available for IND submission.

[0383] Additionally, process development studies including mixing, filterability, and lyophilization cycle development & optimization were conducted to support the manufacturing of CNP Prodrug DP. Using the outcome of these studies, a DP development batch and engineering batch were manufactured to support GMP manufacturing. Both the development batch and engineering batch are on stability. Detailed data on these batches and stability data will be shared during IND submission.

Manufacture

[0384] The manufacturing process is shown in the schematic below and Table 16. and a brief narrative overview is provided which includes controls that represent a combination of manufacturing process parameters, in-process controls/tests, and quality attributes.

CNP Prodrug Drug Product Manufacturing Process Flow Diagram



DP, drug product; DS, drug substance; GMP, Good Manufacturing Practices.

Table 16: Narrative Description of CNP Prodrug Drug Product Manufacturing Process

Step	Process	Description
1	Weighing of GMP DS	DS is equilibrated to room temperature before use. Required amount of DS is weighed after equilibration.
2.	Preparation of formulation buffer (FB)	Formulation buffer components are added to 80% of the required WFI volume in the appropriate container. The solution is stirred for not less than 30 min. The FB solution is filtered into a sterile container.
3.	Preparation of bulk DP solution using FB (40 mg/mL)	FB is added to previously weighed DS to prepare a 40 mg/mL concentrated stock DP solution.
4.	Compounding of DP solution (10 mg/mL) or (30 mg/mL)	Bulk DP solution is diluted to the target concentration using FB. All single-use components are aseptically connected to the filter assembly under the LAF.
5.	Sterile filtration	DP solution is filtered through sterile filters into the sterile bag in preparation for vial filling.
6.	Filling of DP	Vials are filled to target fill volume by weight using density of the DP solution. The filling involves 100% weight check. Vials with acceptable weights are automatically transferred downstream, while rejected vials are automatically removed from the line.
7.	Lyophilization process (Freezing, Primary & Secondary Drying, Stoppering, Unloading)	Filled vials are partially stoppered and loaded onto the lyophilizer. The lyophilization recipe is programmed and executed. After the cycle is completed, all vials are stoppered and unloaded from the lyophilizer.
8.	Crimping of lyophilized DP vials	Lyophilized vials are transferred to the capping area. The capping recipe is activated, and capping process is completed.

Step	Process	Description
9.	Visual inspection of lyophilized DP vials	Lyophilized DP vials are visually inspected to ensure compliance
10.	Batch release testing	Lyophilized DP samples are tested to release the batch. After release, DP samples are put in the appropriate stability chambers for stability study.
11.	Bulk DP packaging and labeling	After batch release, bulk DP is packed in the appropriate container and labeled.

DP, drug product; DS, drug substance; GMP, Good Manufacturing Practices; LAF, laminar air flow; WFI, water for injection.

[0385] The drug product manufacturing process is controlled and monitored carefully to ensure consistency of product composition and quality of the drug product to ensure product safety and efficacy.

Drug Product Specification

[0386] The proposed DP specifications are provided in Table 17:

[0387] Table 23:

Summary of Drug Substance

Batches
Stability Storage Conditions
-20 ± 5°C 5 ± 3°C 25 ± 2°C /60 ± 5% RH
-20 ± 5°C 5 ± 3°C 25 ± 2°C /60 ± 5% RH

RH, relative humidity.

Table 24: CNP Prodrug Drug Substance Batch Analysis

Test	Method	Sample 1	Sample 2
Identity by molecular weight	MS	4959.7 m.u.	4959.3 m.u.
Identity	RP-UPLC	Complies	Complies
Appearance	Visual Inspection	Complies	Complies
CNP Prodrug purity	RP-UPLC	88.2%	96.2%
Total Impurities		11.8% ^b	3.8% ^c
Free CNP		ND	0.45%
CNP Prodrug Content	Elemental Analysis	92.9%	92.4%
Water Content	KF USP <921> Ph. Eur. 2.5.32	3.7%	4.0%
Trifluoroacetic acid content	RP-HPLC	ND (< 0.02%)	ND (< 0.02%)
Residual solvents	GC USP < 467 >	< 1500 ppm < 200 ppm < 2500 ppm	< 300 ppm 111 ppm < 500 ppm
Pd content	ICP-MS USP < 233 >	0.73 ppm	0.27 ppm

Test	Method	Sample 1	Sample 2
Chloride content	Ion Chromatography	3.7%	3.7%
Bacterial endotoxins (LAL)	USP <85> Ph. Eur. 2.6.14	< 0.01 EU/mg	< 0.01 EU/mg
Bioburden	USP <61> Ph. Eur. 2.6.12	TAMC < 9 cfu/g TYMC < 10 cfu/g	TAMC < 9 cfu/g TYMC < 9 cfu/g

CNP, C-type natriuretic peptide; GC, gas chromatography; GMP, Good Manufacturing Practices; ICP-MS, inductively coupled plasma mass spectrometry; KF, Karl Fischer; LAL, Limulus Amebocyte Lysate; MS, mass spectrometry; ND, not detected; Ph. Eur., European Pharmacopeia; RP-HPLC, reversed phase high performance liquid chromatography; RP-UPLC, reversed phase ultra performance liquid chromatography; RRT, relative retention time; USP, United States Pharmacopeia; w/w, weight/weight.

^a Total impurities includes impurities $\geq 0.05\%$ by area.

^b Total impurities includes impurities $\geq 0.10\%$ by area per the DS release acceptance criteria for clinical material.

[0388] Table

[0388] 18 depicts the specifications are based on the available knowledge of the product.

All impurities > 0.10% in area by purity method RP-UPLC are monitored on release and stability.

Drug Product Stability

[0389] Stability studies are ongoing on a non-GMP engineering batch and a GMP batch will be placed on stability once manufactured. The stability studies will be performed using principles from ICH Q1A and stability data will be evaluated using principles per ICH Q1E. The stability protocols for the CNP Prodrug DP are provided in Table 18 and Table . It is intended that the stability data obtained from the engineering batch will be used to support the stability of the clinical DP through the duration of the clinical study. The DP in-process tests provide adequate control of the process for both engineering and clinical batches. There are no manufacturing process differences and container closure differences between engineering and GMP batches.

Table 19: CNP Prodrug Drug Product Stability Protocol – Long Term ($5 \pm 3^\circ\text{C}$)

Test Parameter	Method	Tests ^a	Time Points								
			Months								
			0	3	6	9	12	18	20 ^a	24	36
Appearance	Visual inspection	Lyophilized: White to Light Yellow	x	x	x	x	x	x	x	x	x
		Reconstituted in water: Clear, colorless to yellow liquid									

Test Parameter	Method	Tests ^a	Time Points									
			Months									
			0	3	6	9	12	18	20 ^a	24	36	
Peptide concentration upon reconstitution in water	UV absorbance	10.0 mg/mL (9.0-11.0) and 30.0 mg/mL (27.0-33.0) (CNP equivalent)	x	x	x	x	x	x	x	x	x	
Water Content	Karl Fisher USP <921> Ph. Eur. 2.5.32	TBD	x	x	x	x	x	x	x	x	x	
Peptide Purity	RP-UPLC	Purity: ≥ 90% area	x	x	x	x	x	x	x	x	x	
		Total impurities: ≤ 10% Area	x	x	x	x	x	x	x	x	x	
		Specific impurity (CNP): TBD	x	x	x	x	x	x	x	x	x	
		Other related substances: Report RRT and % area for all peaks ≥ 0.10% area for other related substances.	x	x	x	x	x	x	x	x	x	
pH upon reconstitution in water	USP <791> Ph. Eur. 2.2.3	5.0 – 6.0	x	x	x	x	x	x	x	x	x	
Reconstitution Time	Direct measurement	≤ 300 seconds	x	x	x	x	x	x	x	x	x	
Visible Particulate upon reconstitution in water	USP <790> Ph. Eur. 2.9.20	Essentially free of visible particulates	x	x	x	x	x	x	x	x	x	
Deamidation	SCX-HPLC	Report % area	x	x	x	x	x	x	x	x	x	
Multimer	SEC-HPLC	Report % area	x	x	x	x	x	x	x	x	x	
Particulate Matter upon reconstitution in water	USP <788> Ph. Eur. 2.9.19	≤ 6000 particles/vial at ≥ 10 µm ≤ 600 particles/vial at ≥ 25 µm	x				x		x	x	x	

Test Parameter	Method	Tests ^a	Time Points								
			Months								
			0	3	6	9	12	18	20 ^a	24	36
Container closure integrity ¹	USP <381> Ph. Eur. 3.2.9	No ingress	x ^b				x		x	x	x

CNP, C-type natriuretic peptide; GMP, Good Manufacturing Practices; HPLC-CAD, high performance liquid chromatography with charged aerosol detection; Ph. Eur.: European Pharmacopoeia; RP-UPLC, reversed phase ultra performance liquid chromatography; RRT, relative retention time; SCX-HPLC, strong cation exchange high performance liquid chromatography; SEC-HPLC; size exclusion high performance liquid chromatography; TBD, to be determined; USP: United States Pharmacopeia; UV, ultraviolet.

^a Tests applies to GMP Batches only.

^b Container closure integrity at the T0 is confirmed by a passing sterility result at release.

Table 20: Drug Product Stability Protocol for Accelerated Storage Condition (25 ± 2 °C / 60 ± 5%RH)

Test Parameter	Method	Tests ^a	Time Points			
			Months			
			0	1	3	6
Appearance	Visual inspection	Lyophilized: White to Light Yellow	x	x	x	x
		Reconstituted in water: Clear, colorless to yellow liquid				
Peptide concentration upon reconstitution in water	UV absorbance	10.0 mg/mL (9.0-11.0) and 30.0 mg/mL (27.0-33.0) (CNP equivalent)	x	x	x	x
Water Content	Karl Fisher USP <921> Ph. Eur. 2.5.32	TBD	x	x	x	x
Peptide Purity	RP-UPLC	Purity ≥ 90% area	x	x	x	x
		Total impurities ≤ 10% Area	x	x	x	x
		Specific impurity (CNP) TBD	x	x	x	x
		Other related substance: Report RRT and % area for all peaks ≥ 0.10% area for other related substances.	x	x	x	x
pH upon reconstitution in water	USP <791> Ph. Eur. 2.2.3	5.0-6.0	x	x	x	x
Reconstitution Time	Direct measurement	≤ 300 seconds	x	x	x	x
Visible Particulate upon reconstitution in water	USP <790> Ph. Eur. 2.9.20	Essentially free of visible particulates	x	x	x	x
Deamidation	SCX-HPLC	Report % area	x	x	x	x
Multimer	SEC-HPLC	Report % area	x	x	x	x
Particulate Matter upon reconstitution in water	USP <788> Ph. Eur. 2.9.19	≤ 6000 particles/vial at ≥ 10 µm ≤ 600 particles/vial at ≥ 25 µm	x			x
Container closure integrity	USP < 381 > Ph. Eur. 3.2.9	No ingress	x ^b			x

CNP, C-type natriuretic peptide; GMP, Good Manufacturing Practices; HPLC-CAD, high performance liquid chromatography with charged aerosol detection; Ph. Eur., European Pharmacopeia; RP-UPLC, reversed phase ultra performance liquid chromatography; RRT,

relative retention time; SCX-HPLC, strong cation exchange high performance liquid chromatography; SEC-HPLC; size exclusion high performance liquid chromatography; TBD, to be determined; USP, United States Pharmacopeia; UV, ultraviolet.

^a Tests applies to GMP Batches only.

^b Container closure integrity at the T0 is confirmed by a passing sterility result at release.

Manufacturing Process

[0390] This example demonstrates a process for preparing a process for manufacturing CNP Prodrug, including linear chain and side chain assembly by solid phase synthesis (SPPS), TFA cleavage/deprotection, in-solution cyclization by I₂, reverse phase chromatography (RPC) purification and salt exchange, is described below:

Manual solid phase synthesis (SPPS)

[0391] A manual SPPS of CNP Prodrug was performed on chlorotriyl chloride (CTC) resin. The table below described the coupling time, amino acid equivalent, Fmoc deprotection time, and AA residues acetylation that was needed during chain expansion.

Table 21. SPPS of CNP Prodrug

	<u>Fmoc-AA</u>	<u>AA eq</u>	<u>Coupling (h)</u>	<u>DeFmoc* (min)</u>	<u>Ac</u>	<u>BV (height,cm)</u>	<u>Comments</u>
1	Cys(Trt)	1	ON	/		2.3	
2	Leu-Gly	2	ON	5/20		2.8	
3	Gly	2	5.5	5/20	Y	3.0	
4	Ser(tBu)	2	5	5/20		3.4	
5	Met	2	ON	5/20	Y	3.6	
6	Gly-Ser Psi(Me,Me,)pro	1.5	4.5	5/20	Y	4.2	
7	Ile	2	ON	5/20		4.6	
8	Arg(Pbf)	2	5.5	5/25		5.3	
9	Asp(OtBu)	2	4.5	5/25		5.4	
10	Leu	2	ON	5/25		5.8	
11	Lys(Alloc)	2	5	10/25		5.9	
12	Leu	2	ON	10/25		5.9	
13	Gly	2	4.5	10/25	Y	6.0	
14	Phe	2	ON	10/25		6.1	
15	Cys(Trt)	2	5	10/25		6.1	
16	Gly	2	ON	10/30	Y	5.5	
17	Lys(Boc)	2	5	10/35		5.9	
18	Leu-Ser Psi(Me,Me,)pro	1.5	ON	10/30	Y	6.1	
19	Gly	2	4	5/30	Y	6.1	
20	Lys(Boc)	2	9	10/30		6.15	
21	Lys(Boc)	2	ON	5/30			
22	Ans(Trt)	2	4	10/30			
23	Ala	2	5	5/30		8.4	
24	Gly	2	4	10/30	Y	8.4	
25	Lys(Boc)	2	ON	10/30		8.5	
26	Tyr(tBu)	2	4	10/30		8.6	
27	Lys(Boc)	2	ON	10/30		8.6	

28	Arg(Pbf)	2	4.75	10/30		8.4	
29	Ala	2	ON	10/35		~7 (decrease BV)	
30	Asn(Trt)	2.5	4	10/40		8.6	
31	Pro	2.5	7	10/40			
32	His(Trt)	2	8.5	10/40		9.5	DEPBT
33	Glu(OtBu)	2.5	4.5	10/40		9.5	
34	Gln(Trt)	2	8	10/40		9.6	
35	Gly	2.5	4.5	10/40	Y	9.6	
36	Pro	2	ON	10/40		9.6	
37	Bocylation**	5	2	10/40		9.6	
38	Hydrolyzable linker	1.5	ON	Alloc removal	Y	9.6	Pd/PPh ₃ /DMBA in DMF 2 treatments, 2h +1h
39	AEEA	2	ON	10/40		9.6	
40	AEEA	2	7.5	10/40	Y	9.7	
41	gGlu-OtBu	2	5	10/40		10.0	
42	tBu-C18 diacid	2	ON	10/40		10.2	

*20% Piperidine/DMF with 0.1M Oxyma

** 5 eq Boc₂O +3eq DIPEA, 2 hours

General coupling protocol:

- AA:DIC:Oxymapure=1:1.1:1 and AA was dissolved in DMF at 0.2-0.3 M concentration
- 0.1 M Oxymapure was added to the Fmoc removal steps to minimize Cys racemization during base treatment.
- Preactivation for 0.5-1 hour for AA coupling solution except for Arg, Cys, His, and Met.
- Fmoc-Lys(Alloc)-OH was used at A-11 for side chain deprotection prior to side chain assembly.
- Alloc removal used Pd(PPh₃)₄ /PPh₃/DMBA (0.1eq:0.5eq:5eq in DMF)
- DEPBT was used for His coupling to suppress racemization (His:DEPBT:DIPEA = 2:2.2:3-3.5, pH~9)
- Single AA coupling strategy was employed for the side chain assembly.
- N₂ blanketing was applied throughout the SPPS to minimize Cys and Met oxidation.

1. Cleavage and Cyclization

[0392] For the cleavage, the following conditions were used: TFA: DTT : H₂O : TIPS : NH₄I = 87:7.5:2.5:2.5:0.5, 3 hours at room temperature under nitrogen atmosphere. Reverse precipitation was then performed by adding the cleavage mixture (cooled to 1-15°C) to 4V of 25 % n-Heptane/MTBE (pre-cooled to 4°C) over 15 min.

[0393] The bulk crude was then dissolved in HOAc+20% ACN at 30 mg/mL for 30 minutes. 2% I₂ in ACN was added to start oxidation till orange color persisted then quenched by 1% ascorbic acid/H₂O after completion.

2. Purification

[0394] In addition to a first reverse purification on C18 media (A: 0.1M TEAP pH 2.3 in H₂O, B: 0.1M TEAP pH 2.3 in ACN, 25-45% B in 100 min), a second purification/ salt exchange was implemented in order to convert the TFA salt to an HCl salt media (A: 0.02% HCl in H₂O, B: 0.02% HCl in ACN, 10-15% B in 3 min, 15-50% B in 75 min).

3. Lyophilization

[0395] The final combined pool is filtered through 0.2 µm filter and lyophilized. Typical yield: 6 to 7.5%

Procedure for manufacture

Drug Substance – Additional Information

[0396] The synthesis of the CNP Prodrug peptide applies Solid-Phase Peptide Synthesis (SPPS) technology, applying *N*-a-9-fluorenylmethoxycarbonyl (Fmoc) amino acids derivatives as building blocks to assemble the peptide onto a resin (solid support) and Fmoc protected building block to assemble the side chain out of Lys 27. Post-synthesis, CNP Prodrug drug substance (DS) is cleaved from the resin, cyclized, purified and isolated, as a lyophilized powder.

[0397] The specifications for release and stability testing will conform to the general principles of the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Q6A for new DS.

[0398] The acceptance criterion for purity of clinical batches is proposed to set to ≥ 95% area, with total impurities (≤ 5%).

[0399] Relevant ICH guidelines and United States Pharmacopeia (USP)/European Pharmacopoeia (Ph. Eur.) monographs were consulted for setting acceptance criteria for residual solvents (ie, ICH Q3C), elemental impurities (ie, ICH Q3D, and other elements were included for monitoring purposes only), microbiological quality (ICH Q4B), and bacterial endotoxins (ICH Q4B).

[0400] The endotoxin limit for CNP Prodrug DS based on the USP <85> and Ph. Eur. chapter 5.1.10 for parenteral products for intravenous (IV) administration. The endotoxin level for the CNP Prodrug drug product (DP) will also be controlled based on the clinical dosage and administration route to ensure product safety. The acceptance criteria for bioburden follow USP <61>.

[0401] In conclusion, the selected test parameters and release acceptance criteria are considered appropriate to ensure the correct identity, suitable chemical/microbial purity, and reliable assay value of the CNP Prodrug DS at this stage of clinical development.

Drug Product – Additional Information

[0402] Test parameters and acceptance criteria for release and shelf-life of the CNP Prodrug DP were selected to confirm the identity, purity, quality, and to ensure the product's safety. The acceptance criteria have been developed and justified based on limited manufacturing experience and the general principles of ICH Q6A. The relative retention time and % area of all impurities at $\geq 0.10\%$ will be reported and monitored on release and stability testing. The tests for pH, sterility, endotoxins, osmolality, container content, particulate matter, and container closure integrity are conducted according to ICH Q6A guidelines for parenteral products, and their acceptance criteria are established based on all applicable guidance documents, relevant USP chapters for sterile products, and current manufacturing understanding.

Table 23: Summary of Drug Substance Batches

Stability Storage Conditions
-20 $\pm$ 5°C 5 $\pm$ 3°C 25 $\pm$ 2°C /60 $\pm$ 5% RH
-20 $\pm$ 5°C 5 $\pm$ 3°C 25 $\pm$ 2°C /60 $\pm$ 5% RH

RH, relative humidity.

Table 24: CNP Prodrug Drug Substance Batch Analysis

Test	Method	Sample 1	Sample 2
Identity by molecular weight	MS	4959.7 m.u.	4959.3 m.u.
Identity	RP-UPLC	Complies	Complies
Appearance	Visual Inspection	Complies	Complies
CNP Prodrug purity	RP-UPLC	88.2%	96.2%
Total Impurities		11.8% ^b	3.8% ^c
Free CNP		ND	0.45%
CNP Prodrug Content	Elemental Analysis	92.9%	92.4%
Water Content	KF USP <921> Ph. Eur. 2.5.32	3.7%	4.0%
Trifluoroacetic acid content	RP-HPLC	ND (< 0.02%)	ND (< 0.02%)

Test	Method	Sample 1	Sample 2
Residual solvents	GC USP < 467 >	< 1500 ppm	< 300 ppm
		< 200 ppm	111 ppm
		< 2500 ppm	< 500 ppm
Pd content	ICP-MS USP < 233 >	0.73 ppm	0.27 ppm
Chloride content	Ion Chromatography	3.7%	3.7%
Bacterial endotoxins (LAL)	USP <85> Ph. Eur. 2.6.14	< 0.01 EU/mg	< 0.01 EU/mg
Bioburden	USP <61> Ph. Eur. 2.6.12	TAMC < 9 cfu/g	TAMC < 9 cfu/g
		TYMC < 10 cfu/g	TYMC < 9 cfu/g

CNP, C-type natriuretic peptide; GC, gas chromatography; GMP, Good Manufacturing Practices; ICP-MS, inductively coupled plasma mass spectrometry; KF, Karl Fischer; LAL, Limulus Amebocyte Lysate; MS, mass spectrometry; ND, not detected; Ph. Eur., European Pharmacopeia; RP-HPLC, reversed phase high performance liquid chromatography; RP-UPLC, reversed phase ultra performance liquid chromatography; RRT, relative retention time; USP, United States Pharmacopeia; w/w, weight/weight.

^a Total impurities includes impurities $\geq 0.05\%$ by area.

^b Total impurities includes impurities $\geq 0.10\%$ by area per the DS release acceptance criteria for clinical material.

Table 25: CNP Prodrug Drug Product Preliminary Specification

Test		Method	Testing
Identity		RP-UPLC	Coelute with the reference standard
Appearance		Visual inspection	Lyophilized: White to Light Yellow
			Reconstituted in water: Clear, colorless to yellow liquid
Visible Particulate ¹		USP <790> Ph. Eur. 2.9.20	Essentially free of visible particulates
Peptide concentration		UV absorbance	9.0 – 11.0 mg/mL (10 mg/mL) (CNP equivalent) 27.0 – 33.0 mg/mL (30 mg/mL) (CNP equivalent)
Water Content		Karl Fisher USP <921> Ph. Eur. 2.5.32	TBD
CNP Prodrug Purity	Purity	RP-UPLC	≥ 90% Area
	Total impurities		≤ 10% Area
	Specific Impurity (CNP)		TBD
	Other related substance		Report RRT and % area for all peaks ≥ 0.10% area for other related substances.

Test	Method	Testing
Osmolality ¹	USP <785> Ph. Eur. 2.2.35	240 - 380 mOsmol/kg
pH ¹	USP <791> Ph. Eur. 2.2.3	5.0 - 6.0
Polysorbate 80 Content	HPLC-CAD	25 - 75 µg/mL
Endotoxin Content ¹	USP <85> Ph. Eur. 2.6.14	≤ 59 EU/mL (10 mg/mL) ≤ 177 EU/mL (30 mg/mL)
Sterility	USP <71> Ph. Eur. 2.6.1	No growth
Particulate Matter ¹	USP <788> Ph. Eur. 2.9.19	≤ 6000 particles/vial at ≥ 10 µm ≤ 600 particles/vial at ≥ 25 µm
Reconstitution Time	Direct measurement	≤ 300 seconds
Uniformity of dosage units	USP <905> Ph. Eur. 2.9.40	Conforms
Deamidation	SCX-HPLC	Report % area
Multimer content	SEC-HPLC	Report % area

CNP, C-type natriuretic peptide; HPLC-CAD, high performance liquid chromatography with charged aerosol detection; Ph. Eur., European Pharmacopeia; RP-UPLC, reversed phase ultra performance liquid chromatography; RRT, relative retention time; SCX-HPLC, strong cation exchange high performance liquid chromatography; SEC-HPLC; size exclusion high performance liquid chromatography; USP, United States Pharmacopeia; UV, ultraviolet; TBD, to be determined.

¹ Lyophilized DP is reconstituted in water prior to testing.

Table 26: Overview of CNP Prodrug Non-clinical Studies

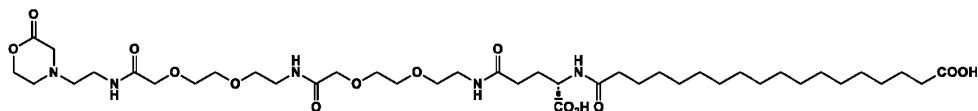
Type of Study	Test System	
Pharmacology		
Ligand affinity assay	Human NPR-B and NPR-C	
Skeletal growth in juvenile WT mice	Male C57BL/6J mice	
Skeletal growth in juvenile WT mice	Male C57BL/6NCrl mice	
Pharmacodynamics in juvenile WT or Noonan (RAF1 L613V) mice	Male Raf1L613V/+ or WT mice	
Pharmacodynamics in WT or Noonan (RIT1 M90I) mice	Male Rit1+/- or WT mice	
1-month repeat-dose pharmacodynamics and cardiovascular safety pharmacology with repeat-dose PK in NHPs	Male cynomolgus monkeys	
GLP acute toxicity and cardiovascular safety pharmacology	Male and female cynomolgus monkeys	
Pharmacokinetics		
Single-dose PK in mice	Male C57BL/6J mice	
Single-dose PK in adult rats	Male and female SD rats	
Single-dose PK in NHPs	Male and female cynomolgus monkeys	
1-month repeat-dose pharmacodynamics and cardiovascular safety pharmacology with repeat-dose PK in NHPs	Male cynomolgus monkeys	
Albumin binding affinity assay	Albumin	
CNP Prodrug hydrolysis in buffer	Phosphate buffered saline, pH 7.4	
Toxicology		
GLP acute toxicity and cardiovascular safety pharmacology with repeat-dose PK in NHPs	Male and female cynomolgus monkeys	
1-month repeat-dose pharmacodynamics	Male cynomolgus monkeys	

GLP, Good Laboratory Practice; NHP, non-human primate; NPR, natriuretic peptide receptor; PK, pharmacokinetics; SD, Sprague-Dawley; WT, wild-type.

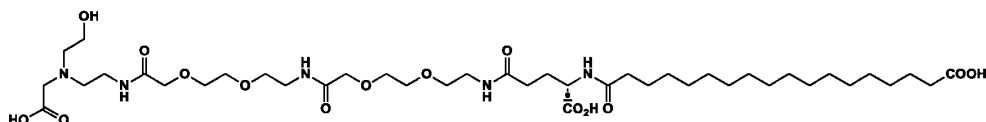
Metabolites

[0403] CNP Prodrug is comprised of CNP bound via the Lys²⁷ side chain to a pH responsive self-immolative linker, a spacer, and C-18 fatty acid albumin binding domain. Upon hydrolysis of the linker, active CNP is formed along with 2 major pharmacologically inactive metabolite isoforms consisting of the albumin-binding C-18 fatty acid and hydrolyzed linker, referred to as Metabolites A and B, respectively. The structure of both inactive metabolite isoforms is shown below.

Structure of Metabolite A



Structure of Metabolite B



[0404] Both Metabolites A and B are expected to be formed collectively *in vivo* in NHPs at a similar proportion to CNP Prodrug as in humans as the rate of CNP Prodrug hydrolysis is constant at physiologic pH. Therefore, the toxicity of each metabolite isoform will be evaluated as part of general toxicity studies of CNP Prodrug. Metabolite A is unstable and rapidly forms Metabolite B by hydrolysis during synthesis and purification; therefore, the major metabolite isoform likely to be detected in plasma of both humans and nonclinical species is Metabolite B. Both metabolite isoforms will be evaluated using 2 *in silico* approaches.

[0405] As genetic toxicology studies of CNP Prodrug are not planned, Metabolite B will be further evaluated in an *in vitro* bacterial reverse mutation assay and an *in vitro* micronucleus assay. Metabolite A is too unstable to produce, as it spontaneously forms Metabolite B, so will not be evaluated in genetic toxicology studies.

[0406] Additional metabolite identification and characterization of metabolite toxicity and absorption, distribution, metabolism, and excretion properties will be conducted, as appropriate, in accordance with FDA Guidance on *Safety Testing of Drug Metabolites* (March 2020).

[0407] Inhibition of CYP enzymes (e.g., CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6 and CYP3A4/5 enzymes) in human liver microsomes by CNP Prodrug or Metabolite B were evaluated *in vitro*. Neither CNP Prodrug nor Metabolite B inhibited the activity of CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, or CYP3A4/5 *in vitro* at concentrations up to 20 μ M. For each tested CYP, no direct, time-dependent (30-minute pre-incubation), or metabolism dependent (30-minute pre-incubation with NADPH) inhibition of activity was observed for either CNP Prodrug or Metabolite B. Evaluation of inhibition of

CYP enzymes (e.g., CYP1A2, CYP2D6 and CYP3A4 enzymes) in human liver microsomes) in cultured human hepatocytes by CNP Prodrug or Metabolite B are ongoing.

[0408] *In silico* screening assessment of Metabolites A and B were conducted by means of Quantitative Structure activity Relationship (QSAR) evaluation. The *in silico* analysis did not identify any hazards relevant to administration of CNP Prodrug. Neither Metabolite A nor B is likely to be mutagenic based on QSAR analysis.

[0409] Evaluations of *in vitro* transporter substrate assessment of CNP Prodrug and lipid-linker in different cell lines are ongoing.

Phase 1 Study

[0410] BioMarin plans to initiate a comprehensive clinical development program, including a Phase 1 study in healthy adult volunteers followed by Phase 2 dose-range finding and a pivotal Phase 3, to support approval of CNP Prodrug in pediatric patients with ACH.

Example 24: Pharmacodynamics of CNP Prodrug

[0411] CNP Prodrug is a pharmacologically inactive prodrug of CNP, that does not bind to NPR-B (internal data). Hydrolysis of the linker forms the active parent, CNP, and inactive metabolite isoforms consisting of the lipid and hydrolyzed linker. Therefore, the pharmacodynamics of CNP Prodrug relies on the pharmacologic activity of released CNP. CNP has an established mechanism of action and has been shown to increase endochondral bone growth in both WT and animal models of FGFR3-mediated chondrodysplasia. It is approved for treatment of ACH in children with open epiphyses. Therefore, pharmacology studies of CNP Prodrug were designed to confirm CNP released from CNP Prodrug retains the desired pharmacologic effect and to establish the sustained CNP exposure needed to achieve endochondral bone growth.

[0412] Pharmacodynamics of CNP Prodrug is being evaluated in mice and NHPs to demonstrate the dose response effect of CNP is retained when active CNP is released continuously from its albumin bound inactive prodrug form into the circulation compared to daily pulsatile delivery of CNP. Completed studies demonstrated the expected dose responsive endochondral skeletal growth effect of CNP is retained with CNP Prodrug treatment, as shown by increases in cellularity and height of the hypertrophic zone of the growth plate and/or increased length of long bones.

[0413] Growth effects of CNP Prodrug in young WT mice were compared to CNP in a study, entitled, "Five Weeks Growth Study of CNP Prodrug Compared to CNP in C57BL/6J mice." A total of 80, 3-week-old male C57BL/6J mice (Jackson Laboratories) were

randomized into 7 treatment groups. Animals were 6.3 to 7.2 g at study initiation. Animals were treated for 5 weeks with CNP by daily SC injection at 0 (vehicle), 40, 150, or 500 µg/kg/day or with CNP Prodrug by SC injection once every other day (QAD) at 0 (vehicle), 80, 300, or 1000 µg/kg/dose. All doses were based on CNP content. The design of the study is shown in Table 24-1.

Table 24-1: Design of the Pharmacodynamics Study of CNP Prodrug in Young Wild-Type Mice

Group	Test Article	Dose (µg/kg based on CNP content)	Dose Frequency	Dose Concentration ^a (µg/mL)	Number of Animals and Sex
A	Vehicle ^b	0	QD	NA	10 M
B	Vehicle ^b	0	QAD	NA	10 M
C	CNP ^c	40	QD	8	10 M
D	CNP ^c	150	QD	30	10 M
E	CNP ^c	500	QD	100	10 M
F	CNP Prodrug ^d	80	QAD	16	10 M
G	CNP Prodrug ^d	300	QAD	60	10 M
H	CNP Prodrug ^d	1000	QAD	200	10 M

CNP, C-type natriuretic peptide; M, male; NA, not applicable; QAD, once every other day; QD, once daily.

^aThe dose volume for each animal was 5 µL/gram bodyweight.

^bVehicle, 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) Polysorbate 80.

^cCNP, in 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) Polysorbate 80.

^dCNP Prodrug, in 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) Polysorbate 80.

[0414] Animals were observed daily for general health. Body weights were collected prior to each dose (daily for Groups A, C, D, and E and QAD for Groups B, F, G, and H). Overall growth was assessed by weekly naso-anal and tail lengths, beginning prior to treatment using a ruler while the mouse was anesthetized with inhaled isoflurane. The femur, tibia, humerus, ulna, and vertebral column (superior cervical to inferior sacral vertebrae) were measured weekly from micro computed tomography (µCT) images performed during inhaled isoflurane anesthesia. The µCT images were also reviewed by the study veterinarian for evidence of abnormalities. After 5 weeks of treatment, animals were euthanized and necropsied.

[0415] There were no effects on body weight gains when differences at Baseline were accounted for. There were no changes in gait or ambulation observed or macroscopic abnormalities at necropsy. Dose-responsive significant increases in naso-anal length were observed in all CNP-treated mice compared to the corresponding vehicle-treated mice. As

shown in Table 25, significant increases in naso-anal length were observed only at 1000 µg/kg QAD for CNP Prodrug-treated mice. Tail lengths were only significantly increased in CNP-treated mice at doses of 150 and 500 µg/kg/day compared to concurrent controls. No effects on tail length were observed in CNP Prodrug-treated mice.

Table 24-2: Naso-anal and Tail Length Growth in CNP and CNP Prodrug-Treated Young WT Mice

Group	Treatment	Increase in Naso-Anal Length from Baseline		Increase in Tail Length from Baseline	
		cm (mean ± SD)	% (mean ± SD)	cm (mean ± SD)	% (mean ± SD)
A	Vehicle, QD	2.31 ± 0.22	34.3 ± 4.2	2.12 ± 0.19	45.1 ± 7.2
C	CNP, 40 µg/kg QD	2.98 ± 0.30	44.7 ± 5.0	2.54 ± 0.21	53.0 ± 6.0
D	CNP, 150 µg/kg QD	3.51 ± 0.29	52.2 ± 5.5	2.94 ± 0.22	60.2 ± 5.6
E	CNP, 500 µg/kg QD	3.50 ± 0.23	51.9 ± 4.8	3.06 ± 0.20	63.3 ± 8.4
B	Vehicle, QAD	2.57 ± 0.21	39.3 ± 2.9	2.37 ± 0.19	53.6 ± 4.7
F	CNP Prodrug, 80 µg/kg QAD	2.89 ± 0.22	44.0 ± 03.4	2.46 ± 0.16	52.7 ± 5.3
G	CNP Prodrug, 300 µg/kg QAD	2.70 ± 0.29	39.0 ± 4.9	2.19 ± 0.23	47.7 ± 5.9
H	CNP Prodrug, 1000 µg/kg QAD	3.38 ± 0.23	50.0 ± 4.4	2.69 ± 0.17	56.8 ± 4.7

ANOVA, analysis of variance; QAD, every other day dosing; QD, daily dosing; SD, standard deviation. **Bolded** values indicate significant difference ($p < 0.05$, by one-way ANOVA with Tukey's post hoc test for multiple comparisons from treatment control for % change from Baseline (Groups C, D, and E compared to Group A and Groups F, G, and H compared to Group B).

[0416] Estimated C_{max} and AUC for Prodrug and CNP are set out in Table 24-2.1.

Table 24-2.1: Estimated C_{max} and AUC for Prodrug and CNP

Group	Treatment	Estimated Vosoritide C_{max} (pM)	Estimated Vosoritide $AUC_{(0-\tau)}^b$ (hr*pM)
A	Vehicle, QD	NA	NA
C	Vosoritide, 40 µg/kg QD	1368 ^c	563 ^c
D	Vosoritide, 150 µg/kg QD	5132 ^c	2113 ^c
E	Vosoritide, 500 µg/kg QD	17105 ^c	7043 ^c
B	Vehicle, QAD	NA	NA
F	CNP Prodrug, 80 µg/kg QAD	49 ^d	1227 ^d
G	CNP Prodrug, 300 µg/kg QAD	186 ^d	4601 ^d
H	CNP Prodrug, 1000 µg/kg QAD	619 ^d	15336 ^d

$AUC_{(0-\tau)}$ presented where $\tau = 120$ min for animals treated with CNP and 48 hours for animals treated with CNP prodrug, representing the typical range of detectable vosoritide in plasma of mice following daily vosoritide treatment and interval between doses, respectively.

^cEstimated exposures are extrapolated from WT male mice administered 600 µg/kg CNP.

^dEstimated exposures are extrapolated from WT mice administered 627 or 1891 µg/kg CNP prodrug (based on CNP content).

[0417] The level of cGMP, a biomarker of CNP binding to the NPR-B receptor, was measured in plasma collected at 0.5, 4, and 24 hours post-dose. While in CNP-treated mice levels of cGMP in plasma were elevated at 0.5 hours post-dose at all doses, levels were similar to vehicle-treated mice by 4 hours post-dose. In contrast, for CNP Prodrug, at the 1000 µg/kg QAD dose level, there was a sustained release of cGMP observed at all time points. When compared to cGMP in CNP-treated mice, levels were approximately a quarter of the peak observed with CNP treatment. There was no detectable cGMP in the plasma of mice treated with 80 or 300 µg/kg QAD CNP Prodrug.

[0418] While significant dose-responsive growth of the femur, tibia, and vertebral column was observed for CNP-treated mice, no significant growth was observed in CNP Prodrug-treated mice. This study demonstrated treatment with 1000 µg/kg QAD CNP Prodrug is pharmacologically active in WT mice, with an effect on naso-anal length and detectable plasma cGMP. Nonetheless, it was concluded that QAD dosing of CNP Prodrug does not allow sufficiently consistent CNP exposure in mice to produce a similar growth effect as CNP given daily.

Growth Effects of CNP Prodrug in WT Mice Dosed Daily

[0419] Increased frequency (daily) dosing of CNP Prodrug was evaluated in a study, entitled, "A 5 Week Study of CNP and CNP Prodrug by Subcutaneous Administration in Juvenile Male Mice."

[0420] A total of 40 male C57BL/6NCrl mice (Charles River Laboratories) were randomized into 4 treatment groups on postnatal day (PND) 21. Animals were treated by daily SC injection of vehicle, 500 µg/kg/day CNP, or CNP Prodrug at 500 or 1600 µg/kg/day. All doses were based on CNP content. Dosing was initiated for all animals on PND 22 and treatment was intended to occur for 5 weeks (to PND 57); however, due to test article effects, treatment was terminated early, after up to 18 days of treatment. The design of the study is shown in Table 24-3.

Table 24-3: Design of the Daily-Dosing Pharmacodynamics Study of CNP Prodrug in Young Wild-Type Mice

Group ¹	Test Article	Dose (µg/kg based on CNP content)	Dose Concentration ^a (µg/mL)
1	Vehicle ^b	0	0
2	CNP ^c	500	100
3	CNP Prodrug _d	500	100
4	CNP Prodrug _d	1600	320

CNP, C-type natriuretic peptide;

¹each group consisted of 10 male mice;

^a The dose volume for each animal was 5 mL/kg bodyweight.

^b Vehicle, 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) polysorbate 80.

^c CNP, in 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) Polysorbate 80.

^d CNP Prodrug, in 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) Polysorbate 80.

[0421] Animals were observed for mortality, clinical observations, body weights. Digital radiographs were taken on PNDs 21 (Pretest), 29 (Study Day 8), and 36 (Study Day 15), while animals were under general anesthesia. In addition, radiographs were taken on PND 41 (Study Day 20) on animals exhibiting suspected fractures, including the remaining controls. At scheduled necropsy, the right femur, right humerus, and right ulna were trimmed of tissues and a caliper was used to measure length and width.

[0422] There were several animals that were euthanized early in the 500 µg/kg/day CNP-treated group (Group 2) and the 1600 µg/kg/day CNP Prodrug-treated group (Group 4). One Group 4 animal was euthanized on Study Day 4 due to poor/deteriorating condition. Due to the short duration treatment, this death was considered to be potentially related to poor adaption to weaning and was not likely related to treatment with CNP Prodrug. One Group 4 animal was euthanized on Study Day 8 due to slight limited use of the right forelimb that was the result of a suspected cage-related injury and is of uncertain relationship to treatment with CNP Prodrug, given the short duration of treatment. On Study Day 15-16, 4/10 Group 2 animals and 5/8 remaining Group 4 animals were euthanized due to suspected fractures and/or clinical signs of slight to moderate firm swollen bilateral hindlimbs and abnormal gait. As a result of these early deaths, the study was terminated on Study Day 20 and all surviving animals were euthanized and necropsied.

[0423] During the dosing period, there were bilateral CNP-related radiographic findings in males at 500 µg/kg/day at the distal tibia and calcaneus. On Study Day 8, all animals presented radiographic increased physis thickness and radiographic mixed reaction of the distal tibia. On Study Day 15, radiographic increased physis thickness and radiographic mixed reaction of the distal tibia were still generally observed in association with increased physis thickness of the calcaneus and suspected or confirmed fracture of the distal tibia in some animals. In addition, there were occasional epiphyseal fractures of the calcaneus. On Study Day 20, all remaining males presented radiographic increased physis thickness and radiographic mixed reaction of the distal tibia and suspected or confirmed fracture at the distal tibia. Most males presented epiphyseal fractures of the calcaneus and/or increased physis thickness of the calcaneus. Radiographic findings are summarized in Table 24.4.

[0424] During the dosing period, there were bilateral CNP Prodrug-related radiographic findings in males at ≥ 500 $\mu\text{g/kg/day}$ at the distal tibia and calcaneus, generally similar to those noted for CNP. On Study Day 8, all males at 1600 $\mu\text{g/kg/day}$ presented radiographic increased physis thickness and radiographic mixed reaction of the distal tibiae. On Study Day 15, radiographic increased physis thickness and radiographic mixed reaction of the distal tibia were generally observed in animals treated with 500 or 1600 $\mu\text{g/kg/day}$ CNP Prodrug or 500 $\mu\text{g/kg/day}$ CNP. These findings were associated with an increased physis thickness of the calcaneus and suspected or confirmed fracture of the distal tibia for some animals treated with 500 $\mu\text{g/kg}$ CNP or 1600 $\mu\text{g/kg/day}$ CNP Prodrug. In addition, one male treated with 500 $\mu\text{g/kg/day}$ CNP Prodrug presented an epiphyseal fracture of the calcaneus. On Study Day 20, prior to necropsy, only those animals with clinically suspected fractures (3/6 500 $\mu\text{g/kg}$ CNP-treated animals and 1/6 500 $\mu\text{g/kg}$ CNP Prodrug-treated animals) as well as 2 representative control animals had radiographs performed. As on Study Days 8 and 15, radiographic increased physis thickness and radiographic mixed reaction, i.e., regions of both increased and decreased density of bone tissue, of the distal tibia and calcaneus were observed. Epiphyseal fracture of the calcaneus was observed in 2 of the CNP-treated animals and the 500 $\mu\text{g/kg/day}$ CNP Prodrug-treated animal. Radiographic findings are summarized in Table 24-4.

Table 24-4: Test Article Related Radiographic Findings on Study Days 8, 15, and 20

Test Material	Vehicle	CNP	CNP Prodrug	CNP Prodrug
Dose ($\mu\text{g/kg/day}$)	0	500	500	1600
Study Day 8				
No. Animals Examined	10	10	10	9
Radiographic Increased Physis Thickness and Radiographic Mixed Reaction				
Distal tibia, Rt and Lt	-	10	-	9
Study Day 15				
No. Animals Examined	10	10	10	8
Radiographic Increase Physis Thickness				
Distal tibia, Rt and/or Lt	-	8	10	6
Calcaneus, Rt and/or Lt	-	6	-	4
Radiographic Mixed Reaction				
Distal tibia, Rt and/or Lt	-	7	5	6
Fracture – clinically suspected or confirmed				
Distal tibia, Rt and/or Lt	-	6	-	8

Test Material	Vehicle	CNP	CNP Prodrug	CNP Prodrug
Dose (µg/kg/day)	0	500	500	1600
Epiphyseal Fracture				
Calcaneus, Rt and/or Lt	-	5	1	-
Study Day 20^b				
No. Animals Examined	2	3	1	0
Radiographic Increase Physis Thickness				
Distal tibia, Rt and Lt	-	3	1	-
Calcaneus, Rt and Lt	-	1	-	-
Radiographic Mixed Reaction				
Distal tibia, Rt and Lt	-	3	1	-
Fracture – clinically suspected or confirmed				
Distal tibia, Rt and/or Lt	-	3	1	-
Epiphyseal Fracture				
Calcaneus, Rt and Lt	-	2	1	-

Lt, left; Rt, right.

^a Radiographs were performed only on surviving animals with suspected fractures due to clinical observations of hindlimb swelling and/or altered gait, along with two control animals for comparison purposes.

[0425] Radiographic bone measurements were conducted on Study Days 8 and 15.

Despite the observed physis thickness increase observed in radiographs presence of fractures, there was no treatment-related effect on the length of the femur, tibia, ulna, humerus, or lumbar spine in mice treated with 500 µg/kg/day CNP Prodrug or CNP compared to controls at either time point. Animals given 1600 µg/kg/day had significantly higher greater femur (+11%) and lumbar spine (+12%) lengths at Study Day 15 compared with controls. No other CNP Prodrug-related effects on the length of the tibia, ulna, or humerus were noted. Interpretation of terminal *ex vivo* measurements was impacted by the reduced number of surviving animals in the 500 ug/kg/day CNP and 1600 ug/kg/day CNP Prodrug-treated groups. At the end of the dosing period, males administered CNP Prodrug at 500 µg/kg/day presented statistically significant longer femur (+7%), humerus (+4%), and ulna length (+7%), compared with controls; however, there was high inter-animal variability, suggesting the findings may be incidental. No treatment effect was detected in histomorphometry, but value of this end point was impacted by the number of early deaths and reduced number of animals surviving to Study Day 20.

[0426] Microscopic findings for animals euthanized on or after Study Day 15 are detailed in Table 24.5. Mildly to severely increased thickness of the physis in the distal tibia was

noted at 500 µg/kg/day CNP and 500 or 1600 µg/kg/day CNP Prodrug, correlating to joint enlargement grossly. Increased thickness of the physis progressed to minimal to marked degeneration/necrosis of the physis in most animals and physeal fracture in a few animals, mostly in the 1600 µg/kg/day CNP Prodrug group. Physeal fractures correlated to joint enlargement observed clinically. Increased thickness physis of the distal tibia was accompanied by 2 secondary changes at the same doses. Minimally to mildly increased bone in the metaphysis, which was considered an elongation of the primary and secondary spongiosa, and minimal to mild cortical resorption, which was considered an elongation of the “cut-back” zone.

[0427] In the proximal tibia, the most important change was minimally decreased thickness of the physis, which was noted at 500 µg/kg/day CNP or CNP Prodrug, with a greater incidence in CNP Prodrug-treated animals. The change correlated to decreased physeal parameters measured by histomorphometry. Minimally increased physis thickness was noted in a few animals at ≥ 500 µg/kg/day CNP Prodrug, and the incidence of the change was dose-related. The change was accompanied by minimally increased bone in the metaphysis in one 1600 µg/kg/day CNP Prodrug-treated animal, which was considered a secondary change. Minimal chondrocyte vacuolation was noted at 500 µg/kg/day CNP. It was characterized by vacuolation of chondrocytes in the hypertrophic zone.

[0428] In the calcaneus, changes were similar to the ones described in the distal tibia and consisted of mildly to moderately increased thickness of the physis, minimal to moderate physeal degeneration/necrosis, fracture, and mild to moderate cortical resorption, all noted in the dorsal aspect of the bone. Fracture correlated to joint enlargement noted grossly.

Table 24-5: Test Article Related Microscopic Findings in Wild-Type Mice

Treatment	Vehicle	CNP	CNP Prodrug	CNP Prodrug
Dose (µg/kg/day)	0	500	500	1600
Animals per Group	10	10	10	10
Bone, tibia (No. Examined)	10	10	10	8
Growth plate closed/Growth plate partially closed, distal	(5) ^a	(0)	(0)	(0)
Physis thickness, increased; distal	(0)	(9)	(10)	(7)
Mild	-	4	6	0
Moderate	-	3	3	1
Marked	-	2	1	2
Severe	-	0	0	4
Degeneration/necrosis; distal, physis	(0)	(6)	(9)	(5)
Minimal	-	4	5	0
Mild	-	1	2	0
Moderate	-	0	1	2
Marked	-	1	1	3

Treatment	Vehicle	CNP	CNP Prodrug	CNP Prodrug
Dose (µg/kg/day)	0	500	500	1600
Animals per Group	10	10	10	10
Fracture; distal, physis	(0)	(1)	(1)	(5)
Bone increased; distal, metaphysis	(0)	(7)	(2)	(7)
Minimal	-	6	2	2
Mild	-	1	0	5
Resorption; cortical, distal	(0)	(3)	(3)	(5)
Minimal	-	3	3	3
Mild	-	0	0	2
Bone increased; cortical, distal	(0)	(0)	(1)	(0)
Minimal	-	-	1	-
Physis thickness, decreased; proximal	(0)	(2)	(7)	(0)
Minimal	-	2	7	-
Physis thickness, increased; proximal	(0)	(0)	(1)	(3)
Minimal	-	-	1	3
Bone increased; proximal, metaphysis	(0)	(0)	(0)	(1)
Minimal	-	-	-	1
Vacuolation; chondrocyte ^b	(0)	(4)	(0)	(0)
Minimal	-	4	-	-
Calcaneus ^c (No. Examined)	0	6	4	0
Physis thickness, increased	(0)	(2)	(2)	(0)
Mild	-	2	1	-
Moderate	-	-	1	-
Degeneration/necrosis; distal, physis	(0)	(1)	(4)	(0)
Minimal	-	1	1	-
Mild	-	0	1	-
Moderate	-	0	2	-
Fracture	(0)	(4)	(2)	(0)
Resorption; cortical	(0)	(0)	(2)	(0)
Minimal	-	-	1	-
Mild	-	-	1	-

^a Numbers in parentheses represented the number of animals with the finding.

^b The incidence of vacuolation reflected the total incidence of the change irrespective of the location.

^c Number of animals examined for this tissue represents the number of animals in which findings were noted in the calcaneus in the section submitted for tibio-tarsal joint examination.

[0429] In conclusion, the increase in dose interval from QAD to daily SC dosing of CNP Prodrug resulted in an increase in plasma levels of CNP that led to adverse effects on the tibia and calcaneus in juvenile WT mice at doses of 500 or 1600 µg/kg/day. Similar effects were seen with a high daily dose of CNP (500 µg/kg/day). These changes reflect exaggerated pharmacologic effects that have been previously observed with CNP in rodents, occasionally in the absence of no or modest effects on bone lengths. The absence of a significant effect on growth in this study despite these effects should be considered in the context of the short treatment period and detectability of subtle growth effects. This fracture effect was not observed radiologically via µCT, as discussed below, in which WT mice of a

different strain were treated with 500 µg/kg/day CNP or 1600 µg/kg/day CNP Prodrug daily for 6 weeks.

Example 25: Mouse Models of Noonan Syndrome

[0430] Noonan Syndrome (NS) is a genetically inherited disease affecting the MAPK signaling pathway in multiple cell and tissue types, with distinct disruptions in heart and bone morphology. Manifestations of NS pathobiology include reduced height and moderate alterations of the facial bones as well as hypertrophic cardiomyopathy (HCM). The genetic etiology of NS involves autosomal-dominant mutations in major signaling components of the RAS signaling cascade such as protein tyrosine phosphatase non-receptor type 11, RIT1, and rapidly accelerating fibrosarcoma 1 (RAF-1) (Saint-Laurent 2024 Eur J Pediatr;183:1011-1019). Mutations in these signaling intermediates result in reduced negative regulation pathway needed for normal ERK/MAPK homeostasis, resulting in elevated MAPK activity. As in ACH, this increased MAPK signaling can be reduced by treatment with CNP or CNP, which antagonizes MAPK signaling at the level of RAF-1. Therefore, these models are useful to demonstrating the efficacy of CNP Prodrug compared to CNP in the context of increased MAPK signaling.

[0431] This study entitled, "Pilot Pharmacodynamic Study of CNP and CNP Prodrug in Juvenile Wild Type or Raf1 L613V Heterozygous Male Mice Dosed Daily via Subcutaneous Administration for 6 Weeks" was conducted to assess effects on growth in the presence of the *Raf1* L613V activating mutation of CNP and CNP Prodrug. These effects were contrasted with a small molecule MAPK kinase inhibitor (MEKi), PD0235901, that has been previously evaluated in this model (Wu 2011, J Clin Invest 121:1009-1025).

[0432] *Raf1*^{+/L613V} mice express one WT copy of the *Raf1* gene, and one copy containing a knock-in missense mutation where the leucine at residue 613 is substituted for valine (L613V). This mutation results in excessive activation of the MAPK pathway. Mice heterozygous for this mutation begin to develop phenotypes like those seen in NS patients by 5 weeks of age, including enlarged hearts, mild flattening of craniofacial features and mild reduction in naso-anal length compared to WT siblings (Wu *supra*).

[0433] In this study, male WT or *Raf1*^{+/L613V} mice of 50% 129S1/SvImJ and 50% C57BL/6J background were bred through crossing of male *Raf1*^{+/L613V} 129S1/SvImJ mice (custom made via clustered regularly interspaced short palindromic repeats [CRISPR] Cas9 technique; Jackson Laboratories, Sacramento, CA) to female WT C57BL6/J mice (JAX stock #000664, Jackson Laboratories, Sacramento, CA). Animals were genotyped on PND 7 via a tail tip snip. A total of 25 WT and 38 *Raf1*^{+/L613V} males weighing 8-12 g were assigned to 8

groups following weaning at 3 weeks of age (PND 21-23). The study design is shown in Table 25-1.

Table 25-1: Design of the Pharmacodynamics Study in WT and *Raf1*^{+/L613V} Noonan's Syndrome Mice

Group	Genotype	Test Article	Dose (µg/kg/day based on CNP content) ^a	Dose Volume (µL/g)	Route	Number of Animals and Sex
A	WT	Vehicle ^b	0	4	SC	5 M
B	WT	CNP ^c	500	4	SC	10 M
C	WT	CNP Prodrug ^d	1600	4	SC	10 M
D	<i>Raf1</i> ^{+/L613V}	Vehicle ^b	0	4	SC	5 M
E	<i>Raf1</i> ^{+/L613V}	CNP ^c	500	4	SC	10 M
F	<i>Raf1</i> ^{+/L613V}	CNP Prodrug ^d	1600	4	SC	10 M
G	<i>Raf1</i> ^{+/L613V}	Vehicle ^e	0	10	IP	4 M/1 F ^g
H	<i>Raf1</i> ^{+/L613V}	MEKi PD0235901 ^f	5 mg/kg/day	10	IP	8 M

CNP, C-type natriuretic peptide; F, female; M, male; MEKi, mitogen-activated protein kinase kinase inhibitor; IP, intraperitoneal; SC, subcutaneous; WT, wild-type.

^a Doses based on CNP content in µg/kg, except for Groups G and H that were treated with PD0235901.

^b Vehicle, 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) polysorbate 80.

^c CNP, Bag 11, in 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) Polysorbate 80.

^d CNP Prodrug, in 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) Polysorbate 80.

^e Vehicle, 5% dimethyl sulfoxide (DMSO) in sterile 1X phosphate buffered saline.

^f MEKi PD0235901, IUPAC name: N-[(2R)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide, in 5% dimethyl sulfoxide (DMSO) in sterile 1X phosphate buffered saline.

^g During Study Week 4, one mouse was found to have been mis-sexed at study initiation and was found to be a female. The animal was housed separately for the remainder of the study.

[0434] Following randomization into study, animals were anesthetized and subjected to a baseline echocardiogram, body weight, and naso-anal length measurement using a standard metric ruler. At 3 weeks of age, *Raf1*^{+/L613V} were approximately 0.46 cm or 6.72% shorter than age-matched WT littermates and body weights were correspondingly lower. Cardiac hypertrophy was present in this model, but a HCM phenotype was not observed at Baseline and did not develop, with or without CNP or CNP Prodrug treatment.

[0435] Treatment was initiated at 4 weeks of age. Wild-type and *Raf1*^{+/L613V} mice were dosed by daily SC injection with vehicle, 500 µg/kg/day CNP, or 1600 µg/kg/day CNP Prodrug. All doses were based on CNP content. Comparator groups of *Raf1*^{+/L613V} mice were by daily intraperitoneal (IP) injection of vehicle or 5 mg/kg/day MEKi. All animals were dosed for 6 weeks.

[0436] Animals were examined daily for body condition, behavior and gait and were weighed daily prior to dosing. During Study Week 5, and naso-anal length was measured, and animals were subjected to an echocardiogram under anesthesia. Prior to euthanasia in Study Week 6, mice in Groups A-F received a full body μ CT scan to assess femur and tibia length and skull morphology. Animals were euthanized on Study Days 49-52 (Study Week 6) and necropsied. The right femur and tibia were collected from all WT animals. Hearts and spleens were weighed.

[0437] There were no test article-related deaths. One animal in Group H (5 mg/kg/day MEKi) did not recover from anesthesia following the Study Week 5 echocardiogram procedure. After 2 weeks of treatment, animals treated with either CNP or CNP Prodrug began to develop visibly elongated skeletal features and minor peripheral bone outgrowths resulting in hunched posture and curled paws. Mice treated with MEKi rapidly gained weight and developed mild obesity by Study Week 5.

[0438] As shown in Figure 25, by 8 weeks of age, the reduced naso-anal length phenotype in $Raf1^{+/L613V}$ mice observed at 3 weeks of age was no longer present as the lengths of vehicle-treated $Raf1^{+/L613V}$ mice were not significantly different from vehicle-treated WT animals. Wild-type mice treated with 1600 μ g/kg/day CNP Prodrug had an increase in naso-anal length of 21.2% and $Raf1^{+/L613V}$ mice naso-anal lengths increased 12.4% compared to vehicle-treated mice. Wild-type mice treated with 500 μ g/kg/day CNP exhibited a 7.8% increase in naso-anal length and $Raf1^{+/L613V}$ mice lengths increased 7.4% compared to vehicle-treated mice. Both WT and $Raf1^{+/L613V}$ mice treated with CNP Prodrug had significantly longer naso-anal lengths than mice treated with 500 μ g/kg/day CNP. Mitogen-activated protein kinase kinase inhibitor treatment was also associated with an increase in naso-anal length compared to vehicle control, however these gains were smaller than those observed with CNP or CNP Prodrug treatment.

[0439] There were no fractures detected by μ CT at the end of the 6-week treatment period, in contrast to the effects observed in C57BL/6NCrl mice. As with naso-anal length, by 9 weeks of age, there was no difference in femur or tibia length found by μ CT in vehicle-treated $Raf1^{+/L613V}$ mice compared to vehicle-treated WT mice. Both 1600 μ g/kg/day CNP Prodrug and 500 μ g/kg/day CNP induced statistically significant growth in the long bones of WT mice (Table 25-2). There was also a statistical difference between the growth effects of CNP Prodrug and CNP in WT mice, where CNP Prodrug at 1600 μ g/kg/day induced greater growth than 500 μ g/kg/day CNP in both femur and tibia ($p < 0.0001$ by 2-way analysis of variance with Tukey's multiple comparisons test).

Table 25-2: Femur and Tibia Lengths in WT and *Raf1*^{+/-L613V} Mice Treated for 6 Weeks

Genotype	Treatment	Mean Femur Length (cm)	Mean Tibia Length (cm)
WT	Vehicle	1.48	1.72
	500 µg/kg/day CNP	1.64	1.81
	1600 µg/kg/day CNP Prodrug	1.82	1.98
<i>Raf1</i> ^{+/-L613V}	Vehicle	1.428	1.717
	500 µg/kg/day CNP	1.573	1.573
	1600 µg/kg/day CNP Prodrug	1.610	1.803

ANOVA, analysis of variance; WT, wild-type.

Bold values are significantly different from control by two-way ANOVA with Tukey's multiple comparison test.

[0440] *Raf1*^{+/-L613V} mice skulls exhibited mild enlargement of intercanthal distance, reduced skull width, and reduced skull length compared to their WT siblings at 9 weeks of age. These data compare with reported skull phenotypes seen in other published reports for this model (Wu 2011) and relate to the skull phenotype observed clinically in patients with NS and ACH. As shown in Table 25-3, treatment with 1600 µg/kg/day CNP Prodrug or 500 µg/kg/day CNP improved this phenotype, non-significantly reducing the mean intercanthal distance and significantly increasing skull width and length. As with the femur and tibia, CNP Prodrug effects were greater for skull length and width than for CNP. Only skull length was significantly increased in WT mice treated with 1600 µg/kg/day CNP Prodrug compared to vehicle treated WT mice.

Table 25-3: Skull Morphology in WT and *Raf1*^{+/-L613V} Mice Treated for 6 Weeks

Genotype	Treatment	Mean Inter-Canthal Distance (cm)	Mean Skull Width (cm)	Mean Skull Length (cm)
WT	Vehicle	0.437	1.040	2.283
	500 µg/kg/day CNP	0.459	1.015	2.315
	1600 µg/kg/day CNP Prodrug	0.461	1.024	2.353
<i>Raf1</i> ^{+/-L613V}	Vehicle	0.552	1.087	2.209
	500 µg/kg/day CNP	0.482	1.064	2.275
	1600 µg/kg/day CNP Prodrug	0.492	1.057	2.282

ANOVA, analysis of variance; WT, wild-type.

Bold values are significantly different from genotype-matched control by two-way ANOVA with Tukey's multiple comparison test.

[0441] There were no test article related effects on echocardiography parameters, confirming neither CNP nor CNP Prodrug impacted the function of either normal or hypertrophic hearts.

[0442] In conclusion, daily doses of 500 µg/kg/day of CNP and 1600 µg/kg/day induced characteristic overgrowth in both WT (50% 129S1/SvilmJ and 50% C57BL/6J) and *Raf1*^{+/-L613V} mice on the same background. The skull phenotype of *Raf1*^{+/-L613V} mice was normalized with treatment with CNP and, to a greater degree, with CNP Prodrug. Animals did not present with fractures either clinically or by µCT imaging, despite longer treatment. These animals initiated treatment at 4 weeks of age rather than at 3 weeks and have a different strain background, which may have influenced these differences. Treatment with CNP or CNP Prodrug did not lead to adverse effects on the heart despite the hypertrophy phenotype.

Effect of CNP Prodrug on Increased MAPK Signaling at the Level of RIT1

[0443] This study was conducted to assess effects on growth of CNP and CNP Prodrug in the presence of the activating M90I mutation of *Rit1* (*Rit*^{+/-} mice). These effects were contrasted with a small molecule MEKi, PD0235901.

[0444] Mutations in *RIT1* are less frequently associated with short stature in NS than mutations to *RAF1* associated with NS (Yaoita 2016). The M90I mutation in *RIT1* increases signaling along the ERK1/2 MAPK pathway, which may be ameliorated by treatment with CNP. Mice heterozygous for the M90I mutation (*Rit1*^{+/-}) have been previously shown to have shorter body lengths at 4 weeks of age and increased spleen and heart to body weight ratios compared to WT littermates (Castel 2019).

[0445] In this study, 30 WT and 45 *Rit1*^{+/-} 8-week-old male mice were randomized into 10 groups. Animals were dosed daily by SC injection with vehicle or CNP at 500 µg/kg/day or QAD with vehicle or CNP Prodrug at 3280 µg/kg/dose. Comparator groups of *Rit*^{+/-} mice were treated by daily IP injection of vehicle or 5 mg/kg/day MEKi. All animals were dosed for 10 weeks. The design of the study is shown in Table 25-4.

Table 25-4: Design of the Pharmacodynamics Study in WT and *Rit1*^{+/-} Noonan's Syndrome Mice

Group	Genotype	Test Article	Dose (µg/kg/day based on CNP content) ^a	Frequency	Dose Volume (µL/g)	Route	Number of Animals and Sex
1	WT	Vehicle ^b	0	QD	4	SC	5 M
2	WT	Vehicle ^b	0	QAD	4	SC	5 M
3	WT	CNP ^c	500	QD	4	SC	10 M
4	WT	CNP Prodrug ^d	3280	QAD	4	SC	10 M
5	<i>Rit1</i> ^{+/-}	Vehicle ^b	0	QD	4	SC	5 M
6	<i>Rit1</i> ^{+/-}	Vehicle ^b	0	QAD	4	SC	5 M
7	<i>Rit1</i> ^{+/-}	Vehicle ^e	0	QD	10	IP	5 M
8	<i>Rit1</i> ^{+/-}	CNP ^c	500	QD	4	SC	10 M

Group	Genotype	Test Article	Dose (µg/kg/day based on CNP content) ^a	Frequency	Dose Volume (µL/g)	Route	Number of Animals and Sex
9	<i>Rit1^{+/-}</i>	CNP Prodrug ^d	3280	QAD	4	SC	10 M
10	<i>Rit1^{+/-}</i>	MEKi PD0235901 ^f	5 mg/kg/day	QD	10	IP	10 M

CNP, C-type natriuretic peptide; DMSO, dimethyl sulfoxide; IP, intraperitoneal; M, male; MEKi, mitogen-activated protein kinase inhibitor; QAD, every other day; QD, daily; SC, subcutaneous; WT, wild-type.

^a Doses based on CNP content in µg/kg, except for Groups G and H that were treated with PD0235901.

^b Vehicle, 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) polysorbate 80.

^c CNP, Bag 11, in 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) Polysorbate 80.

^d CNP Prodrug, in 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) Polysorbate 80.

^e Vehicle, 5% DMSO in sterile 1X phosphate buffered saline.

^f MEKi PD0235901, IUPAC name: N-[(2R)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]-benzamide, in 5% DMSO in sterile 1X phosphate buffered saline.

[0446] Animals were weighed prior to each dose. Naso-anal lengths, tail lengths, radiographic measurements of long bones (tibia, femur, ulna, humerus) and vertebrae, and echocardiograms were collected at Baseline, prior to treatment initiation, after 6 weeks of treatment. Radiographic measurements were also collected at Study Week 10 prior to termination. In Study Week 10, animals were euthanized and necropsied. Terminal body weights, naso-anal lengths, and tail lengths were measured and the heart, lung, liver, and spleen were weighed.

[0447] During the 10-week treatment period, visibly noticeable skeletal growth occurred in CNP and CNP Prodrug treated WT and, to a lesser degree, *Rit1^{+/-}* mice. The study was originally planned for 14 weeks of dosing but was terminated at 10 weeks due to the skeletal growth phenotype observed in CNP and CNP Prodrug-treated animals and the absence of a HCM phenotype, making further dosing futile.

[0448] As with *Raf1^{+/-}L613V* mice, *Rit1^{+/-}* mice did not exhibit a HCM phenotype, however hearts were enlarged relative to body weight, which was not meaningfully affected by treatment with CNP Prodrug or CNP. There were no adverse test article effects on cardiac function as assessed by echocardiography.

[0449] Naso-anal lengths were significantly increased from treatment and genotype-matched vehicle-treated controls for both 500 µg/kg/day CNP and 3280 µg/kg/dose QAD CNP Prodrug (Figure 26). In WT animals, treatment with 3280 µg/kg/dose QAD CNP Prodrug resulted in significantly longer naso-anal lengths than animals treated with CNP.

There was no difference in dose-response in *Rit^{+/+}* mice. Treatment with MEKi also resulted in increased naso-anal length, albeit to a lesser degree than achieved with either CNP or CNP Prodrug treatment. A similar trend was observed with tail lengths.

[0450] Figure 27 shows representative images at Baseline, 6 weeks, and 10 weeks of treatment for a vehicle-treated and CNP Prodrug-treated WT mice. CNP Prodrug treatment resulted in noticeable increases in long bone lengths and growth of the spinal column is evident. Early data on this study shows a clear treatment related effect of CNP Prodrug on skeletal growth, as animals reached the characteristic overgrowth phenotype observed previously for CNP in mice.

CNP Prodrug Pharmacodynamics in NHPs

[0451] Male cynomolgus monkeys were treated with CNP Prodrug 1 month to evaluate pharmacologic activity, as assessed by radiographic and microscopic evaluation of bones and assessment of biomarkers of osteogenesis. Pharmacokinetics and CV safety pharmacology were also evaluated. Animals were 2 years old at study initiation and weighed 2.7 to 3.3 kg. The study design is shown in Table 25-5.

Table 25-5: Design of the 1-Month Pharmacology Study in Cynomolgus Monkeys

Group ¹	Test Article	Dose ($\mu\text{g/kg/week}$, based on CNP content)	Dose Concentration ^a ($\mu\text{g/mL}$ CNP content)
1	Vehicle ^b	0	0
2	CNP Prodrug ^c	30	30
3	CNP Prodrug ^c	150	150

CNP, C-natriuretic peptide; M, male; USP, United States Pharmacopeia; w/v, weight/volume.

¹each group consisted of 4 male monkeys

^a 1 mL/kg dose volume.

^b 0.005 mol/L citrate buffer solution, pH 5.5, containing 5.25% (w/v) sucrose, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) polysorbate 80 prepared in sterile water for injection, USP.

^c CNP Prodrug formulated in 0.005 mol/L citrate buffer solution, pH 5.5, containing 5.25% (w/v) sucrose, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) polysorbate 80 prepared in sterile water for injection, USP.

[0452] Animals were given vehicle or CNP Prodrug at 30 or 150 $\mu\text{g/kg/dose}$ (based on CNP content) by SC injection once weekly on Study Days 1, 8, 15, 22, and 29. Doses were delivered in the back in the scapular area, alternating between the left (Study Days 1 and 15) and right (Study Days 8 and 22) sides. The final dose on Day 29 was delivered to a naïve site in the mid back.

[0453] Animals were monitored daily for mortality or evidence of gross toxicity and detailed clinical observations and body weights were performed weekly during the dosing period. Food consumption was monitored qualitatively. Clinical chemistry and hematology samples were collected weekly and on the day of necropsy. Biomarkers of chondrocyte hypertrophic differentiation (Collagen X and fragment of the NC1 domain of Collagen X [Pro-C10 HP]), and bone turnover/formation, ie, type II collagen degradation (type II collagen neoepitope [T2CM]), osteoblast activity (bone-specific alkaline phosphatase [BAP] and fragment of N-terminal type I collagen [Pro C1]), and type II collagen formation (fragment of N-terminal type IIB procollagen [Pro C2]) were measured in serum from fasted animals prior to treatment initiation, prior to dosing on Study Days 1, 8, 15, 22, and 29 and prior to necropsy on Study Day 31. Telopeptide of type I collagen (CTx-Ia), a biomarkers of type I collagen degradation, and telopeptide of type II collagen (CTx-II), a biomarker of type II collagen degradation, and cGMP, a biomarker of CNP cellular activity, were measured in urine collected overnight from fasted animals prior to treatment initiation and one and five days after each dose on Study Days 2, 6, 9, 13, 16, 20, 23, 27, and 30. Atrial natriuretic peptide (ANP) levels in plasma were measured in PK samples collected after each dose to confirm sustained supraphysiologic levels of CNP did not interfere with ANP clearance via NPR-C.

[0454] Prior to initiation of dosing, on Study Day 21, and prior to necropsy on Study Day 31, digital radiographs of femora/tibiae/fibulae, spine, and radii/ulnae were performed on all

animals under anesthesia. Radiographs were evaluated qualitatively and the physal closure was scored for the right proximal tibia/fibula and radius/ulna and for the right distal and proximal femora. Tibia and ulna length (right and left) were derived from the radiographs. Body length and tail length were also measured directly on anesthetized animals at the same time radiographs flexible measuring tape.

[0455] On Study Day 31, all animals were euthanized and underwent a full necropsy. The sternum, costochondral junction of the sixth rib, proximal left tibia, injection sites, and gross lesions were collected and examined microscopically. Histomorphometry was performed on the left proximal tibial physis for each animal to measure the thickness of the total epiphyseal growth plate and proliferative and hypertrophic zones.

[0456] All animals survived to scheduled necropsy. There were no test article-related clinical observations or effects on body weight/body weight gains, hematology, or clinical chemistry. ANP levels in plasma were below the limit of quantification (BLQ) at most timepoints for CNP Prodrug-treated animals, precluding definitive conclusions on trends; however, the result suggests continuous delivery of CNP via CNP Prodrug treatment did not result in competition for the shared clearance receptor, NPR-C.

[0457] Change from Baseline in normalized cGMP was increased in dosed groups (Groups 2 and 3) from the second dose onwards, on the second day from weekly dosing (Days 9, 16, 23, and 30). Change from Baseline in normalized cGMP was generally lower on the sixth day after weekly dosing (Study Days 13, 20 and 27) compared to the second day after weekly dosing (Study Days 9, 16, 23, and 30). Increases in normalized cGMP were higher in Group 3 (150 µg/kg/week) compared to Group 2 (30 µg/kg/week), suggesting that CNP pharmacological activity was sustained in a dose-dependent manner through 30 days of dosing.

[0458] There were no detectable treatment effects on Collagen X, Pro-C10 HP, T2CM, Pro-C1, or Pro C2 in serum. Changes from Baseline of BAP concentrations were similar in all 3 groups except on Study Day 29 and Day 31 (on the day of or 2 days after the 5th dose), when positive changes were observed in animals dosed with 150 µg/kg/week CNP Prodrug. Changes from Baseline in CTxIa concentration normalized to urine creatinine was similar in all 3 groups and no observable trend was noted. CTx-II concentrations in urine were BLQ for most timepoints in all groups.

[0459] There were no apparent treatment-related radiographic changes or exacerbation of pre-existing radiographic findings. There were no meaningful treatment related changes in

tibia, ulna, body, or tail lengths when compared to vehicle-treated animals, likely due to the short duration of the study.

[0460] At necropsy, there were no treatment related macroscopic findings and all gross lesions were attributed to surgical implantation of the telemetry device. There were no CNP Prodrug related findings at the injection sites. Minimal increased cellularity of the hypertrophic zone of the physis was observed in 150 µg/kg/week treated animals in the sternum (4/4), proximal tibia (2/4), and the sixth rib costochondral junction (1/4), demonstrating pharmacologic activity in the hypertrophic chondrocytes, as expected and previously observed with CNP treatment. A small increase in mean thickness of the hypertrophic zone of the proximal tibial physis was observed in animals administered 150 µg/kg/week compared to concurrent controls. Although this change was not statistically significant, it was considered CNP Prodrug-related and correlated microscopically to increased cellularity of the hypertrophic zone in 2 animals at this dose. There were no CNP Prodrug-related changes at 150 µg/kg/week for total epiphyseal growth plate thickness and proliferative zone thickness and there were no changes at 30 µg/kg/week for any of the measured parameters, when compared to concurrent control animals.

[0461] In conclusion, CNP Prodrug was well-tolerated in male NHPs when given weekly at 30 or 150 µg/kg/week for a total of 5 doses over 1 month. Pharmacologic activity (ie, binding to NPR-B) was evident by increases in normalized urinary cGMP levels in urine at both dose levels; however, effects on growth plate cellularity and hypertrophic zone thickness were only observed in animals given 150 µg/kg/week CNP Prodrug, suggesting the 30 µg/kg/week dose level was subtherapeutic. Effects on the growth plate did not translate to increases in bone, body, or tail length during the short duration of this study.

[0462] As with the primary and secondary pharmacodynamics, the active component of CNP Prodrug, CNP, also informs the safety pharmacology profiling of CNP Prodrug. Safety pharmacology studies of CNP Prodrug have focused on effects on CV endpoints, as the altered PK of CNP (ie, blunting of C_{max}) mitigates the observed effects of CNP on heart rate and blood pressure. Safety pharmacology evaluations of CNP are briefly summarized below followed by a detailed discussion of the safety pharmacology studies of CNP Prodrug.

Safety Pharmacology of CNP

[0463] No CNP-related respiratory or central nervous system (CNS) parameter changes were observed after a single SC administration of CNP at 30, 100 or 300 µg/kg to rats. The no-observed-effect level (NOEL) was 300 µg/kg in these studies.

[0464] In NHPs, as expected based on CNP mechanism of action in the vasculature, direct CNP administration induced vascular smooth muscle relaxation and subsequent dose-responsive decreases in blood pressure and compensatory increases in heart rate. Overall, little or no changes in blood pressure/heart rate were observed in the conscious NHPs given ≤ 10 $\mu\text{g/kg}$ CNP. An approximately 25% increase in heart rate was observed in conscious NHPs given 28 $\mu\text{g/kg}$ CNP. In the GLP CV safety pharmacology study, No overt CV-related clinical signs were observed in conscious NHPs given 50 $\mu\text{g/kg}$ while a $< 10\%$ decrease in blood pressure and an approximately 37% increase in heart rate were noted. The presence of overt CV-related clinical signs was observed in some conscious NHPs given ≥ 200 $\mu\text{g/kg}$ CNP. These clinical signs consisted of reduced activity in 5/8 animals from 40 to 60 minutes post-dose on Day 1 in the pilot CV study and of idiosyncratic short and repeated bouts of sternal or lateral recumbency in 3/8 animals during the first hour post-dose in the Phase 2 of the main CV study. In the 28-day repeat-dose toxicity study no overt CV-related clinical signs were noted in the NHPs treated with up to 300 $\mu\text{g/kg}$ CNP. The discrepancy between studies regarding the presence of overt CV-related observations was likely related to the method of administration (remote or manual) and to the amount of time technical staff were present in the room based on the size of the animal cohorts. Attenuation of the effects on blood pressure decreases, but not in the heart rate increases, was observed after daily repeat CNP administrations and was possibly due to NPR-B desensitization in the vasculature at high repeat doses delivered. A CV naïve-like response was observed following at least a 2-day washout period. It is possible that, because NPR-B distribution in the vasculature is mostly restricted to the peripheral vasculature, the severity of the measurable and observable hemodynamic changes was overall limited. Based on the absence of measurable changes in heart rate and blood pressure and CV related clinical observation, the NOEL of subcutaneously administered CNP to conscious NHPs was 10 $\mu\text{g/kg}$ in this study. No overt CV-related clinical observations were observed in the conscious monkey given a SC administration of ≤ 50 $\mu\text{g/kg}$ CNP. Across all studies, no prolongation of QT interval was observed.

[0465] As CNP was not shown to have any effects on the CNS or pulmonary function, no CNS or respiratory safety pharmacology studies of CNP Prodrug are planned.

[0466] The CV effects of CNP were expected to be ameliorated by the blunting of CNP C_{max} with continuous release from CNP Prodrug rather than daily pulsatile treatment. This was confirmed in 2 studies in cynomolgus monkeys, a non-GLP study and a GLP-compliant study.

[0467] As described above, male cynomolgus monkeys were dosed weekly by SC injection of vehicle or CNP Prodrug at 30 or 150 µg/kg/week (based on CNP content) for a total of 5 doses. Animals' heart rate, systemic arterial pressures (systolic, diastolic, pulse pressure, and mean arterial pressure), ECGs parameters (PR, RR, QRS, QT intervals, and corrected QT interval [QTc]), and body temperature were monitored remotely via surgically implanted DSI PhysioTel® Digital M11 telemetry devices continuously for 24 hours prior to initiation of dosing, then for a 20-hour period after each dose, starting at 25 hours post-dose. A qualitative evaluation of the ECG was also conducted at 36 hours post-dose by a veterinary cardiologist. No effects on any CV parameters were observed. This study confirmed that repeated pharmacologically active doses of CNP Prodrug, as expected, did not result in the reduction in blood pressure and increased heart rate typically observed with CNP as a single SC injection.

[0468] A GLP-compliant study designed to evaluate toxicity, described further below, and pharmacologic effects of CNP Prodrug and formed CNP, on the CV system in male and female cynomolgus monkeys was carried out

[0469] In this study, male and female cynomolgus monkeys were dosed with vehicle or escalating doses of 150, 300, and 500 µg/kg/dose CNP Prodrug (based on CNP content) by SC injection, with each dose given two weeks apart (Study Days 1, 15, and 29) to avoid accumulation. The design of the study is shown in Table 25-6.

Table 25-6: Design of the Ascending Dose Toxicity and CV Safety Pharmacology Study of CNP Prodrug in Cynomolgus Monkeys

Dose Occasion	Dose (µg/kg based on CNP content)	Dose concentration (µg/mL CNP content) ^a	Number of Animals and Sex
Day 1	0 ^b	0	2 M/2 F
	150 ^c	500	6 M/6 F
Day 15	0 ^b	0	2 M/2 F
	300 ^c	1000	6 M/6 F
Day 29	0 ^b	0	2 M/2 F
	500 ^c	1666.67	6 M/6 F

CNP, C-type natriuretic peptide; CV, cardiovascular; F, female; M, male.

^a Dose volume of 0.3 mL/kg.

^b CNP Prodrug Vehicle (10 mM Histidine pH 5.5, 48 mg/mL Trehalose Dihydrate, 12.4 mg/mL D-Mannitol, 0.73 mg/mL L-Methionine, 0.05 mg/mL Polysorbate 80 in Sterile Water for Injection)

^c CNP Prodrug, formulated in 10 mM Histidine pH 5.5, 48 mg/mL Trehalose Dihydrate, 12.4 mg/mL D-Mannitol, 0.73 mg/mL L-Methionine, 0.05 mg/mL Polysorbate 80 in Sterile Water for Injection.

[0470] Animals' heart rate, systemic arterial pressures (systolic, diastolic, pulse pressure, and mean arterial pressure), ECGs parameters (PR, RR, QRS, QT intervals, and QTc), and body temperature were monitored remotely via surgically implanted DSI PhysioTel® Digital

M11 telemetry devices continuously for 24 hours prior to initiation of dosing and then up to 72 hours after each dose on Study Days 1, 15, and 29.

[0471] There were no CNP Prodrug-related changes in systemic blood pressures (mean arterial pressure, systolic and diastolic blood pressures, pulse pressure) and heart rate, as assessed up to 72 hours post-dose at 150, 300, and 500 µg/kg. An apparent trend for decreased group means heart rate measurements was noted in CNP Prodrug-treated females at 500 µg/kg (Dose 3), more pronounced during the dark cycles, relative to the controls, with the changes occasionally attaining statistical significance. However, the individual heart rate measurements of the CNP Prodrug-treated females at 500 µg/kg remained comparable to their own pre-dose and/or pre-study values. This trend was attributed in part to the additional individual variability resulting from the increased heart rate measurements of one control animal on this occasion.

[0472] Together, these studies confirm that by reducing the steep peaks of CNP exposure seen with daily delivery, CV effects of CNP can be eliminated while maintaining pharmacologic activity on endochondral bone growth.

Example 26: Pharmacokinetics of CNP Prodrug

[0473] Pharmacokinetics of CNP has been extensively evaluated. The characterization of the nonclinical PK of subcutaneously administered CNP Prodrug was conducted in small animals (mice and rats) and large animals (NHPs). These studies were conducted (or are ongoing) in conjunction with toxicity studies that included 3 single-dose studies and 2 repeat-dose studies. Additionally, studies are planned to characterize metabolic stability, plasma protein binding and drug-drug interaction potential of CNP Prodrug and its metabolite (lipid-linker; Metabolite B) released after hydrolysis.

Pharmacokinetics in Mice

[0474] In a single-dose PK study in 10-week old male C57BL/6J mice, single SC injections of 500 µg/kg or 1600 µg/kg of CNP Prodrug were administered. Plasma concentration from each mouse was measured at single time point and for each time point approximately 3 mice were used. Average plasma concentration at each time point was used for non-compartment analysis to determine PK parameters. After SC injection, CNP Prodrug was slowly absorbed in plasma with median T_{max} for a 500 µg/kg dose of 8 hours and 4 hours for a 1600 µg/kg dose. CNP Prodrug had a mean $t_{1/2}$ of approximately 10.5 hours for both dose groups. Dose normalized area under the plasma concentration-time curve (AUC) and C_{max} values were similar in both dose groups, suggesting plasma exposure of CNP Prodrug increased proportionally with dose over a range of 500 to 1600 µg/kg. CNP

was slowly released into plasma with a mean T_{max} of 4 hours for the 500 µg/kg dose and 12 hours for the 1600 µg/kg dose. Similar to CNP Prodrug, released CNP from CNP Prodrug had a longer mean $t_{1/2}$ of 9.6 hours compared to a SC dose of CNP for the 500 µg/kg dose and 9.9 hours for the 1600 µg/kg dose. Dose normalized plasma concentration were similar in both groups. Dose normalized CNP AUC and C_{max} were similar in both dose groups, suggesting plasma exposure of CNP increased proportionally with dose between doses of 500 to 1600 µg/kg. Percent metabolite ratio (released CNP/CNP Prodrug) at C_{max} of was 0.07% for the 500 µg/kg dose and 0.1 % for the 1600 µg/kg dose. Percent metabolite ratio of AUC of released CNP compared with CNP Prodrug was 0.06% for the 500 µg/kg dose and 0.08% for the 1600 µg/kg dose.

Pharmacokinetics in Rats

[0475] In a single-dose PK study in adult rats, CNP Prodrug was administered at 560 µg/kg IV and 150 µg/kg, 280 µg/kg, 560 µg/kg, or 1680 µg/kg SC. After an IV dose of CNP Prodrug, CNP was slowly released, reaching T_{max} at 4 hours. Mean $t_{1/2}$ of released CNP was 10.8 hours, which was similar to CNP Prodrug (8.9 hours). Released CNP metabolite ratio of area under the plasma concentration-time curve from time 0 to infinity ($AUC_{0-\infty}$) was 0.58%. Released CNP mean $t_{1/2}$ was similar in both males (10.7 hours) and females (10.9 hours). However, exposure (AUC and C_{max}) of released CNP was slightly higher in males (60900 h*pM and 2800 pM, respectively) compared to females (45800 h*pM and 2380 pM, respectively). After a SC dose, CNP Prodrug was slowly absorbed in plasma with median T_{max} at 8 hours for all dose groups. The CNP Prodrug mean $t_{1/2}$ ranged from 6.72 hours for the 150 µg/kg SC dose to 10.5 hours for the 1680 µg/kg SC dose. Dose proportional increase in CNP Prodrug exposure were observed with the mean dose normalized exposure ($AUC_{0-\infty}/\text{dose}$) of CNP Prodrug being similar for all SC doses ranging from 150 µg/kg to 1680 µg/kg and dose normalized plasma concentration curves were virtually overlapping. Bioavailability of CNP Prodrug SC doses ranged from 17.67% to 21.71%. No consistent trend for bioavailability between sexes was observed. With the slow release of CNP, the median CNP T_{max} was slightly delayed relative to CNP Prodrug (T_{max} approximately 10-12 hours post-dose). The $t_{1/2}$ for released CNP was similar to CNP Prodrug, ranging from 7.6 to 12.8 hours. Released CNP mean exposure ($AUC_{0-\infty}$) and as C_{max} increased with increasing dose of 150 µg/kg to 280 µg/kg and 560 µg/kg to 1680 µg/kg but was similar for dose 280 µg/kg and 560 µg/kg. Percent metabolite ratio of AUC_{0-t} was higher for the 2 lower dose groups (0.461% for the 150 µg/kg dose and 0.494% for the 280 µg/kg dose) compared to the 2 higher dose groups (0.294% for the 560 µg/kg and 0.323% for the 1680 µg/kg dose). No consistent trend based on sex in exposure of CNP Prodrug and released CNP was

observed. CNP Prodrug C_{max} and AUC increased proportionally with increase in dose from dose range 280 $\mu\text{g/kg}$ to 1680 $\mu\text{g/kg}$. For released CNP, C_{max} and AUC increased proportionally from dose 150 $\mu\text{g/kg}$ to 280 $\mu\text{g/kg}$ and 560 $\mu\text{g/kg}$ to 1680 $\mu\text{g/kg}$. *Pharmacokinetics in NHPs*

[0476] In a repeat-dose study in NHPs, CNP Prodrug was administered by weekly SC injection of 30 $\mu\text{g/kg/week}$ or 150 $\mu\text{g/kg/week}$, based on CNP content, for 1 month. After SC administration, CNP Prodrug was slowly absorbed with a median T_{max} of 24 hours after the first dose on Study Day 1 and 16 hours after multiple weekly doses on Study Day 22 at 30 $\mu\text{g/kg}$ QW and at 48 hours after the first dose on Study Day 1 and 28 hours after multiple weekly doses on Study Day 22 at the 150 $\mu\text{g/kg/week}$ dose. Compared to rodents, CNP Prodrug had a longer mean $t_{1/2}$ of 60 to 65.3 hours, which was consistent for both 30 $\mu\text{g/kg}$ QW and 150 $\mu\text{g/kg}$ QW dose levels. After 3 weeks of 30 $\mu\text{g/kg}$ QW dosing, 2.09, 2.36, 3.37, and 3.15-fold accumulation of CNP Prodrug was observed in C_{max} , AUC_{last} , $AUC_{0-\infty}$ and trough plasma concentration (C_{trough}) at 168 hours, respectively. After 3 weeks of 150 $\mu\text{g/kg}$ QW dosing, 1.24, 1.5, 1.42 and 1.67 -fold accumulation in C_{max} , AUC_{last} , $AUC_{0-\infty}$ and C_{trough} at 168 hours, respectively of CNP Prodrug was observed. The apparent clearance of drug, apparent volume of distribution based upon the terminal phase and $t_{1/2}$ values were similar on Day 1 and Day 22 indicating that CNP Prodrug PK remains consistent after repeat dosing in NHPs.

[0477] Plasma concentrations of released CNP were not quantifiable for the majority of animals treated with 30 $\mu\text{g/kg}$ QW on Study Days 1 and 22. Demonstrating a slow release of CNP, at 150 $\mu\text{g/kg}$ QW, plasma concentrations of released CNP had a median T_{max} of 8 hours after the first dose and 48 hours. After 3 weekly doses of 150 $\mu\text{g/kg}$ QW, 1.88, 2.62, 4.05 and 2.92-fold accumulation of released CNP in C_{max} , AUC_{0-t} , $AUC_{0-\infty}$ and C_{trough} at 168 hours, respectively, was observed. Because of slow release rate and quick clearance of released CNP, $t_{1/2}$ of CNP was similar to CNP Prodrug (65.3 hours on Study Day 1 and 63.8 hours on Study Day 22 at 150 $\mu\text{g/kg}$ QW). Percent metabolite ratio of AUC_{0-t} was could only be computed for 150 $\mu\text{g/kg}$ QW group. After a single dose, the metabolite ratio of AUC_{0-t} was 0.1% and after three weekly doses, on Day 22, it was 0.14%.

[0478] A single-dose PK study was conducted in cynomolgus monkeys in which male and female 2 -year-old cynomolgus monkeys were given a single SC injection of 101 $\mu\text{g/kg}$, 202 $\mu\text{g/kg}$, and 336 $\mu\text{g/kg}$ CNP Prodrug. Sample analysis from this study is currently ongoing. Toxicokinetics are also being evaluated in the ongoing 26-week repeat-dose pharmacology

and toxicology study in NHPs wherein animals are receiving weekly SC injections of 75 µg/kg/week, 200 µg/kg/week, and 350 µg/kg/week CNP Prodrug.

Single-dose Toxicity of CNP Prodrug in NHPs

[0479] A GLP-compliant study was designed to evaluate toxicity and pharmacologic effects of CNP Prodrug and formed CNP, on the CV system in male and female cynomolgus monkeys.

[0480] In this study, male and female cynomolgus monkeys were dosed with vehicle or escalating doses of 150, 300, and 500 µg/kg/dose CNP Prodrug (based on CNP content) by SC injection, with each dose given two weeks apart (Study Days 1, 15, and 29) to avoid accumulation. Each dose was delivered into a unique injection site on the back. Animals in this study were transferred from a prior study following an approximately 7-week washout between studies and, therefore, were not naïve to CNP Prodrug treatment. As a result, although this study is referred to as a single-dose study, animals were not naïve to CNP Prodrug treatment at any dose level. The design of the study is shown in Table 26-1.

Table 26-1: Design of the Ascending Dose Toxicity and CV Safety Pharmacology Study of CNP Prodrug in Cynomolgus Monkeys

Dose Occasion	Dose (µg/kg based on CNP content)	Dose concentration (µg/mL CNP content) ^a	Number of Animals and Sex
Day 1	0 ^b	0	2 M/2 F
	150 ^c	500	6 M/6 F
Day 15	0 ^b	0	2 M/2 F
	300 ^c	1000	6 M/6 F
Day 29	0 ^b	0	2 M/2 F
	500 ^c	1666.67	6 M/6 F

CNP, C-type natriuretic peptide; CV, cardiovascular; F, female; M, male.

^a Dose volume of 0.3 mL/kg.

^b CNP Prodrug Vehicle (10 mM Histidine pH 5.5, 48 mg/mL Trehalose Dihydrate, 12.4 mg/mL D-Mannitol, 0.73 mg/mL L-Methionine, 0.05 mg/mL Polysorbate 80 in Sterile Water for Injection)

^c CNP Prodrug, formulated in 10 mM Histidine pH 5.5, 48 mg/mL Trehalose Dihydrate, 12.4 mg/mL D-Mannitol, 0.73 mg/mL L-Methionine, 0.05 mg/mL Polysorbate 80 in Sterile Water for Injection

[0481] Animals were observed daily at cageside for mortality or gross toxicity and detailed physicals were performed at least weekly during the dosing phase as well as following the 72-hour telemetry monitoring period after each dose. Animals were weighed twice weekly, with one weight collected the day prior to each dose. Funduscopic and biomicroscopic ophthalmology examinations were performed prior to dose initiation and on Study Day 32 (3 days after the final dose). Hematology and clinical chemistry clinical pathology parameters were evaluated approximately 96 hours after each dose of CNP Prodrug. Toxicokinetic samples were collected prior to (0 hour) and 72, 84, and 96 hours after each dose. Ant-drug antibody samples were collected prior to study initiation and on Study Day 33, but only the

Baseline sample will be measured due to expected drug interference in the sample on Study Day 33.

[0482] On Study Day 33, 4 days after animals received the 500 µg/kg dose, all animals underwent a detailed necropsy. The brain, epididymides, adrenal glands, pituitary glands, prostate, thyroid and parathyroid gland, kidneys, liver with gall bladder, ovaries, spleen, testes, and thymus were weighed (when present). A comprehensive list of tissues was collected from each animal for microscopic examination (Table 26-2). A coronal section of the proximal left tibia was collected for histomorphometry of the epiphyseal growth plate.

Table 26-2: Tissues Collected for Microscopic Evaluation in CNP Prodrug-Treated Cynomolgus Monkeys

Aorta	Kidney	Spinal Cord
Brain	Cecum	Spleen
Epididymides	Colon	Stomach
Eye	Liver	Testes
Gallbladder	Lung	Thymus
Adrenal Gland	Axillary Lymph Node	Trachea
Mammary Gland	Mesenteric Lymph Node	Urinary Bladder
Parathyroid Gland	Skeletal Muscle	Uterus/Cervix
Pituitary Gland	Optic Nerve	Vagina
Prostate Gland	Sciatic Nerve	Bone:
Submandibular Salivary Gland	Ovary	Sternum
Seminal Vesicle	Pancreas	Femoral acetabula (bilateral)
Thyroid Gland	Injection Sites (3)	Proximal Femur head (left)
Gut-associated Lymphoid Tissue	Skin	Costochondral junction of sixth rib
Heart	Duodenum	Proximal Tibia (left)
	Ileum	
	Jejunum	

[0483] All animals survived to their scheduled necropsy. There were no test article-related effects on ophthalmology, body temperature, hematology, clinical chemistry, or organ weights. The only test article-related clinical observations were at or adjacent to the injection site, which consisted of slight soft or moderate firm swelling in the lumbar region of the back of 2 male animals 3-4 days after the 500 µg/kg dose (Study Days 32 and 33). Red skin discoloration in the lumbar area was also noted for one female and one male on Study Day 33 (4 days after the 500 µg/kg dose). At necropsy on Study Day 33, dark foci were noted in some animals at all injection sites and nodule and/or mass were observed in a few animals at the 300 and 500 µg/kg injection sites (Table 26-3).

Table 26-3: Macroscopic Findings Adjacent to the Injection Sites in CNP Prodrug-Treated Cynomolgus Monkeys

Site and Gross Finding	Number of Animals with Finding			
	Vehicle-Treated N=2		150/300/500 µg/kg CNP Prodrug-Treated N=6	
	Males	Females	Males	Females
Injection Site 1 (150 µg/kg)				
Focus, dark	0	0	3	2
Nodule	0	0	0	0
Mass	0	0	0	0
Injection Site 2 (300 µg/kg)				
Focus, dark	0	0	2	1
Nodule	0	0	1	0
Mass	0	0	1	1
Injection Site 3 (500 µg/kg)				
Focus, dark	0	0	3	0
Nodule	0	0	1	0
Mass	0	0	1	0

[0484] The injection site (left scapular, Site 1) used for the 150 µg/kg CNP Prodrug dose was associated with mild granulomatous inflammation of the SC tissue in treated males. The injection sites used for the 300 µg/kg CNP Prodrug dose (right scapular; Site 2) and 500 µg/kg CNP Prodrug (mid-dorsal, Site 3) doses were associated with mild to marked granulomatous inflammation. The incidence and severity of these findings is summarized in Table 26-4. Granulomatous inflammation within the SC tissue in treated animals was characterized variably by a core of eosinophilic necrotic debris and/or empty cavities rimmed by numerous epithelioid macrophages and multinucleated giant cells with fewer neutrophils which in turn were rimmed by lymphocytes and fibrous connective tissue. This change correlated to the macroscopic observations of dark focus, nodule and/or mass. Moderate to marked severity at the localized injection site findings, corresponding to a dose of ≥ 300 µg/kg CNP Prodrug were considered adverse under these study conditions. Based on the histology findings in the 150 µg/kg injection site and the features of the histology, a foreign body reaction was determined to be the more likely process responsible for these injection site findings rather than a delayed-type hypersensitivity.

Table 26-4: Summary of Injection Site Related Microscopic Findings in CNP Prodrug-Treated Cynomolgus Monkeys

Site and Microscopic Finding	Number of Animals with Finding/Number Examined			
	Vehicle-Treated		150/300/500 µg/kg CNP Prodrug-Treated	
	Males	Females	Males	Females
Injection Site 1 (150 µg/kg) Inflammation, granulomatous; subcutaneous tissue mild	0/0 ^a	0/0 ^a	2/3	0/2
Injection Site 2 (300 µg/kg) Inflammation, granulomatous; subcutaneous tissue mild moderate marked	0/0 ^a	0/0 ^a	2/4 0/4 1/4	0/2 1/2 0/2
Injection Site 3 (500 µg/kg) Inflammation, granulomatous; subcutaneous tissue mild marked	0/2 0/2	0/2 0/2	2/6 1/6	0/6 0/6

^a Injection sites 1 and 2 were only observed microscopically if associated with gross lesions.

[0485] In the bones (proximal femur [femoral head and/or greater trochanter], acetabulum, rib, sternum and/or tibia), there was a minimal increase in cellularity of the hypertrophic zone of the physis in CNP Prodrug treated animals of both sexes with increased incidence in males compared to females. This was characterized by an increased number and/or size of chondrocytes or their lacunar spaces within the zone of hypertrophy. The change correlated to increased thickness of the growth plate and the hypertrophic zone of the physis of the proximal tibia on histomorphometric analysis. These findings were of a minimal nature, did not result in architectural alteration within growth plates or associated bones and joints, and as such were not considered adverse under these study conditions.

[0486] Mean thickness of the total growth plate and the hypertrophic zone of the proximal tibial physis was increased in CNP Prodrug treated animals from both sexes compared to concurrent controls. The increase of the total epiphyseal growth plate thickness was 5% and 9%, in males and females, respectively. In males, this resulted from increased individual measurements from most animals, while in females, the increase was mostly due to 2 animals exceeding the control range. the hypertrophic zone thickness increase was 32% and 22%, in males and females, respectively. In both sexes, this resulted from increased individual measurements from almost all animals, correlating to increased cellularity of the

hypertrophic zone noted microscopically. The proliferative zone was not affected by CNP Prodrug treatment, as expected based on previous studies of CNP. These changes in the growth plate are likely mostly attributed to the 500 µg/kg dose of CNP Prodrug but may be a cumulative effect of the ascending doses of CNP Prodrug given in this study.

[0487] In conclusion, CNP Prodrug did not cause adverse systemic effects when given as a single ascending SC injections of 150, 300, or 500 µg/kg CNP Prodrug with a 14-day interval between each dose. Therefore, the NOAEL for systemic effects was identified as 500 µg/kg. Due to the severity of effects at injection sites 2 and 3, the localized NOAEL was determined to be 150 µg/kg. CNP has previously been shown to be mildly more irritating than vehicle at the injection site, so in this study, CNP Prodrug appears to be less tolerable at the SC injection site. In the 1-month repeat-dose pharmacology study of CNP Prodrug in male NHPs, doses of 30 or 150 µg/kg/week CNP Prodrug did not result in microscopic changes at the SC injection site, in contrast to the effects observed at the 150 µg/kg injection site in this study. The reproducibility of the injection site findings in this study is being evaluated in a 26-week repeat-dose pharmacology and toxicology study in NHPs at doses up to 350 µg/kg/week CNP Prodrug (based on CNP content) to support repeat-dose clinical studies. In the proposed Phase 1 study, all participants will be naïve to treatment at the time of injection and will receive only a single dose of CNP Prodrug.

Repeat-dose Toxicity of CNP Prodrug 1-Month Repeat-dose Toxicity in Male NHPs

[0488] A non-GLP repeat-dose pharmacology study of CNP Prodrug was conducted in male NHPs that included some assessments of safety/tolerability. Male cynomolgus monkeys were dosed weekly by SC injection of vehicle or CNP Prodrug at 30 or 150 µg/kg/week (based on CNP content) for a total of 5 doses. Injections were given in the back in 3 injection sites, with Sites 1 and 2 receiving 2 doses (Study Days 1 and 15 and Study Days 8 and 22, respectively). The final injection on Study Day 29 was given in a naïve site, Site 3.

[0489] Toxicity was assessed by twice daily observations at cageside for mortality and gross toxicity, weekly detailed clinical observations, weekly body weights, daily qualitative food consumption, and clinical pathology (hematology and clinical chemistry) prior to each dose and prior to necropsy on Study Day 31. On Study Day 31, animals underwent a detailed necropsy. All 3 injection sites were collected and examined microscopically.

[0490] Weekly doses of up to 150 µg/kg/week CNP Prodrug were well tolerated with no apparent adverse effects observed. There were no test article-related effects on clinical

observations, body weight, food consumption, clinical chemistry, hematology, or the injection sites.

Chronic Repeat-Dose Toxicity in NHPs

[0491] The 6-month duration was selected to allow for comparability to a similar previous study of CNP in juvenile NHPs. Although a 10-month study of CNP was also conducted in NHPs, that study was conducted in older animals to assess impacts on sexual maturation and did not include extensive skeletal endpoints to evaluate pharmacologic effects. The longer duration of the 10-month study in NHPs of CNP did not identify any unique effects related to CNP and the lower NOAEL reflected the older age and larger size of the animals that increased CNP plasma levels during the duration of the study rather than an impact of longer treatment.

[0492] In this study, male and female 2 to 3-year-old cynomolgus monkeys are being given a weekly SC dose of vehicle or 75, 200, or 350 µg/kg/dose CNP Prodrug (based on CNP content) for 26 weeks. Following a 26-week treatment period, animals treated with vehicle or 350 µg/kg/week CNP Prodrug will undergo a 6-week recovery period to determine the reversibility of any observed effects. The design of the study is shown in Table 26-5.

Table 26-5: Design of the 26-Week Pharmacology and Toxicology Study in Cynomolgus Monkeys

Group	Treatment	Dose ^a (µg/kg CNP Content)	Number of Animals and Sex	
			Main Study	Recovery Study
1	Vehicle ^b	0	5 M/5 F	3 M/3 F
2		75	5 M/5 F	NA
3		200	5 M/5 F	NA
4		350	5 M/5 F	3 M/3 F

CNP, C-type natriuretic peptide; F, female; M, male; NA, not applicable.

^a 0.3 mL/kg Dose Volume.

^b CNP Prodrug Vehicle (10 mM Histidine pH 5.5, 48 mg/mL Trehalose Dihydrate, 12.4 mg/mL D-Mannitol, 0.73 mg/mL L-Methionine, 0.05 mg/mL Polysorbate 80 in Sterile Water for Injection).

^c CNP Prodrug, formulated in 10 mM Histidine pH 5.5, 48 mg/mL Trehalose Dihydrate, 12.4 mg/mL D-Mannitol, 0.73 mg/mL L-Methionine, 0.05 mg/mL Polysorbate 80 in Sterile Water for Injection

[0493] Doses are being delivered by SC injection in 4 rotating injection sites on the back and the final dose will be given in a fifth, naïve, injection site. Animals are observed twice daily for mortality and evidence of gross toxicity and cageside daily prior to dosing and approximately 45 minutes after the completion of dosing for the group on dosing days or once daily on non-dosing days. Detailed physicals are conducted weekly. Animals are weighed weekly and food consumption is monitored qualitatively daily. Funduscopic and biomicroscopic ophthalmic examinations are being conducted pre-study, during Study Week 15 and 26, and at the end of the recovery period (Study Week 32). Prior to study initiation, animals were implanted with DSI PhysioTel® Digital M11 telemetry implants, allowing for

monitoring of blood pressure, ECG waveforms, and body temperature once pre-study and on Study Days 15, 57, 106, and 162 as well as during the recovery phase on Study Week 32. The pre-study and recovery evaluations will be conducted for 24 hours, starting as close as possible to the expected time of monitoring during the dosing period. During the dosing phase, animals will be monitored for 2 hours pre-dose and at least 24 hours post-dose. Clinical pathology (hematology, coagulation, clinical chemistry, urinalysis, and urine chemistry) will be evaluated twice pre-study then on Study Days 4, 46, 81, 123, 151, and 184 of the dosing phase and during the recovery phase on Study Day 225. Densitometry scans and assessments will be performed using DXA and peripheral quantitative computed tomography (pQCT). Dual energy X-ray absorptiometry scans of the whole body and right proximal femur will be conducted prior to study initiation, on Study Weeks 13/14 and 25/26 on all animals, and during the recovery phase during Study Week 32. The right proximal tibia will be scanned by pQCT once prior to study initiation, on Study Weeks 13/14 and 25/26 on all animals and on Study Week 32 during the recovery phase. Radiographs of bilateral femora, tibiae, radii and ulnae and the lumbar and thoracic spine will be conducted prior to study initiation, during Study Weeks 12/13 and 24/25 and during the recovery phase during Study Week 31. Radiographs will be evaluated qualitatively for abnormalities and for skeletal maturity. Tibia, femur, and ulna lengths (right and left) will be derived from the radiograph measurements. Toxicokinetic of CNP Prodrug and formed CNP will be evaluated on Study Days 1, 92, and 176 at 0, 8, 12, 24, 36, 48, 96, and 168 hours post-dose.

[0494] On Study Day 184, 5 males and 5 females/group will be euthanized and undergo a detailed necropsy. After a 6-week recovery, 3 males and 3 females in Groups 1 and 4 will be euthanized and undergo a detailed necropsy. Organ weights and a comprehensive list of tissues will be collected from all animals and tissues will be evaluated microscopically. The width and length of the skull and foramen magnum will be measured *ex vivo*. *Ex vivo* μ CT and a 3-point bend test of the femur will also be conducted. The proximal left tibia will be collected for histomorphometry.

Table 26.6 Pharmacokinetics: A 10-Day Pharmacokinetic Study of CNP Prodrug by a Single Intravenous or Subcutaneous Administration in Non-Human Primates

		Test Article: CNP Prodrug		
Species	Cynomolgus monkeys			
Feeding Condition:	Ad libitum			
Sample (e.g., whole blood, plasma, serum):	Plasma			
Analyte:	CNP Prodrug and vosoritide			
Assay:	LC-MS/MS			
Vehicle/Formulation:	10mM Histidine pH 5.5, Trehalose Dihydrate (48 mg/mL), D-Mannitol (12.4 mg/mL), L-Methionine (0.73 mg/mL), Polysorbate 80 (0.05 mg/mL)			
Additional Dosing Information: Animals were dosed by subcutaneous injection at 102.3, 205.8, and 352.8 µg/kg and by intravenous injection at 100.5 µg/kg. These were the actual doses delivered due to a formulation calculation error where all doses were only ~66% of the nominal dose levels, which were 150, 300, and 500 µg/kg CNP content for subcutaneous injection and 150 µg/kg CNP for intravenous injection.				
Method of Administration:	Intravenous	Subcutaneous	Subcutaneous	Subcutaneous
Dose (µg/kg CNP content)	100.5	102.3	205.8	352.8
Sex	Combined			
C ₀ (pmol/L)	307000 ±138000	NA	NA	NA
C _{max} (pmol/L)	288000 ±115000	116000 ±20500	154000 ±74800	324000 ±116000
C _{max} /Dose (pmol/L/(µg/kg))	2870 ±1140	1140 ±201	750 ±364	919 ±329
t _{max} ^a (hr)	1.00 (1.00 - 2.00)	8.00 (4.00 - 24.0)	12.0 (8.00 - 24.0)	8.00 (4.00 - 12.0)
t _{last} ^a (hr)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)
AUC _{tlast} (hr*pmol/L)	12100000 ±4380000	10700000 ±2260000	14900000 ±6910000	28500000 ±14800000
AUC _{tlast} /Dose (hr*pmol/L/(µg/kg))	120000 ±43600	104000 ±22100	72500 ±33600	80800 ±41900
AUC _{0-inf} (hr*pmol/L)	12300000 ±7350000	13600000 ±NA	16700000 ±9050000	31100000 ±18300000
t _{1/2} (hr)	62.2 ±3.07	61.7 ±NA	59.2 ±5.45	60.9 ±7.52
F (%)	NA	86.5	60.2	67.2

				Test Article: CNP Prodrug
CI (L/hr/kg)	0.00270 ±0.00195	0.00185 ±NA	0.00395 ±0.00250	0.00335 ±0.00129
Vz (L/kg)	0.243 ±0.174	0.164 ±NA	0.328 ±0.180	0.292 ±.124
Males				
C ₀ (pmol/L)	391000 ±73000	NA	NA	NA
C _{max} (pmol/L)	339000 ±52400	113000 ±31600	119000 ±48200	237000 ±42000
C _{max} /Dose (pmol/L/(µg/kg))	3370 ±522	1110 ±309	579 ±234	673 ±119
t _{max} ^a (hr)	1.00 (1.00 - 1.00)	8.00 (4.00 - 8.00)	12.0 (8.00 - 24.0)	8.00 (4.00 - 8.00)
t _{last} ^a (hr)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)
AUC _{tlast} (hr*pmol/L)	12600000 ±960000	9430000 ±2530000	11200000 ±5160000	18200000 ±2040000
AUC _{tlast} /Dose (hr*pmol/L/(µg/kg))	126000 ±9550	92100 ±24700	54500 ±25100	51700 ±5770
AUC _{0-inf} (hr*pmol/L)	12200000 ±NA	NA	6830000 ±NA	20600000 ±NA
t _{1/2} (hr)	59.0 ±NA	NA	52.2 ±NA	63.8 ±NA
F (%)	NA	73.4	43.4	41.2
CI (L/hr/kg)	0.00200 ±NA	NA	0.00730 ±NA	0.00420 ±NA
Vz (L/kg)	0.171 ±NA	NA	0.554 ±NA	0.386 ±NA
Females				
C ₀ (pmol/L)	224000 ±146000	NA	NA	NA
C _{max} (pmol/L)	238000 ±150000	119000 ±5570	190000 ±89200	411000 ±97100
C _{max} /Dose (pmol/L/(µg/kg))	2370 ±1490	1160 ±54.4	921 ±434	1160 ±275
t _{max} ^a (hr)	2.00 (1.00 - 2.00)	24.0 (4.00 - 24.0)	12.0 (12.0 - 24.0)	12.0 (8.00 - 12.0)
t _{last} ^a (hr)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)

				Test Article: CNP Prodrug
AUC_{last} (hr*pmol/L)	11600000 ±6800000	11900000 ±1360000	18600000 ±7160000	38800000 ±15000000
AUC_{last}/Dose (hr*pmol/L/(µg/kg))	115000 ±67700	116000 ±13300	90600 ±34800	110000 ±42500
AUC_{0-Inf} (hr*pmol/L)	12400000 ±NA	13600000 ±NA	20000000 ±7590000	41500000 ±NA
t_{1/2} (hr)	63.8 ±NA	61.7 ±NA	61.5 ±3.51	58.0 ±NA
F (%)	NA	101	78.6	95.5
Cl (L/hr/kg)	0.00305 ±NA	0.00185 ±NA	0.00283 ±0.00137	0.00250 ±NA
V_z (L/kg)	0.279 ±NA	0.164 ±NA	0.253 ±0.121	0.198 ±NA
Released vosoritide Pharmacokinetic Parameters				
Combined				
C_{max} (pmol/L)	195 ±88.6	54.9 ±3.84	79.6 ±37.0	195 ±67.8
C_{max}/Dose (pmol/L/(µg/kg))	1.94 ±0.882	0.537 ±0.0376	0.387 ±0.180	0.552 ±0.192
t_{max}^a (hr)	1.00 (1.00 - 4.00)	16.0 (4.00 - 24.0)	10.0 (4.00 - 12.0)	8.00 (4.00 - 8.00)
t_{last}^a (hr)	120 (72.0 - 168)	48.0 (24.0 - 72.0)	72.0 (24.0 - 120)	120 (72.0 - 168)
AUC_{last} (hr*pmol/L)	11200 ±8390	2100 ±1080	4480 ±3150	13400 ±8010
AUC_{last}/Dose (hr*pmol/L/(µg/kg))	112 ±83.5	20.6 ±10.5	21.7 ±15.3	37.9 ±22.7
AUC₀₋₂₄ (hr*pmol/L)	20300 ±8300	NA	9650 ±NA	18300 ±9070
AUC_{0-240hr}/Dose (hr*pmol/L/(µg/kg))	202 ±82.5	NA	46.9 ±NA	51.8 ±25.7
vosoritide: CNP Prodrug C_{max} (%)	0.0683 ±0.0131	0.0486 ±0.0107	0.0518 ±0.00315	0.0606 ±0.00798
vosoritide: CNP Prodrug AUC_{last} (%)	0.0923 ±0.0586	0.0187 ±0.00664	0.0267 ±0.00935	0.0455 ±0.00667
Males				

				Test Article: CNP Prodrug
C_{max} (pmol/L)	258 ±53.0	56.1 ±3.86	60.8 ±25.0	149 ±30.8
C_{max}/Dose (pmol/L/(µg/kg))	2.57 ±0.528	0.549 ±0.0377	0.295 ±0.121	0.422 ±0.0873
t_{max}^a (hr)	1.00 (1.00 - 1.00)	8.00 (4.00 - 24.0)	12.0 (8.00 - 12.0)	8.00 (4.00 - 8.00)
t_{last}^a (hr)	168 (168 - 168)	24.0 (24.0 - 48.0)	72.0 (24.0 - 72.0)	120 (72.0 - 120)
AUC_{tlast} (hr*pmol/L)	17700 ±6880	1350 ±820	2540 ±1790	7520 ±1010
AUC_{tlast}/Dose (hr*pmol/L/(µg/kg))	176 ±68.5	13.2 ±8.02	12.4 ±8.72	21.3 ±2.87
AUC₀₋₂₄ (hr*pmol/L)	20300 ±8300	NA	NA	8550 ±NA
AUC_{0-240hr}/Dose (hr*pmol/L/(µg/kg))	202 ±82.5	NA	NA	24.2 ±NA
vosoritide: CNP Prodrug C_{max} (%)	0.0757 ±0.00552	0.0522 ±0.0156	0.0509 ±0.000405	0.0630 ±0.0108
vosoritide: CNP Prodrug AUC_{tlast} (%)	0.138 ±0.0461	0.0136 ±0.00441	0.0206 ±0.00838	0.0416 ±0.00732
Females				
C_{max} (pmol/L)	132 ±70.2	53.7 ±4.19	98.5 ±41.7	241 ±65.0
C_{max}/Dose (pmol/L/(µg/kg))	1.31 ±0.699	0.525 ±0.0409	0.479 ±0.203	0.682 ±0.184
t_{max}^a (hr)	2.00 (1.00 - 4.00)	24.0 (4.00 - 24.0)	4.00 (4.00 - 12.0)	8.00 (4.00 - 8.00)
t_{last}^a (hr)	72.0 (72.0 - 72.0)	72.0 (48.0 - 72.0)	120 (72.0 - 120)	168 (120 - 168)
AUC_{tlast} (hr*pmol/L)	4720 ±1580	2860 ±736	6410 ±3230	19200 ±7530
AUC_{tlast}/Dose (hr*pmol/L/(µg/kg))	47.0 ±15.7	27.9 ±7.20	31.1 ±15.7	54.5 ±21.3
AUC₀₋₂₄ (hr*pmol/L)	NA	NA	9650 ±NA	21500 ±7780
AUC_{0-240hr}/Dose (hr*pmol/L/(µg/kg))	NA	NA	46.9 ±NA	61.0 ±22.0

Test Article: CNP Prodrug				
vosoritide: CNP Prodrug C_{max} (%)	0.0609 ±0.0152	0.0451 ±0.00222	0.0527 ±0.00469	0.0583 ±0.00516
vosoritide: CNP Prodrug AUC_{last} (%)	0.0467 ±0.0150	0.0238 ±0.00360	0.0329 ±0.00592	0.0494 ±0.00356

Note: SD and CV are not calculated or not reported when mean concentration equals zero or N<3.
 Bioavailability (%F) = (AUC/Dose Group 2, 3, or 4 - SC / AUC/Dose Group 1 - IV) * 100.

AUC_{last}, Area under the concentration-time curve from time zero to 96 hours by the linear trapezoidal method for increasing concentrations and log trapezoidal method; AUC_{last}/Dose, The AUC_{last} divided by pMol/kg of the dose administered; AUC_(0-inf), Area under the concentration-time curve from time zero to infinity following the last measurable concentration; C₀, Concentration at time 0 for an initial IV bolus dose extrapolated from initial measured plasma concentrations. C_{max}, Maximum observed concentration; C_{max}/Dose, The C_{max} divided by pMol/kg of the dose administered; Cl, Apparent clearance; CNP, C-type natriuretic peptide (39 amino acid); F, Bioavailability; T_{max}, Time of maximum observed concentration; t_{1/2}, Elimination half-life; NA, Not available; V_z, Apparent volume of distribution.

^a Median (minimum - maximum).

Table 26.7 Pharmacokinetics: A 1-Month Study of CNP Prodrug by Subcutaneous Injection in Monkeys

Test Article: CNP Prodrug	
Species	Cynomolgus monkey
Feeding Condition:	Ad libitum
Sample (Analyte):	Plasma (CNP Prodrug and vosoritide [PK] and ANP), serum (Collagen type X, BAP, Pro-C1, Pro-C2, and T2CM), and urine (cGMP, CTx-I, and CTx-II)
Assay:	LC-MS/MS (plasma), CLIA or ECLA (serum), and ELISA (serum and urine)
Vehicle/Formulation:	0.005 mol/L citrate buffer solution, pH 5.5, containing 5.25% (w/v) sucrose, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) polysorbate 80 prepared in sterile water for injection, USP
Method of Administration:	Subcutaneous Injection

Dose (µg/kg CNP content)	0 (Control)	30	150
Number of Animals/Group^a:	M	M	M
Main:	4	4	4
<u>vosoritide</u>			
Day 1			
t _{max} (h)	NA	NC	7

Dose (µg/kg CNP content)	0 (Control)	30	150
Number of Animals/Group ^a :	M	M	M
Main:	4	4	4
C _{max} (pM)	NA	NC	81.9
AUC _{tlast} (hr*pM)	NA	NC	6630
AUC ₀₋₁₆₈ (hr*pM)	NA	NC	6630
AUC _(0-∞) (hr*pM)	NA	NC	9660
t _{1/2} (h)	NA	NC	65.3
Day 22			
t _{max} (h)	NA	16	48
C _{max} (pM)	NA	20.2	160
AUC _{tlast} (hr*pM)	NA	815	17900
AUC ₀₋₁₆₈ (hr*pM)	NA	NC	20200
AUC _(0-∞) (hr*pM)	NA	NC	30500
t _{1/2} (h)	NA	NC	61.2
Accumulation Ratio			
R _(Cmax)	NA	NC	1.88
R _(AUC_{tlast})	NA	NC	2.62
R _(AUC_{0-∞})	NA	NC	4.05
R _(C₁₆₈)	NA	NC	2.92
<u>CNP Prodrug</u>			
Day 1			
t _{max} (h)	NA	22	38
C _{max} (pM)	NA	8300	86800
AUC _{tlast} (hr*pM)	NA	813000	8230000
AUC ₀₋₁₆₈ (hr*pM)	NA	813000	8230000

Dose (µg/kg CNP content)	0 (Control)	30	150
Number of Animals/Group ^a :	M	M	M
Main:	4	4	4
AUC _(0-∞) (hr*pm)	NA	491000	9670000
t _{1/2} (h)	NA	60	65.3
Day 22			
t _{max} (h)	NA	14.5	28
C _{max} (pm)	NA	15500	105000
AUC _{tlast} (hr*pm)	NA	1670000	12500000
AUC ₀₋₁₆₈ (hr*pm)	NA	1670000	12500000
AUC _(0-∞) (hr*pm)	NA	NC	12000000
t _{1/2} (h)	NA	NC	63.8
Accumulation Ratio			
R _(Cmax)	NA	2.09	1.24
R _(AUC_{tlast})	NA	2.36	1.50
R _(AUC_{0-∞})	NA	3.37	1.42
R _(C₁₆₈)	NA	3.15	1.67
Normalized cGMP/Creatinine			
Day 9	559	700	819
Day 13	715	727	748
Day 16	537	732	848
Day 20	663	668	723
Day 23	557	568	730
Day 27	641	646	660
Day 30	589	658	755
Change from baseline Normalized cGMP/Creatinine			
Day 9	-40.5	96.1	223
Day 13	103	123	152

Dose (µg/kg CNP content)	0 (Control)	30	150
Number of Animals/Group ^a :	M	M	M
Main:	4	4	4
Day 16	-105	83.9	252
Day 20	76	63.9	127
Day 23	-48	-80.2	134
Day 27	54.2	42.5	63.9
Day 30	-10	53.8	159
Change from baseline BAP (ng/mL)			
Day 29	-88.5	-34.4	-40.7
Day 31	-71.6	-39.9	-64.2
Additional Information:	Results of ANP in plasma and Collagen type X in serum were generally or completely BLQ and CTx-II normalized to urine creatinine concentrations at most timepoints were above the ULQ.		

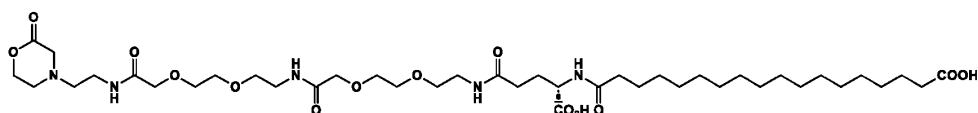
ANP, Atrial natriuretic peptide; AUC₀₋₁₆₈, the area under the concentration time curve from time zero to 168 hours following dosing; AUC_(0-∞), area under the concentration-time curve from time zero extrapolated to infinity; AUC_{tlast}, the area under the concentration versus time curve from the start of dose administration to the last observed quantifiable concentration; cGMP, cyclic guanosine monophosphate; BAP, Bone-specific Alkaline Phosphatase; BLQ, Below the limit of quantitation; cGMP, cyclic guanosine monophosphate; C_{max}, maximum observed concentration measured after dosing; CNP, C-type natriuretic peptide (39 amino acid); CTx-I, Telopeptide of Type I collagen; CTx-II, Telopeptide of Type II collagen; M, male; NA, Not applicable; NC, Not calculated; Pro-C1, N terminal Pro-peptide of Type I collagen; Pro-C2, Released N-terminal Pro-peptide of Type II collagen; R_(AUC_{0-∞}), the area under the curve from time zero extrapolated to infinity after repeat doses divided by the area under the curve from time zero extrapolated to infinity during the initial dosing interval; R_(AUC_{tlast}), the area under the curve from 0 to T2 after repeat doses divided by the area under the curve from 0 to T2 during the initial dosing interval; R_(C₁₆₈), the trough concentration at 168 hours after repeat doses divided by the trough concentration at 168 hours during the initial dosing interval; R_(C_{max}), the C_{max} after repeat doses divided by the C_{max} during the initial dosing interval; t_{max}, the time after dosing at which the maximum concentration was observed; t_{1/2}, terminal half-life; T2CM, Neo-epitope of MMP-1 and MMP-13 mediated degradation of Type II collagen; ULQ, Upper limit of quantitation.

^a The n was between 0 and 4 for each parameter/group

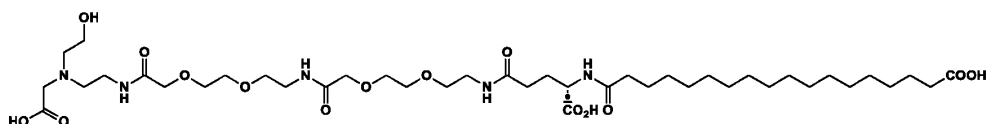
Toxicology of CNP Prodrug Inactive Metabolite Isoforms

[0495] CNP Prodrug is comprised of CNP bound via the Lys²⁷ side chain to a pH responsive self-immolative linker, a spacer, and C-18 fatty acid albumin binding domain. Upon hydrolysis of the linker, active CNP is formed along with 2 major pharmacologically inactive metabolite isoforms consisting of the albumin-binding C-18 fatty acid and hydrolyzed linker, referred to as Metabolites A and B. Metabolite A is unstable and rapidly forms Metabolite B; therefore, the major metabolite isoform that is likely to be detected in plasma of both humans and nonclinical species is Metabolite B.

Structure of Metabolite A



Structure of Metabolite B



[0496] A hydrolysis assay of the CNP Prodrug and lipid linker in Phosphate buffered saline, pH 7.4 and 9.4 is conducted. Both Metabolites A and B are expected to be formed *in vivo* following hydrolysis of the bond to Lys²⁷ of active CNP. Formation would be based on the rate of vosoritide release from CNP Prodrug via hydrolysis of the linker at a constant rate (zero order kinetics) under physiological conditions

[0497] The stability of BMN 333 and lipid-linker in human liver microsomes was evaluated. When incubated with human liver microsomes, a time-dependent loss of CNP Prodrug was observed. However, this loss was also observed in the absence of nicotinamide adenine dinucleotide phosphate (NADPH), indicating the loss of CNP Prodrug was not due to CYP activity. Metabolite B (lipid-linker) was stable in human liver microsomes ($\pm$ NADPH) for up to 1 hour, also suggesting it is not a CYP substrate.

[0498] Both Metabolites A and B are expected to be formed *in vivo* in NHPs at a similar proportion to CNP Prodrug as in humans, as the rate of CNP Prodrug hydrolysis is constant at physiologic pH, allowing the evaluation of toxicity of each to be incorporated into the general toxicity studies of CNP Prodrug, as recommended by FDA Guidance on *Safety Testing of Drug Metabolites* (March 2020).

[0499] An *in silico* screening assessment of Metabolites A and B will be conducted by means of evaluation by Derek (v. 6.3.0), Leadscape Model Applier (v. 2023-0.0.15), and Organisation for Economic Co-operation and Development (OECD) Quantitative Structure-activity Relationship (QSAR) Toolbox (v. 4.6). Derek and OECD QSAR Toolbox will be utilized for the purposes of QSAR and read-across assessment of the metabolite isoforms.

[0500] Metabolite B will be evaluated by *in vitro* assays for mutagenicity and clastogenicity. Mutagenicity will be assessed in a non-GLP bacterial reverse mutation assay using *S. typhimurium* strains T98, TA100, TA1535, and TA1537 and *E. coli* strain WP2 uvrA using the plate incorporation method, with or without metabolic activation with rat liver post-mitochondrial fraction (S-9). Clastogenicity will be assessed in an *in vitro* micronucleus assay in human peripheral blood lymphocytes with or without metabolic activation with rat S-9. Both assays will use internationally recognized and validated techniques in full accordance with the ICH Guideline S2(R1): *Guidance on Genotoxicity Testing and Data Interpretation for Pharmaceuticals Intended for Human Use* (June 2012).

[0501] Together, these studies have demonstrated that CNP Prodrug treatment has the same primary pharmacodynamic effect as CNP: a dose-responsive increase in the hypertrophic zone of epiphyseal growth plates. As with CNP treatment, this effect on the growth plate leads to endochondral bone growth, as demonstrated by increased bone lengths and body length increases. As observed in the CNP nonclinical program, high doses, particularly in WT rodents, leads to exaggerated pharmacologic effects including overgrowth related DJD and/or fracture. These effects are currently being evaluated in a more translatable model to human patients, juvenile NHPs dosed weekly for 26 weeks.

[0502] Nonclinical PK studies have confirmed a long half-life in plasma in NHPs and a markedly shorter $t_{1/2}$ in rodents, consistent with the expected effect on $t_{1/2}$ with the albumin-binding lipid region of CNP Prodrug. The low metabolite ratio and similar T_{max} and $t_{1/2}$ for released CNP to CNP Prodrug are consistent with the slow release of CNP from CNP Prodrug but an unchanged clearance mechanism for CNP.

[0503] A GLP ascending dose toxicity and CV safety pharmacology study of CNP Prodrug in NHPs has been conducted that identified a NOAEL for systemic toxicity of 500 µg/kg (based on CNP content). This study confirmed that with CNP Prodrug, the desired primary pharmacodynamics of CNP are retained; however, CNP related effects on hemodynamics (ie, reduce blood pressure and increased heart rate) are absent at doses up to 500 µg/kg CNP Prodrug in NHPs. CNP Prodrug related changes at the injection site, consisting of

granulomatous inflammation at 150 µg/kg CNP Prodrug became adverse due to the presence of necrosis at single doses $\geq$ 300 µg/kg CNP Prodrug in NHPs; however, the reproducibility of these findings is questionable given the absence of test article related injection site lesions in male NHPs dosed weekly with up to 150 µg/kg CNP Prodrug for 1 month and is being further investigated in the 6-month repeat-dose pharmacology and toxicology study in NHPs at doses up to 350 µg/kg QW prior to initiation of repeat-dose clinical studies.

[0504] Overall, these studies inform and support the clinical evaluation of CNP Prodrug in an initial Phase 1 single ascending dose clinical study in healthy adult participants with a 10X safety margin between the NOAEL in NHPs for local tolerance and the planned starting dose of 15 µg/kg, based on dose.

[0505] Single Dose Pharmacokinetic Study of CNP Prodrug in Adult Wild Type Male Mice

[0506] Study Title: Pilot Pharmacokinetic Study of CNP Prodrug in Adult Wild Type Male Mice Following a Single Subcutaneous Bolus Dose of Either 500 or 1600 µg/kg

[0507] The non-GLP study was designed to characterize the pharmacokinetic properties of CNP Prodrug and released vosoritide in adult wild type mice after a single subcutaneous (SC) dose of CNP Prodrug.

[0508] Methods

[0509] A total of 51 male C57BL/6J mice (Jackson Laboratories, Bar Harbor, ME, USA) were 10 weeks old with body weights ranging from 20 to 30 grams at initiation of dosing. Animals were divided into 17 groups (3/group) where 8 groups were dosed 627 µg/kg (500 µg/kg target dose) CNP Prodrug (Groups A, C, E, G, J, L, N and Q), 8 groups were dosed 1891 µg/kg (1600 µg/kg target dose) CNP Prodrug (Groups B, D, F, H, K, M, P, and R), and 1 group (Group S) was untreated. A terminal blood sample was collected via cardiac puncture from 3 mice/timepoint at 1, 4, 8, 12, 24, 36, 48, or 60 hrs following subcutaneous administration of test article as specified by animal group. Samples were analyzed using UPLC-MS/MS analysis. LLOQ for vosoritide and CNP Prodrug was 50 and 2500 pM, respectively. For NCA analysis, the dose levels were converted from mass to molar units using the molecular weight (MW) of vosoritide (4102.78 g/mol). The design of the study is shown in Table 26.8.

Table 26.8: Study Design of the Single Dose Pharmacokinetic Study of CNP Prodrug in Adult Wild Type Male Mice

Group ¹	Test Article	CNP Target Dose ^a (µg/kg)	CNP Actual Dose ^a (µg/kg)	Dose Route	PK sampling time (500 µL)
A	CNP Prodrug ^b	500	627	SC	1 hr
B	CNP Prodrug ^b	1600	1891	SC	1 hr
C	CNP Prodrug ^b	500	627	SC	4 hr
D	CNP Prodrug ^b	1600	1891	SC	4 hr
E	CNP Prodrug ^b	500	627	SC	8 hr
F	CNP Prodrug ^b	1600	1891	SC	8 hr
G	CNP Prodrug ^b	500	627	SC	12 hr
H	CNP Prodrug ^b	1600	1891	SC	12 hr
J	CNP Prodrug ^b	500	627	SC	24 hr
K	CNP Prodrug ^b	1600	1891	SC	24 hr
L	CNP Prodrug ^b	500	627	SC	36 hr
M	CNP Prodrug ^b	1600	1891	SC	36 hr
N	CNP Prodrug ^b	500	627	SC	48 hr
P	CNP Prodrug ^b	1600	1891	SC	48 hr
Q	CNP Prodrug ^b	500	627	SC	60 hr
R	CNP Prodrug ^b	1600	1891	SC	60 hr
S	NA	0	0	NA	NA

CNP, C-natriuretic peptide; hr, hour; M, male; NA, not applicable; PK, pharmacokinetics; SC, subcutaneous. ¹each group consisted of 3 male mice; ^aDoses are based on CNP content in CNP Prodrug. ^bCNP Prodrug formulated in 0.005 mol/L citrate buffer solution, pH 5.5 containing 5.8% (w/v) trehalose dihydrate, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) Polysorbate 80.

[0510] Results

[0511] The summary of PK parameters of CNP Prodrug and released vosoritide obtained from the non compartmental analysis (NCA) of average plasma concentrations at each timepoint after SC doses of 627 and 1891 µg/kg CNP Prodrug are presented in Table 26.9. Plasma concentration profiles of released vosoritide (BMN 111) and CNP Prodrug (BMN 333) are presented in Figure 28.

[0512] Shown in Figure 28 is plasma CNP Prodrug and released vosoritide concentration-time profiles separated by Dose (A) and separated by analyte (B). Each plot shows a

trendline of either CNP Prodrug or released vosoritide concentration in plasma and 95% confidence intervals (CI) generated by the LOESS algorithm. Each data point represents the plasma concentration of CNP Prodrug and released vosoritide from a single mouse.

[0513] After SC injection, CNP Prodrug was slowly absorbed in plasma with median Tmax of 4-8 hrs following the 627 µg/kg dose and 4 hrs following the 1891 µg/kg dose. CNP Prodrug showed a long mean $t_{1/2}$ of approximately 10.5 hrs for both dose groups, likely due to albumin binding. The dose-normalized area under the plasma concentration-time curve (AUC) and Cmax values were similar in both dose groups, suggesting that the plasma exposure of CNP Prodrug increased proportionally with dose over a range of 627 to 1891 µg/kg. This also suggests that the albumin binding was not saturated at the high dose of CNP Prodrug.

[0514] Vosoritide was slowly released into plasma with a mean Tmax of 4 hrs for the 627 µg/kg dose and 12 h for the 1891 µg/kg dose. Released vosoritide was quickly cleared from plasma as demonstrated by a short half-life of vosoritide following SC administration ($t_{1/2}$ < 30 mins. Therefore, with slow release and fast clearance, released vosoritide showed flip-flop kinetics. Thus, similar to CNP Prodrug, released vosoritide from CNP Prodrug had a longer mean $t_{1/2}$ of 9.6 hrs for the 627 µg/kg dose and 9.9 hrs for the 1891 µg/kg dose compared to a $t_{1/2}$ of 15 min following SC dose of vosoritide. Dose-normalized plasma concentrations were similar in both groups. Dose-normalized released vosoritide AUC and Cmax were similar in both dose groups, suggesting plasma exposure of vosoritide increased proportionally with dose between doses of 627 to 1891 µg/kg CNP Prodrug.

[0515] The vosoritide to CNP Prodrug ratio for Cmax was 0.07% for the 627 µg/kg dose and 0.1 % for the 1891 µg/kg dose. The vosoritide to CNP Prodrug ratio for AUC was 0.06% for the 627 µg/kg dose and 0.08% for the 1891 µg/kg dose.

[0516] Overall, after SC administration in mice, CNP Prodrug was slowly absorbed with Tmax approximately 4-8 h and had a long $t_{1/2}$ of approximately 10.5 h, consistent with elimination protection through albumin binding. Released vosoritide had a similar Tmax and long $t_{1/2}$ of approximately 10 h, indicating slow and consistent release from CNP Prodrug with no evidence of dose dumping. Released vosoritide had a similar plasma concentration vs time curve to CNP Prodrug but with consistently lower plasma concentration (> 1000-fold lower) due to the slow release relative to the faster clearance of free vosoritide. The vosoritide to CNP Prodrug ratios in Cmax and AUC_{last} were less than 1% for doses tested. Plasma exposure of CNP Prodrug and released vosoritide increased proportionally with dose over a range of 627 to 1891 µg/kg.

Dose ($\mu\text{g/kg}$ CNP content) ^a	Analyte	$t_{1/2}$ (h)	T_{max} (h)	C_{max} (pM)	C_{max} /Dose ($\text{kg}^*\text{pM}/\mu\text{g}$)	AUC_{last} (h^*pM)	AUC_{last} /Dose ($\text{h}^*\text{kg}^*\text{pM}/\mu\text{g}$)	$AUC_{0-\infty}$ (h^*pM)
627	vosoritide	9.6	4	331	0.528	7720	12.3	7980
	CNP Prodrug	10.5	8	496000	791	12100000	19298	12600000
1891	vosoritide	9.91	12	1170	0.619	30000	15.9	30900
	CNP Prodrug	10.4	4	1210000	640	35600000	18826	37000000

Dose ($\mu\text{g/kg}$ CNP content) ^a	$AUC_{0-\infty}$ /Dose ($\text{h}^*\text{kg}^*\text{pM}/\mu\text{g}$)	V_z/F (L/kg)	CL/F (L/h/Kg)	R C_{max} (%)	R $AUC_{0-\infty}$ (%)
627	12.7	NC	NC	0.07	0.06
	20096	0.184	0.0122		
1891	16.3	NC	NC	0.10	0.08
	19566	0.186	0.0124		

AUC_{last} , Area under the concentration-time curve from time zero to 60 hours by the linear trapezoidal method for increasing concentrations and log trapezoidal method; $AUC_{\text{last}}/\text{Dose}$, The AUC_{last} divided by pMol/kg of the dose administered; $AUC_{(0-\infty)}$, Area under the concentration-time curve from time zero to infinity following the last measurable concentration; $AUC_{(0-\infty)}/\text{Dose}$, The $AUC_{0-\infty}$ divided by pMol/kg of the dose administered; $AUC_{(0-\infty)}$ Ratio, Metabolite ratio of $AUC_{(0-\infty)}$ of vosoritide to CNP Prodrug; C_{max} , Maximum observed concentration; $C_{\text{max}}/\text{Dose}$, The C_{max} divided by pMol/kg of the dose administered; CL/F , Apparent clearance; C_{max} Ratio, Metabolite ratio of C_{max} of vosoritide to CNP Prodrug; CNP, C-type natriuretic peptide (39 amino acid); F, Bioavailability; T_{max} , Time of maximum observed concentration; $t_{1/2}$, Elimination half-life; NC, Not calculated; V_z/F , Apparent volume of distribution.

^a Actual doses delivered. Nominal doses were 500 and 1600 $\mu\text{g/kg}$ CNP content.

Table 26.9 Summary of PK parameters of CNP Prodrug and released vosoritide obtained from the non compartmental analysis (NCA) of average plasma concentrations at each timepoint after SC doses of 627 and 1891 $\mu\text{g/kg}$ CNP Prodrug

[0517] Single Dose Pharmacokinetic Study of CNP Prodrug in Adult Wild Type Rats The non-GLP study was designed to characterize the pharmacokinetic properties of CNP Prodrug and released vosoritide in adult wild type rats after a single intravenous (IV) or SC dose of CNP Prodrug.

[0518] Pharmacokinetics: A 1-Day Pharmacokinetic Study of CNP Prodrug by Intravenous or Subcutaneous Administration in Adult Rats

		Test Article: CNP Prodrug (or vosoritide)
Species	Sprague Dawley Rats	
Feeding Condition:	Ad libitum	
Sample:	Plasma	
Analyte:	CNP Prodrug and vosoritide	
Assay:	LC-MS/MS	
Vehicle/Formulation:	0.005 mol/L citrate buffer solution, pH 5.5, containing 5.25% (w/v) sucrose, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) polysorbate 80 prepared in sterile water for injection, USP	

Method of Administration	Subcutaneous		Subcutaneous		Intravenous		Subcutaneous		Subcutaneous		Subcutaneous		Subcutaneous	
Dose (µg/kg CNP content)	80		240		560		150		280		560		1680	
No. of animals/Sex	5M	5F	5M	5F	5M	5F	5M	5F	5M	5F	5M	5F	5M	5F
Analyte	vosoritide ^a		vosoritide ^a		vosoritide ^b		vosoritide ^b		vosoritide ^b		vosoritide ^b		vosoritide ^b	
PK parameters:														
T _{max} (h)	0.17	0.0833	0.17	0.0833	4	2	11.2	10.4	10.4	8	11.2	6.4	8.8	12
C _{max} (pM)	308	758	907	2280	2800	2380	186	132	269	242	279	200	806	422
C _{max} /Dose ^c (kg*pM/pMol)	0.0158	0.0389	0.0155	0.039	0.0205	0.0174	0.0009	0.00061	0.00094	0.00054	0.0005	0.00047	0.00097	0.0003
AUC _{last} (h*pM)	49.1	166	299	779	60400	44200	2680	2260	4450	3670	5840	3890	17800	12400
AUC _{last} /Dose ^c (h*kg*pM)/pMol)	0.00252	0.000851	0.000512	0.0133	0.442	0.324	0.0733	0.0617	0.0651	0.0537	0.0428	0.0285	0.0436	0.0302
AUC _(0-∞) (h*pM)	175	215	310	833	60900	45800	3840	2260	NA	5410	7440	5630	22600	14000
AUC _(0-∞) /Dose ^c (h*kg*pM)/pMol)	0.00898	0.011	0.00529	0.0142	0.446	0.335	0.105	0.0618	NA	0.0793	0.0545	0.0412	0.0552	0.0343
t _{1/2} (h)	0.0906	0.12	0.243	0.187	10.7	10.9	8.12	7.76	NA	7.6	10	9.75	10.5	15.1
Cl/F (L/h/kg)	111	111	232	81.1	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

Method of Administration	Subcutaneous		Subcutaneous		Intravenous		Subcutaneous		Subcutaneous		Subcutaneous		Subcutaneous	
Dose (µg/kg CNP content)	80		240		560		150		280		560		1680	
No. of animals/Sex	5M	5F	5M	5F	5M	5F	5M	5F	5M	5F	5M	5F	5M	5F
V _z /F (L/kg)	14.5	21.3	59.3	19.8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
MR C _{max} (%)	NA	NA	NA	NA	0.5	0.424	0.735	0.465	0.691	0.758	0.402	0.285	0.448	0.177
MR AUC _{tlast} (%)	NA	NA	NA	NA	0.607	0.51	0.458	0.465	0.479	0.508	0.326	0.262	0.442	0.204
MR AUC _(0-∞) (%)	NA	NA	NA	NA	0.612	0.527	0.725	0.496	NA	0.557	0.386	0.295	0.466	0.223
Dose Ratios ^a :														
C _{max} Ratio	1		3.0		NA		1		1.6		1.5		3.9	
AUC _{0-t} (h*µM)	108		539		NA		2470		4060		4860		15100	
AUC _{0-t} Ratio	1		5.0		NA		1		1.6		2.0		6.1	
AUC _(0-∞) Ratio	1		3.3		NA		1		1.9		2.3		6.6	
Analyte	NA		NA		CNP Prodrug ^e		CNP Prodrug ^e		CNP Prodrug ^e		CNP Prodrug ^e		CNP Prodrug ^e	
PK parameters:														
T _{max} (h)	NA	NA	NA	NA	NA	NA	8.8	8	9.6	8	10.4	7.2	8	8
C _{max} (µM)	NA	NA	NA	NA	565000	561000	26100	28400	38800	31600	68800	71700	179000	238000
C _{max} /Dose ^c (kg*µM/µMol)	NA	NA	NA	NA	4.14	4.11	0.714	0.776	0.569	0.463	0.504	0.526	0.437	0.582
AUC _{tlast} (h*µM)	NA	NA	NA	NA	995000	867000	577000	493000	910000	727000	1780000	1450000	3920000	6000000
AUC _{tlast} /Dose ^c (h*kg*µM)/pMol)	NA	NA	NA	NA	72.9	63.5	15.8	13.5	13.3	10.7	13	10.6	9.57	14.7
AUC _(0-∞) (h*µM)	NA	NA	NA	NA	996000	868000	582000	512000	919000	743000	1860000	1490000	4150000	6080000
AUC _(0-∞) /Dose ^c (h*kg*µM)/pMol)	NA	NA	NA	NA	72.9	63.6	15.9	14	13.5	10.9	13.7	10.9	10.1	14.8
t _{1/2} (h)	NA	NA	NA	NA	8.89	8.82	6.31	7.13	6.44	8.06	9.96	8.55	11.1	9.86
F (%)	NA	NA	NA	NA	NA	NA	21.83	22.03	18.46	17.13	18.72	17.22	13.88	23.35
Cl (L/h/kg)	NA	NA	NA	NA	0.0138	0.0157	NA	NA	NA	NA	NA	NA	NA	NA
V _z (L/kg)	NA	NA	NA	NA	0.179	0.201	NA	NA	NA	NA	NA	NA	NA	NA

Method of Administration	Subcutaneous		Subcutaneous		Intravenous		Subcutaneous		Subcutaneous		Subcutaneous		Subcutaneous	
Dose (µg/kg CNP content)	80		240		560		150		280		560		1680	
No. of animals/Sex	5M	5F	5M	5F	5M	5F	5M	5F	5M	5F	5M	5F	5M	5F
CL/F (L/h/kg)	NA	NA	NA	NA	NA	NA	0.0647	0.0738	0.0863	0.095	0.0743	0.109	0.109	0.0713
V _z /F (L/kg)	NA	NA	NA	NA	NA	NA	0.594	0.764	0.792	1.12	1.08	1.32	1.68	1.04
Dose Ratios ^d :														
C _{max} Ratio	NA		NA		NA		1		1.3		2.6		7.7	
AUC _{0-t} (h·pM)	NA		NA		NA		535000		818000		1610000		4960000	
AUC _{0-t} Ratio	NA		NA		NA		1		1.5		3.0		9.3	
AUC _(0-∞) Ratio	NA		NA		NA		1		1.5		3.0		9.3	

AUC_{last}, Area under the concentration-time curve from time zero to 96 hours by the linear trapezoidal method for increasing concentrations and log trapezoidal method; AUC_{0-t}, The area under the concentration time curve from time zero to t, where t denotes a specific sampling time following dosing; AUC_{last}/Dose, The AUC_{last} divided by pMol/kg of the dose administered; AUC_(0-∞), Area under the concentration-time curve from time zero to infinity following the last measurable concentration; AUC_(0-∞)/Dose, The AUC_{0-∞} divided by pMol/kg of the dose administered; AUC_{last} Ratio (MR), Metabolite ratio of AUC_{last} of vosoritide to CNP Prodrug; AUC_(0-∞) Ratio, Metabolite ratio of AUC_(0-∞) of vosoritide to CNP Prodrug; C_{max}, Maximum observed concentration; C_{max}/Dose, The C_{max} divided by pMol/kg of the dose administered; CL/F, Apparent clearance; C_{max} Ratio, Metabolite ratio of C_{max} of vosoritide to CNP Prodrug; CNP, C-type natriuretic peptide (39 amino acid); F, Bioavailability; T_{max}, Time of maximum observed concentration; t_{1/2}, Elimination half-life; NA, Not available; V_z/F, Apparent volume of distribution.

^aVosoritide (including oxidized forms), following dose administration of vosoritide.

^bVosoritide (including oxidized forms), following dose administration of CNP Prodrug.

^cWhere applicable, CNP Prodrug dose in pMol/kg was used for calculating AUC_{last}/dose, AUC_{0-∞}/dose and C_{max}/Dose.

^dDose proportionality ratios based on mean plasma pharmacokinetics following subcutaneous administration; sex combined.

^eCNP Prodrug (including oxidized forms), following dose administration of CNP Prodrug.

Methods

[0519] A total of 70 Sprague Dawley rats (35 males and 35 females; Charles River Labs International Inc., Kingston, NY, USA), were 9 weeks old with body weights ranging from 267 to 359 grams (males) and 193 and 237 grams (females) at study initiation and were divided into 7 groups (5 male and 5 female/group). In Group 1 and Group 2, rats were administered a SC dose of 80 and 240 µg/kg of vosoritide, respectively. In Group 3, rats were administered an IV dose of 560 µg/kg CNP Prodrug. In Groups 4, 5, 6, and 7, rats were dosed SC at 150, 280, 560, and 1680 µg/kg CNP Prodrug, respectively. Unlike studies in mice, plasma sampling for this study in rat was not terminal, which allows more timepoints to be assessed per animal, but less sample volume collected per timepoint.

[0520] The design of the study is shown in Table 26.10, and bioanalytical sample collection for pharmacokinetics are presented in Table 26.11. Plasma concentrations of vosoritide, vosoritide (ox), CNP Prodrug and CNP Prodrug (ox) were analyzed using LC-MS/MS (method: CNP Prodrug-24-041). The lower limit of quantitation (LLOQ) of the assay for vosoritide and vosoritide (ox) was 12.5 pM. However, due to limited sample volume, a subset of samples was diluted resulting in an effective LLOQ of 25 pM after 2-fold dilution or 50 pM after 4-fold dilution. The LLOQ of the assay for CNP Prodrug and CNP Prodrug (ox) was 250 pM. However, due to limited sample volume, a subset of samples was diluted resulting in an effective LLOQ 500 pM after 2-fold dilution. For PK analysis, non-oxidized and oxidized forms of CNP Prodrug and released vosoritide were combined at each time point.

[0521] A NCA approach consistent with the SC or IV route of administration was used for PK parameter estimation. All parameters were generated from CNP Prodrug and released vosoritide (including oxidized forms) individual concentrations in plasma after a single dose (from Day 1). Nominal doses (pM/kg) and times were used for NCA and calculating dose-normalized concentrations. For the PK analyses, the dose levels were converted from mass to molar units using the molecular weight (MW) of vosoritide (4102.78 g/mol).

Table 26.10: Study Design of the Single Dose Pharmacokinetic Study of CNP Prodrug in Adult Wild Type Rats

Group	Treatment	Dose Route	Dose Level (µg/kg CNP content)	Dose Volume ^a (mL/kg)	Dose Concentration (µg/mL)	Number of Animals and Sex
1	Vosoritide	SC	80	2	40	5M/5F
2	Vosoritide	SC	240	2	120	5M/5F
3	CNP Prodrug	IV	560	2	280	5M/5F
4	CNP Prodrug	SC	150	2	75	5M/5F
5	CNP Prodrug	SC	280	2	140	5M/5F
6	CNP Prodrug	SC	560	2	280	5M/5F
7	CNP Prodrug	SC	1680	2	840	5M/5F

F, female; IV, intravenous; M, male; SC, subcutaneous.

^a Based on the most recent body weight measurement.**Table 26.11: Plasma PK Sample Collection**

Group	Time Post-Dose on Day 1													
	5 min	15 min	30 min	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr	48 hr	72 hr	96 hr	120 hr	168 hr
1 and 2	X	X	X	X	X	-	-	-	-	-	-	-	-	-
3 to 5	-	-	-	X	-	X	X	X	X	X	X	X	X	-
6 and 7	-	-	-	X	-	X	X	X	X	X	-	X	X	X

Results

[0522] The mean vosoritide plasma concentration vs time profiles combined, and gender separated after administration of CNP Prodrug are presented in 9. The summary of PK parameters of vosoritide and CNP Prodrug obtained from the NCA of plasma concentrations after administration of vosoritide or CNP Prodrug are presented in Tables 26.12 and 26.13. Dose-normalized C_{max} and AUC of CNP Prodrug and vosoritide are presented in Table 26.14.

[0523] A comparison of the CNP Prodrug and released vosoritide PK profiles following IV administration of CNP Prodrug demonstrated that vosoritide was slowly released, as indicated by vosoritide reaching T_{max} at 4 hrs. Due to slow release and fast clearance of released vosoritide, mean $t_{1/2}$ of released vosoritide was 10.8 hrs, which was similar to CNP Prodrug (8.9 hrs). The released vosoritide to CNP Prodrug ratio was 0.58% for $AUC_{0-\infty}$ and 0.47% for C_{max} . No gender difference was observed during the elimination phase of released vosoritide as mean $t_{1/2}$ was similar in both males (10.7 hrs) and females (10.9 hrs).

[0524] After SC dose of vosoritide, it was quickly absorbed in plasma with a median T_{max} for both 80 $\mu\text{g/kg}$ dose and 240 $\mu\text{g/kg}$ dose of 5 mins (0.0833 h). The vosoritide had short mean terminal half-life of 0.113 h and 0.203 h for 80 $\mu\text{g/kg}$ dose and 240 $\mu\text{g/kg}$ dose, respectively.

[0525] Following SC administration of CNP Prodrug, the median Male and female combined CNP Prodrug T_{max} was 8 hrs for all SC dose groups, indicating slow absorption into plasma following SC administration. Similar to the IV dose group, after SC dose, CNP Prodrug showed an extended mean $t_{1/2}$ ranging from 6.72 to 10.5 hrs. Mean bioavailability of CNP Prodrug following SC administration was low, ranging from 17.67% to 21.71% across dose groups. No consistent effect of sex on bioavailability was observed.

[0526] With the slow release of vosoritide, the median vosoritide T_{max} was slightly delayed relative to CNP Prodrug (T_{max} approximately 10-12 hrs). The $t_{1/2}$ for released vosoritide was similar to CNP Prodrug, ranging from 7.6- 12.8 hrs. The long $t_{1/2}$ for released vosoritide from CNP Prodrug compared to short $t_{1/2}$ (0.113-0.203 hrs) groups administered SC vosoritide confirms that $t_{1/2}$ for released vosoritide was determined by slow release rate of vosoritide instead of its clearance rate (flip-flop kinetics).

[0527] CNP Prodrug C_{max} and AUC increased proportionally with dose from 280 $\mu\text{g/kg}$ to 1680 $\mu\text{g/kg}$ (Table 26.15). For released vosoritide, mean C_{max} and mean AUC increased proportionally with dose from 150 to 280 $\mu\text{g/kg}$ and 560 to 1680 $\mu\text{g/kg}$ but was similar for 280 and 560 $\mu\text{g/kg}$ doses. In comparison, animals administered vosoritide, C_{max} increased with dose and a slightly higher but comparable increase in AUC was observed with increase in dose. Percent metabolite ratio of released vosoritide AUC_{0-t} ranged from 0.294% to 0.494% and C_{max} ranged from 0.312% to 0.725% after SC administration of CNP Prodrug.

[0528] Overall, after SC administration in rats, CNP Prodrug was slowly absorbed with T_{max} of approximately 8h and had a long $t_{1/2}$ of approximately 6.72 to 10.5 h, consistent with elimination protection through albumin binding. Released vosoritide had a similar T_{max} (approximately 10-12 h) and long $t_{1/2}$ of ~10 h, indicating slow and consistent release from CNP Prodrug with no evidence of dose dumping. In comparison to vosoritide after SC of

vosoritide was quickly absorbed (T_{max} approximately 5 mins) with very short $t_{1/2}$ of 0.113-0.203 h. The slow release of vosoritide relative to faster clearance resulted in the observed flip-flop kinetics of released vosoritide, where the rate of clearance was dictated by the rate of release from CNP Prodrug. As a result, released vosoritide had a similar plasma concentration vs time curve to CNP Prodrug but with consistently lower plasma concentrations (> 1000-fold lower). The vosoritide to CNP Prodrug ratios in C_{max} and AUC_{0-t} were less than 1% for doses tested. Plasma exposure of CNP Prodrug increased proportionally with dose over a range of 150 to 1680 $\mu\text{g/kg}$. No notable sex difference was observed for CNP Prodrug and released vosoritide. Mean bioavailability of CNP Prodrug following SC administration was low, ranging from 17.67% to 21.71% across dose groups.

[0529] Table 26.12 Summary Pharmacokinetic Parameters for Vosoritide after a Single IV or SC Dose of vosoritide or CNP Prodrug in Rats

Group	Sex	AUC _{0-∞} (h* μ M)	AUC _{0-∞} (h* μ M)	C _{max} (μ M)	T _{max} (h)	t _{1/2} (h)
1	M	49.1 (68.4)	175	308 (344)	0.0833 (0.0833,0.25)	0.0906
	F	166 (90.8)	215 (102)	758 (405)	0.0833 (0.0833,0.0833)	0.12 (0.0341)
	M+F	108 (97.7)	205 (85.5)	533 (426)	0.0833 (0.0833,0.25)	0.113 (0.0316)
2	M	299 (100)	310 (189)	907 (173)	0.0833 (0.0833,0.25)	0.243 (0.218)
	F	779 (344)	833 (372)	2280 (521)	0.0833 (0.0833,0.0833)	0.187 (0.0709)
	M+F	539 (348)	683 (404)	1600 (813)	0.0833(0.0833,0.25)	0.203(0.109)
3	M	60400 (6370)	60900 (6520)	2800 (157)	4 (4,4)	10.7 (0.463)
	F	44200 (4380)	45800 (4710)	2380 (204)	1 (1,4)	10.9 (0.753)
	M+F	53400 (10000)	54400 (9710)	2620 (279)	4 (1,4)	10.8 (0.556)
4	M	2680 (922)	3840	186 (45)	12 (8,12)	8.12
	F	2260 (443)	2260 (312)	132 (25.8)	12 (8,12)	7.76 (0.261)
	M+F	2470 (717)	2790 (938)	159 (44.9)	12 (8,12)	7.88 (0.279)
5	M	4450 (2180)	NA	269 (104)	12 (8,12)	NA
	F	3670 (690)	5410	242 (66.8)	8 (4,12)	7.6
	M+F	4060 (1580)	5410	255 (83.9)	10 (4,12)	7.6
6	M	5840 (1710)	7440 (2130)	279 (72)	12 (8,12)	10 (0.614)
	F	3890 (1860)	5630 (691)	200 (84.6)	4 (4,12)	9.75 (1.64)
	M+F	4860 (1980)	6350 (1540)	240 (85)	10 (4,12)	9.87 (1.21)
7	M	17800 (7490)	22600 (7450)	806 (399)	8 (4,12)	10.5 (0.653)
	F	12400 (4070)	14000 (4080)	422 (197)	12 (12,12)	15.1 (4.26)
	M+F	15100 (6370)	18300 (6960)	614 (359)	12 (4,12)	12.8 (3.63)

Group	Sex	MR C _{max} (%)	MR AUC _{last} (%)	MR AUC _{0-∞} (%)
1	M	NA	NA	NA
	F	NA	NA	NA
	M+F	NA	NA	NA
2	M	NA	NA	NA
	F	NA	NA	NA
	M+F	NA	NA	NA
3	M	0.5 (0.063)	0.607 (0.029)	0.612 (0.031)
	F	0.424 (0.035)	0.51 (0.036)	0.527 (0.042)
	M+F	0.467 (0.063)	0.565 (0.06)	0.576 (0.056)
4	M	0.735 (0.237)	0.458 (0.139)	0.725
	F	0.465 (0.02)	0.465 (0.068)	0.496 (0.011)
	M+F	0.6 (0.213)	0.461 (0.103)	0.572 (0.133)
5	M	0.691 (0.059)	0.479 (0.051)	NA
	F	0.758 (0.11)	0.508 (0.048)	0.557
	M+F	0.725 (0.091)	0.494 (0.049)	0.557
6	M	0.402 (0.035)	0.326 (0.068)	0.386 (0.029)
	F	0.285 (0.032)	0.262 (0.03)	0.295 (0.023)
	M+F	0.344 (0.069)	0.294 (0.06)	0.331 (0.055)
7	M	0.448 (0.063)	0.442 (0.066)	0.466 (0.002)
	F	0.177 (0.032)	0.204 (0.028)	0.223 (0.005)
	M+F	0.312 (0.15)	0.323 (0.134)	0.344 (0.141)

F, female; M, male; NA, not applicable. MR: ratio of released vosoritide and CNP Prodrug.
 Data are presented as Mean (SD), T_{max} is presented as Median (Min,Max); SD are not calculated or not reported when mean concentration equals zero or N < 3.
 Secondary parameters (t_{1/2}, and/or AUC_{0-inf}) reported as NA when it could not be calculated due to insufficient plasma concentration-time data in any animal in the group.

[0530] Table 26.13: Summary Pharmacokinetic Parameters for CNP Prodrug after a Single IV or SC Dose in Rats

Gro up	Se x	AUC _(0-∞) ^a (h* μ M)	AUC _{0-∞} ^a (h* μ M)	C _{max} ^a (μ M)	T _{max} ^a (h)	t _{1/2} ^a (h)	V _z or V _z /F ^b (L/kg)	CL or CL/F ^b (L/h/kg)	F (%)
3	M	9950000 (957000)	9960000 (957000)	565000 (53400)	NA	8.89 (1.28)	0.179 (0.0421)	0.0138 (0.00132)	NA
	F	8670000 (320000)	8680000 (320000)	561000 (33300)	NA	8.82 (0.802)	0.201 (0.0243)	0.0157 (0.000593)	NA
	M +F	9400000 (980000)	9410000 (980000)	564000 (42400)	NA	8.86 (1.02)	0.188 (0.0349)	0.0146 (0.00144)	NA
4	M	577000 (109000)	582000 (109000)	26100 (5450)	8 (4,12)	6.31 (0.619)	0.594 (0.154)	0.0647 (0.0125)	21. 83
	F	493000 (120000)	512000 (102000)	28400 (5020)	8 (8,8)	7.13 (0.57)	0.764 (0.194)	0.0738 (0.0148)	22. 03
	M +F	535000 (117000)	547000 (106000)	27200 (5080)	8 (4,12)	6.72 (0.707)	0.679 (0.188)	0.0692 (0.0138)	21. 71
5	M	910000 (388000)	919000 (395000)	38800 (15100)	8 (8,12)	6.44 (0.607)	0.792 (0.338)	0.0863 (0.0378)	18. 46
	F	727000 (153000)	743000 (152000)	31600 (5720)	8 (4,12)	8.06 (1.21)	1.12 (0.354)	0.095 (0.0198)	17. 13
	M +F	818000 (294000)	831000 (297000)	35200 (11400)	8 (4,12)	7.25 (1.24)	0.955 (0.369)	0.0907 (0.0288)	17. 67
6	M	1780000 (257000)	1860000 (253000)	68800 (12900)	12 (8,12)	9.96 (1.91)	1.08 (0.304)	0.0743 (0.00999)	18. 72
	F	1450000 (596000)	1490000 (621000)	71700 (33900)	8 (4,12)	8.55 (1.27)	1.32 (0.612)	0.109 (0.0539)	17. 22
	M +F	1610000 (466000)	1680000 (488000)	70300 (24200)	8 (4,12)	9.25 (1.7)	1.2 (0.473)	0.0915 (0.0408)	17. 85
7	M	3920000 (1250000)	4150000 (1410000)	179000 (80500)	8 (8,8)	11.1 (1.33)	1.68 (0.408)	0.109 (0.0374)	13. 88
	F	6000000 (1650000)	6080000 (1540000)	238000 (99300)	8 (8,8)	9.86 (1.94)	1.04 (0.487)	0.0713 (0.0195)	23. 35
	M +F	4960000 (1770000)	5110000 (1720000)	209000 (90800)	8 (8,8)	10.5 (1.69)	1.36 (0.542)	0.0899 (0.0343)	18. 11

F, female; M, Male; NA, not applicable.; CNP Prodrug PK parameters in Group 1 and 2 was not reported as animals were administered vosoritide.

Secondary parameters (Cl or Cl/F, V_z or V_z/F, t_{1/2}, and/or AUC_{0-∞}) reported as NA when it could not be calculated due to insufficient plasma concentration-time data in any animal in the group.

^aData are presented as Mean (SD), T_{max} is presented as Median (Min, Max); ^b CL and V_z were calculated for IV dose groups whereas CL/F and V_z/F were calculated for SC dosed groups.

[0531] Table 26.14: Summary of Dose-Normalized Exposures of Vosoritide and CNP Prodrug after a Single IV or SC Dose of vosoritide or CNP Prodrug

Group	Gender	Vosoritide			CNP Prodrug		
		AUC _(0-∞) /Dose (h*kg* μ M/ μ g)	AUC _{0-∞} /Dose (h*kg* μ M/ μ g)	C _{max} /Dose (kg* μ M/ μ g)	AUC _(0-∞) /Dose (h*kg* μ M/ μ g)	AUC _{0-∞} /Dose (h*kg* μ M/ μ g)	C _{max} /Dose (kg* μ M/ μ g)
1	M	0.00252 (0.00351)	0.00898	0.0158 (0.0177)	NA	NA	NA
	F	0.00851 (0.00466)	0.011 (0.00523)	0.0389 (0.0208)	NA	NA	NA
	M+ F	0.00552 (0.00501)	0.0105 (0.00439)	0.0273 (0.0219)	NA	NA	NA
2	M	0.00512 (0.00171)	0.00529 (0.00322)	0.0155 (0.00296)	NA	NA	NA
	F	0.0133 (0.00589)	0.0142 (0.00636)	0.039 (0.00891)	NA	NA	NA
	M+ F	0.00922 (0.00595)	0.0117 (0.00691)	0.0273 (0.0139)	NA	NA	NA
3	M	0.442 (0.0467)	0.446 (0.0478)	0.0205 (0.00115)	72.9 (7.01)	72.9 (7.01)	4.14 (0.392)
	F	0.324 (0.0321)	0.335 (0.0345)	0.0174 (0.00149)	63.5 (2.34)	63.6 (2.34)	4.11 (0.244)
	M+ F	0.392 (0.0736)	0.399 (0.0711)	0.0192 (0.00205)	68.9 (7.18)	68.9 (7.18)	4.13 (0.311)
4	M	0.0733 (0.0252)	0.105	0.00509 (0.00123)	15.8 (2.99)	15.9 (2.99)	0.714 (0.149)
	F	0.0617 (0.0121)	0.0618 (0.00853)	0.00361 (0.000705)	13.5 (3.27)	14 (2.79)	0.776 (0.137)
	M+ F	0.0675 (0.0196)	0.0762 (0.0257)	0.00435 (0.00123)	14.6 (3.19)	15 (2.91)	0.745 (0.139)
5	M	0.0651 (0.032)	NA	0.00394 (0.00153)	13.3 (5.69)	13.5 (5.78)	0.569 (0.222)
	F	0.0537 (0.0101)	0.0793	0.00354 (0.000979)	10.7 (2.25)	10.9 (2.23)	0.463 (0.0839)
	M+ F	0.0594 (0.0231)	0.0793	0.00374 (0.00123)	12 (4.31)	12.2 (4.35)	0.516 (0.168)
6	M	0.0428 (0.0125)	0.0545 (0.0156)	0.00205 (0.000527)	13 (1.88)	13.7 (1.85)	0.504 (0.0946)
	F	0.0285 (0.0136)	0.0412 (0.00506)	0.00147 (0.00062)	10.6 (4.37)	10.9 (4.55)	0.526 (0.248)
	M+ F	0.0356 (0.0145)	0.0465 (0.0113)	0.00176 (0.000623)	11.8 (3.41)	12.3 (3.57)	0.515 (0.178)
7	M	0.0436 (0.0183)	0.0552 (0.0182)	0.00197 (0.000975)	9.57 (3.06)	10.1 (3.43)	0.437 (0.196)
	F	0.0302 (0.00993)	0.0343 (0.00995)	0.00103 (0.000482)	14.7 (4.03)	14.8 (3.77)	0.582 (0.243)
	M+ F	0.0369 (0.0156)	0.0447 (0.017)	0.0015 (0.000878)	12.1 (4.31)	12.5 (4.21)	0.51 (0.222)

F, female; M, Male; NA, not applicable.

Data are presented as Mean (SD); SD are not calculated or not reported when mean concentration equals zero or N < 3. Secondary parameters (Cl or Cl/F, Vz or Vz/F, t_{1/2}, and/or AUC_{0-∞}) reported as NA when it could not be calculated due to insufficient plasma concentration-time data in any animal in the group.

[0532] Table 26.15. Dose Proportionality Based on Mean Plasma PK Parameters
Obtained From Single SC Dose of CNP Prodrug in Rats

Analyte	CNP Dose (µg/kg) ^a	Dose proportionality	C _{max} (pM)	C _{max} Ratio	AUC _{0-t} (h*pM)	AUC _{0-t} Ratio	AUC _{0-∞} (h*pM)	AUC _{0-∞} Ratio
CNP Prodrug	150	1	27200	1	535000	1	547000	1
	280	1.87	35200	1.3	818000	1.5	831000	1.5
	560	3.73	70300	2.6	1610000	3.0	1680000	3.0
	1680	11.20	209000	7.7	4960000	9.3	5110000	9.3
Released Vosoritide	150	1	159	1	2470	1	2790	1
	280	1.87	255	1.6	4060	1.6	5410	1.9
	560	3.73	240	1.5	4860	2.0	6350	2.3
	1680	11.20	614	3.9	15100	6.1	18300	6.6

^aSingle SC administration of CNP Prodrug.

[0533] Study Title: A 10-Day Pharmacokinetic Study of CNP Prodrug by a Single Intravenous or Subcutaneous Administration in Non-Human Primates

[0534] The non-GLP study was designed to characterize the pharmacokinetic properties of CNP Prodrug and released vosoritide in NHPs after a single intravenous (IV) or SC dose of CNP Prodrug.

Table 26.16

		Test Article: CNP Prodrug		
Species	Cynomolgus monkeys			
Feeding Condition:	Ad libitum			
Sample (e.g., whole blood, plasma, serum):	Plasma			
Analyte:	CNP Prodrug and vosoritide			
Assay:	LC-MS/MS			
Vehicle/Formulation:	10mM Histidine pH 5.5, Trehalose Dihydrate (48 mg/mL), D-Mannitol (12.4 mg/mL), L-Methionine (0.73 mg/mL), Polysorbate 80 (0.05 mg/mL)			
Additional Dosing Information: Animals were dosed by subcutaneous injection at 102.3, 205.8, and 352.8 µg/kg and by intravenous injection at 100.5 µg/kg. These were the actual doses delivered due to a formulation calculation error where all doses were only ~66% of the nominal dose levels, which were 150, 300, and 500 µg/kg CNP content for subcutaneous injection and 150 µg/kg CNP for intravenous injection.				
Method of Administration:	Intravenous	Subcutaneous	Subcutaneous	Subcutaneous
Dose (µg/kg CNP content)	100.5	102.3	205.8	352.8
Sex	Combined			
C ₀ (pmol/L)	307000 ±138000	NA	NA	NA
C _{max} (pmol/L)	288000 ±115000	116000 ±20500	154000 ±74800	324000 ±116000
C _{max} /Dose (pmol/L/(µg/kg))	2870 ±1140	1140 ±201	750 ±364	919 ±329
t _{max} ^a (hr)	1.00 (1.00 - 2.00)	8.00 (4.00 - 24.0)	12.0 (8.00 - 24.0)	8.00 (4.00 - 12.0)
t _{last} ^a (hr)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)
AUC _{tlast} (hr*pmol/L)	12100000 ±4380000	10700000 ±2260000	14900000 ±6910000	28500000 ±14800000
AUC _{tlast} /Dose (hr*pmol/L/(µg/kg))	120000 ±43600	104000 ±22100	72500 ±33600	80800 ±41900
AUC _{0-inf} (hr*pmol/L)	12300000 ±7350000	13600000 ±NA	16700000 ±9050000	31100000 ±18300000
t _{1/2} (hr)	62.2 ±3.07	61.7 ±NA	59.2 ±5.45	60.9 ±7.52
F (%)	NA	86.5	60.2	67.2
CI (L/hr/kg)	0.00270 ±0.00195	0.00185 ±NA	0.00395 ±0.00250	0.00335 ±0.00129

				Test Article: CNP Prodrug
V_z (L/kg)	0.243 ±0.174	0.164 ±NA	0.328 ±0.180	0.292 ±.124
Males				
C₀ (pmol/L)	391000 ±73000	NA	NA	NA
C_{max} (pmol/L)	339000 ±52400	113000 ±31600	119000 ±48200	237000 ±42000
C_{max}/Dose (pmol/L/(µg/kg))	3370 ±522	1110 ±309	579 ±234	673 ±119
t_{max}^a (hr)	1.00 (1.00 - 1.00)	8.00 (4.00 - 8.00)	12.0 (8.00 - 24.0)	8.00 (4.00 - 8.00)
t_{last}^a (hr)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)
AUC_{tlast} (hr*pmol/L)	12600000 ±960000	9430000 ±2530000	11200000 ±5160000	18200000 ±2040000
AUC_{tlast}/Dose (hr*pmol/L/(µg/kg))	126000 ±9550	92100 ±24700	54500 ±25100	51700 ±5770
AUC_{0-Inf} (hr*pmol/L)	12200000 ±NA	NA	6830000 ±NA	20600000 ±NA
t_{1/2} (hr)	59.0 ±NA	NA	52.2 ±NA	63.8 ±NA
F (%)	NA	73.4	43.4	41.2
CI (L/hr/kg)	0.00200 ±NA	NA	0.00730 ±NA	0.00420 ±NA
V_z (L/kg)	0.171 ±NA	NA	0.554 ±NA	0.386 ±NA
Females				
C₀ (pmol/L)	224000 ±146000	NA	NA	NA
C_{max} (pmol/L)	238000 ±150000	119000 ±5570	190000 ±89200	411000 ±97100
C_{max}/Dose (pmol/L/(µg/kg))	2370 ±1490	1160 ±54.4	921 ±434	1160 ±275
t_{max}^a (hr)	2.00 (1.00 - 2.00)	24.0 (4.00 - 24.0)	12.0 (12.0 - 24.0)	12.0 (8.00 - 12.0)
t_{last}^a (hr)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)	240 (240 - 240)
AUC_{tlast} (hr*pmol/L)	11600000 ±6800000	11900000 ±1360000	18600000 ±7160000	38800000 ±15000000

				Test Article: CNP Prodrug
AUC_{tlast}/Dose (hr*pmol/L/(µg/kg))	115000 ±67700	116000 ±13300	90600 ±34800	110000 ±42500
AUC_{0-Inf} (hr*pmol/L)	12400000 ±NA	13600000 ±NA	20000000 ±7590000	41500000 ±NA
t_{1/2} (hr)	63.8 ±NA	61.7 ±NA	61.5 ±3.51	58.0 ±NA
F (%)	NA	101	78.6	95.5
Cl (L/hr/kg)	0.00305 ±NA	0.00185 ±NA	0.00283 ±0.00137	0.00250 ±NA
V_z (L/kg)	0.279 ±NA	0.164 ±NA	0.253 ±0.121	0.198 ±NA
Released vosoritide Pharmacokinetic Parameters				
	Combined			
C_{max} (pmol/L)	195 ±88.6	54.9 ±3.84	79.6 ±37.0	195 ±67.8
C_{max}/Dose (pmol/L/(µg/kg))	1.94 ±0.882	0.537 ±0.0376	0.387 ±0.180	0.552 ±0.192
t_{max}^a (hr)	1.00 (1.00 - 4.00)	16.0 (4.00 - 24.0)	10.0 (4.00 - 12.0)	8.00 (4.00 - 8.00)
t_{last}^a (hr)	120 (72.0 - 168)	48.0 (24.0 - 72.0)	72.0 (24.0 - 120)	120 (72.0 - 168)
AUC_{tlast} (hr*pmol/L)	11200 ±8390	2100 ±1080	4480 ±3150	13400 ±8010
AUC_{tlast}/Dose (hr*pmol/L/(µg/kg))	112 ±83.5	20.6 ±10.5	21.7 ±15.3	37.9 ±22.7
AUC₀₋₂₄ (hr*pmol/L)	20300 ±8300	NA	9650 ±NA	18300 ±9070
AUC_{0-240hr}/Dose (hr*pmol/L/(µg/kg))	202 ±82.5	NA	46.9 ±NA	51.8 ±25.7
vosoritide: CNP Prodrug C_{max} (%)	0.0683 ±0.0131	0.0486 ±0.0107	0.0518 ±0.00315	0.0606 ±0.00798
vosoritide: CNP Prodrug AUC_{tlast} (%)	0.0923 ±0.0586	0.0187 ±0.00664	0.0267 ±0.00935	0.0455 ±0.00667
	Males			
C_{max} (pmol/L)	258 ±53.0	56.1 ±3.86	60.8 ±25.0	149 ±30.8

				Test Article: CNP Prodrug
C_{max}/Dose (pmol/L/(µg/kg))	2.57 ±0.528	0.549 ±0.0377	0.295 ±0.121	0.422 ±0.0873
t_{max}^a (hr)	1.00 (1.00 - 1.00)	8.00 (4.00 - 24.0)	12.0 (8.00 - 12.0)	8.00 (4.00 - 8.00)
t_{last}^a (hr)	168 (168 - 168)	24.0 (24.0 - 48.0)	72.0 (24.0 - 72.0)	120 (72.0 - 120)
AUC_{tlast} (hr*pmol/L)	17700 ±6880	1350 ±820	2540 ±1790	7520 ±1010
AUC_{tlast}/Dose (hr*pmol/L/(µg/kg))	176 ±68.5	13.2 ±8.02	12.4 ±8.72	21.3 ±2.87
AUC₀₋₂₄ (hr*pmol/L)	20300 ±8300	NA	NA	8550 ±NA
AUC_{0-240hr}/Dose (hr*pmol/L/(µg/kg))	202 ±82.5	NA	NA	24.2 ±NA
vosoritide: CNP Prodrug C_{max} (%)	0.0757 ±0.00552	0.0522 ±0.0156	0.0509 ±0.000405	0.0630 ±0.0108
vosoritide: CNP Prodrug AUC_{tlast} (%)	0.138 ±0.0461	0.0136 ±0.00441	0.0206 ±0.00838	0.0416 ±0.00732
Females				
C_{max} (pmol/L)	132 ±70.2	53.7 ±4.19	98.5 ±41.7	241 ±65.0
C_{max}/Dose (pmol/L/(µg/kg))	1.31 ±0.699	0.525 ±0.0409	0.479 ±0.203	0.682 ±0.184
t_{max}^a (hr)	2.00 (1.00 - 4.00)	24.0 (4.00 - 24.0)	4.00 (4.00 - 12.0)	8.00 (4.00 - 8.00)
t_{last}^a (hr)	72.0 (72.0 - 72.0)	72.0 (48.0 - 72.0)	120 (72.0 - 120)	168 (120 - 168)
AUC_{tlast} (hr*pmol/L)	4720 ±1580	2860 ±736	6410 ±3230	19200 ±7530
AUC_{tlast}/Dose (hr*pmol/L/(µg/kg))	47.0 ±15.7	27.9 ±7.20	31.1 ±15.7	54.5 ±21.3
AUC₀₋₂₄ (hr*pmol/L)	NA	NA	9650 ±NA	21500 ±7780
AUC_{0-240hr}/Dose (hr*pmol/L/(µg/kg))	NA	NA	46.9 ±NA	61.0 ±22.0
vosoritide: CNP Prodrug C_{max} (%)	0.0609 ±0.0152	0.0451 ±0.00222	0.0527 ±0.00469	0.0583 ±0.00516

Test Article: CNP Prodrug				
vosoritide:	0.0467	0.0238	0.0329	0.0494
CNP Prodrug	±0.0150	±0.00360	±0.00592	±0.00356
AUC_{last} (%)				

Note: SD and CV are not calculated or not reported when mean concentration equals zero or N<3.
 Bioavailability (%F) = (AUC/Dose Group 2, 3, or 4 - SC / AUC/Dose Group 1 - IV) * 100.

AUC_{last}, Area under the concentration-time curve from time zero to 96 hours by the linear trapezoidal method for increasing concentrations and log trapezoidal method; AUC_{last}/Dose, The AUC_{last} divided by pMol/kg of the dose administered; AUC_(0-inf), Area under the concentration-time curve from time zero to infinity following the last measurable concentration; C₀, Concentration at time 0 for an initial IV bolus dose extrapolated from initial measured plasma concentrations. C_{max}, Maximum observed concentration; C_{max}/Dose, The C_{max} divided by pMol/kg of the dose administered; Cl, Apparent clearance; CNP, C-type natriuretic peptide (39 amino acid); F, Bioavailability; T_{max}, Time of maximum observed concentration; t_{1/2}, Elimination half-life; NA, Not available; V_z, Apparent volume of distribution.

^a Median (minimum - maximum). (Sample table taken from M4S: The Safety Appendices)

[0535] Methods

[0536] A total of 24 NHPs (12 male and 12 female; Charles River Laboratories, Laval, QC, Canada) were 22 to 36 months old with body weights ranging from 1.6 to 3.0 kg and were divided into 4 groups (3 males and 3 females per group). In Group 1, NHPs were administered a single IV dose of 100.5 µg/kg CNP Prodrug and in Groups 2, 3, and 4, NHPs were administered a single SC dose of 102.3, 205.8, and 352.8 µg/kg CNP Prodrug, respectively. These dose levels reflect ~70% of the intended nominal doses (which were 150, 150, 300, and 500 µg/kg, respectively) due to a formulation calculation error.

[0537] The design of the study is shown in Table 12. Blood CNP Prodrug and CNP Prodrug (ox) concentrations were analyzed using LC-MS/MS. The lower limit of quantitation (LLOQ) of the assay for vosoritide and vosoritide (ox) was 25 pM. The lower limit of quantitation (LLOQ) of the assay for CNP Prodrug and CNP Prodrug (ox) was 2000 pM. For PK analysis, non-oxidized and oxidized forms of CNP Prodrug and released vosoritide were combined at each time point.

[0538] A NCA approach consistent with the SC or IV route of administration was used for PK parameter estimation. All parameters were generated from CNP Prodrug and released vosoritide individual concentrations in plasma after single dose (from Day 1). For the PK analyses, the dose levels were converted from mass to molar units using the molecular weight (MW) of vosoritide (4102.78 g/mol).

Table 26.17: Design of the Single Dose PK Study in Cynomolgus Monkeys

Group	Test Material	Dose Route	Nominal CNP Dose Level ^a (µg/kg)	Actual CNP Dose Level ^a (µg/kg)	Actual Dose Level (pmol/kg)	Number of Animals and Sex
1	CNP Prodrug	IV	150	100.5	24496	3M/3F
2	CNP Prodrug	SC	150	102.3	24934	3M/3F
3	CNP Prodrug	SC	300	205.8	50161	3M/3F
4	CNP Prodrug	SC	500	352.8	85990	3M/3F

F, female; IV, intravenous; M, male; SC, subcutaneous.

^a All study samples were outside of the ± 10% acceptance criterion for mean concentration. It was determined during the Test Facility investigation that formulations were not adjusted for CNP content. The calculated sample concentrations were consistent with the active vosoritide concentration of the formulations (approximately 30% lower than the target concentrations), which were prepared without the purity and CNP correction of 0.672. The intended nominal doses were 150, 150, 300, and 500 µg/kg.

[0539] Results

[0540] The mean CNP Prodrug and vosoritide plasma concentration vs time profiles are presented in Figures 30A and 30B. The mean CNP Prodrug and vosoritide plasma concentration vs time profiles by male and female animals are presented in Figure 31. The summary of PK parameters of vosoritide and CNP Prodrug after single IV dose of 100.5 µg/kg and SC doses of 102.3, 205.8, and 352.8 µg/kg CNP Prodrug are presented in Table and Table , respectively.

[0541] Systemic exposure to CNP Prodrug appeared to be independent of sex. Following a single IV bolus administration at 100.5 µg/kg and a single SC administration at 102.3 and 205.8 µg/kg, similar exposure was observed for males and females, whereas a trend for higher exposure was observed for females when compared to males following a single SC administration at 352.8 µg/kg. Although C_{max} and AUC_{last} ratios suggest slightly higher exposure for females at 352.8 µg/kg (SC) and given the variability observed for females, no consistent nor notable difference could be concluded. Individual plasma concentration-time profiles, C_{max} , and AUC values were similar between males and females at 100.5/102.3 µg/kg (IV bolus and SC) and 205.8 µg/kg (SC). F:M AUC_{last} ratios was 0.917 following the IV bolus administration. Following 102.3, 205.8, and 352.8 µg/kg SC administration, F:M C_{max} ratios were 1.05, 1.59, and 1.73 and F:M AUC_{last} ratios were 1.26, 1.66, and 2.13, respectively.

[0542] The median T_{max} values for CNP Prodrug in both males and females were similar (individual T_{max} values for CNP Prodrug ranged from 4 to 24 hrs post-dose for the SC groups). In males, median T_{max} values for CNP Prodrug were observed by 8 hrs post-dose at 102.3 and 352.8 µg/kg, and 12 hrs post-dose at 205.8 µg/kg (individual T_{max} values for CNP Prodrug ranged from 4 to 24 hrs post-dose for the SC groups). In females, median T_{max} values for CNP Prodrug were observed by 24 hrs post-dose at 102.3 µg/kg and 12 hrs post-dose at 205.8 and 352.8 µg/kg (individual T_{max} values for CNP Prodrug ranged from 4 to 24 hrs post-dose for the SC groups).

[0543] The absolute bioavailability (%F) following a single SC administration of CNP Prodrug ($AUC_{last}/Dose$) was 86.5%, 60.2%, and 67.2% at 102.3, 205.8, and 352.8 µg/kg, respectively for the sex-combined data. For the females the absolute bioavailability following a single SC administration of CNP Prodrug was 101%, 78.6%, and 95.5% at 102.3, 205.8, and 352.8 µg/kg, respectively. While for the males, the absolute bioavailability following a single SC administration of CNP Prodrug was 73.4%, 43.4%, and 41.2% at 102.3, 205.8, and 352.8 µg/kg, respectively. The difference observed between males and females could

be related to the inter-animal variability and was generally less than 2-fold, and therefore, these results should be viewed with caution.

[0544] Following a single SC administration of CNP Prodrug to male and female NHP, mean C_{max} and AUC_{last} values of CNP Prodrug increased with increasing dose from 102.3 to 352.8 $\mu\text{g/kg}$ with individual C_{max} values overlapping between 102.3 and 205.8 $\mu\text{g/kg}$ and individual AUC_{last} values overlapping between 102.3 and 352.8 $\mu\text{g/kg}$. For combined males and females, a 1:2.0:3.4-fold increase in CNP Prodrug dose resulted in an approximate 1:1.3:2.8-fold increase in mean CNP Prodrug C_{max} values and an approximate 1:1.4:2.7-fold increase in mean CNP Prodrug AUC_{last} values. For males, a 1:2.0:3.4-fold increase in CNP Prodrug dose resulted in an approximate 1:1.1:2.1-fold increase in mean CNP Prodrug C_{max} values and an approximate 1:1.2:1.9-fold increase in mean CNP Prodrug AUC_{last} values. For females, a 1:2.0:3.4-fold increase in CNP Prodrug dose resulted in an approximate 1:1.6:3.5-fold increase in mean CNP Prodrug C_{max} values and an approximate 1:1.6:3.3-fold increase in mean CNP Prodrug AUC_{last} values.

[0545] Due to insufficient plasma concentration-time data with less than 3 half-lives, secondary parameters (Cl or Cl/F , V_z or V_z/F , and $t_{1/2}$) could not be determined for all male and female profiles. For limited animals, secondary parameters were determined, and Cl or Cl/F , V_z or V_z/F , and $t_{1/2}$ values were similar in males and females.

[0546] Systemic exposure to released vosoritide appeared to be higher in males compared to females after a single IV bolus administration of CNP Prodrug at 100.5 $\mu\text{g/kg}$. Individual vosoritide plasma concentrations, C_{max} , and AUC values were greater in males than in females with F:M C_{max} ratio of 0.512 and AUC_{last} ratio of 0.267. When CNP Prodrug was administered SC, the systemic exposure of the release vosoritide appeared to be independent of sex at 102.3 and 205.8 $\mu\text{g/kg}$ whereas a trend for higher exposure was observed for females when compared to males following a single SC administration at 352.8 $\mu\text{g/kg}$. Although C_{max} and AUC_{last} ratios suggest slightly higher exposure for females at 352.8 $\mu\text{g/kg}$ (SC) and given the variability noted for females, no consistent nor notable difference could be concluded. There were no consistent differences in concentration-time profiles at 102.3, 205.8, and 352.8 $\mu\text{g/kg}$ with individual C_{max} and AUC values overlapping between males and females (F:M C_{max} ratios ranged from 0.956 to 1.62 and F:M AUC_{last} ratios ranged from 2.11 to 2.56).

[0547] The median T_{max} values for released vosoritide in both males and females were similar and was similar to CNP Prodrug (individual T_{max} values for CNP Prodrug ranged from 4 to 24 hrs post-dose for the SC groups). In males, median T_{max} values for released vosoritide were observed by 1 hour post-dose at 100.5 $\mu\text{g/kg}$ (IV), 8 hrs post-dose at 102.3

and 352.8 µg/kg, and 12 hrs post-dose at 205.8 µg/kg (individual T_{max} values for released vosoritide ranged from 4 to 24 hrs post-dose for the SC groups). The median T_{max} values for released vosoritide in female plasma concentrations were observed by 2 hrs post-dose at 100.5 µg/kg (IV), 24 hrs post-dose at 102.3 µg/kg, 4 hrs post-dose at 205.8 µg/kg, and 8 hrs post-dose at 352.8 µg/kg (individual T_{max} values for released vosoritide ranged from 1 to 24 hrs post-dose). The $t_{1/2}$ could not be determined in males and females NHP for all profiles due to adjusted R^2 (Goodness of fit statistic) being lower than 0.8 or insufficient plasma concentration-time data with less than 3 half-lives.

[0548] Following once SC administration of CNP Prodrug to male and female NHP, mean C_{max} and AUC_{tlast} values of released vosoritide increased with increasing dose from 102.3 to 352.8 µg/kg. For males, a 1:2.0:3.4-fold increase in CNP Prodrug dose resulted in an approximate 1:1.1:2.7-fold increase in mean released vosoritide C_{max} values and an approximate 1:1.9:5.6-fold increase in mean released vosoritide AUC_{tlast} values. For females, a 1:2.0:3.4-fold increase in CNP Prodrug dose resulted in an approximate 1:1.8:4.5-fold increase in mean released vosoritide C_{max} values and an approximate 1:2.2:6.7-fold increase in mean released vosoritide AUC_{tlast} values.

[0549] The mean systemic exposure to released vosoritide (vosoritide: CNP Prodrug C_{max} and vosoritide: CNP Prodrug AUC_{tlast}) was similar in males and females. Following a single IV bolus administration of CNP Prodrug at 100.5 µg/kg, the mean systemic exposure to released vosoritide (vosoritide: CNP Prodrug C_{max} and vosoritide: CNP Prodrug AUC_{tlast}) was approximately 0.076%, 0.061% and 0.068% (C_{max}) and 0.138%, 0.047% and 0.092% (AUC_{tlast}) of the systemic exposure of CNP Prodrug in male, female and combined NHP, respectively. Following a single SC administration at 102.3, 205.8, and 352.8 µg/kg, the systemic exposure to released vosoritide was 0.049%, 0.052%, and 0.061% (C_{max}) and was approximately 0.019%, 0.027%, and 0.046% (AUC_{tlast}), respectively for the male and female combined NHP.

[0550] Conclusions

[0551] Overall, after SC administration in NHPs, CNP Prodrug was slowly absorbed with T_{max} ~8h (4-12h) and had a long $t_{1/2}$ of ~ 59.2- 61.7 h, consistent with albumin binding. Released vosoritide had a similar T_{max} (4-24 hrs), suggesting no dose dumping of vosoritide. The $t_{1/2}$ of released vosoritide could not be consistently estimated in this study, however released vosoritide had a similar plasma concentration vs time curve to CNP Prodrug, but with consistently lower plasma concentration (> 1000-fold lower), which is indicative of flip-flop kinetics where clearance is driven by the relatively slow rate of release from CNP Prodrug. Plasma exposure of CNP Prodrug increased proportionally with dose over the

evaluated range of 102.3 to 352.8 µg/kg. The vosoritide to CNP Prodrug ratios in C_{max} and AUC_{last} was less than 1% for doses tested. Mean bioavailability of CNP Prodrug following SC administration was high, ranging from 67% to 87% across dose groups. Bioavailability was higher in females compared to males in this study, but the difference was < 2-fold.

[0552] Table 2: Summary Pharmacokinetic Parameters of Released vosoritide in Male and Female NHP Plasma Following IV or SC Administration of CNP Prodrug

Group	Sex	C _{max} (pM)	C _{max} /Dose (pM/(μg/kg))	T _{max} ^a (hr)	AUC _{0-24hr} (hr·pM)	AUC _{0-24hr} /Dose (hr·pM/(μg/kg))	AUC _{0-24hr} (hr·pM)	AUC _{0-24hr} /Dose (hr·pM/(μg/kg))	vosoritide: CNP Prodrug C _{max} (%)	vosoritide: CNP Prodrug AUC _{0-24hr} (%)
1 (100.5 μg/kg, IV)	M	258 (53.0)	2.57 (0.528)	1 (1-1)	17700 (6880)	176 (68.5)	20300 (8300)	202 (82.5)	0.0757 (0.00552)	0.138 (0.0461)
	F	132 (70.2)	1.31 (0.699)	2 (1-4)	4720 (1580)	47 (15.7)	NA	NA	0.0609 (0.0152)	0.0467 (0.015)
	M+F	195 (88.6)	1.94 (0.882)	1 (1-4)	11200 (8390)	112 (83.5)	20300 (8300)	202 (82.5)	0.0683 (0.0131)	0.0923 (0.0586)
2 (102.3 μg/kg SC)	M	56.1 (3.86)	0.549 (0.0377)	8 (4-24)	1350 (820)	13.2 (8.02)	NA	NA	0.0522 (0.0156)	0.0136 (0.00441)
	F	53.7 (4.19)	0.525 (0.0409)	24 (4-24)	2860 (736)	27.9 (7.2)	NA	NA	0.0451 (0.00222)	0.0238 (0.0036)
	M+F	54.9 (3.84)	0.537 (0.0376)	16 (4-24)	2100 (1080)	20.6 (10.5)	NA	NA	0.0486 (0.0107)	0.0187 (0.00664)
3 (205.8 μg/kg SC)	M	60.8 (25.0)	0.295 (0.121)	12 (8-12)	2540 (1790)	12.4 (8.72)	NA	NA	0.0509 (0.000405)	0.0206 (0.00838)
	F	98.5 (41.7)	0.479 (0.203)	4 (4-12)	6410 (3230)	31.1 (15.7)	9650	46.9	0.0527 (0.00469)	0.0329 (0.00592)
	M+F	79.6 (37.0)	0.387 (0.180)	10 (4-12)	4480 (3150)	21.7 (15.3)	9650	46.9	0.0518 (0.00315)	0.0267 (0.00935)
4 (352.8 μg/kg SC)	M	149 (30.8)	0.422 (0.0873)	8 (4-8)	7520 (1010)	21.3 (2.87)	8550	24.2	0.0630 (0.0108)	0.0416 (0.00732)
	F	241 (65.0)	0.682 (0.184)	8 (4-8)	19200 (7530)	54.5 (21.3)	21500 (7780)	61.0 (22.0)	0.0583 (0.00516)	0.0494 (0.00356)

Group	Sex	C _{max} (pM)	C _{max} /Dose (pM/(μg/kg))	T _{max} ^a (hr)	AUC _{last} (hr*pM)	AUC _{last} /Dose (hr*pM/(μg/kg))	AUC _{0-240hr} (hr*pM)	AUC _{0-240hr} /Dose (hr*pM/(μg/kg))	vosoritide: CNP Prodrug C _{max} (%)	vosoritide: CNP Prodrug AUC _{last} (%)
	M+F	195 (67.8)	0.552 (0.192)	8 (4-8)	13400 (8010)	37.9 (22.7)	18300 (9070)	51.8 (25.7)	0.0606 (0.00798)	0.0455 (0.00667)

NA, not available. SD and CV are not calculated or not reported when mean concentration equals zero or N < 3. AUC_{last} and AUC_{last}/Dose are equivalent to AUC_{0-240hr} and AUC_{0-240hr}/Dose. Secondary parameters (t_{1/2}, and/or AUC_{0-inf}) reported as NA when it could not be calculated due to insufficient plasma concentration-time data in any animal in the group; ^a Median (minimum - maximum).

[0553] Table 26.19: Summary Pharmacokinetic Parameters of CNP Prodrug in Male and Female NHP Plasma Following IV or SC Administration of CNP Prodrug

Group	Sex	C _{max} (pM)	C _{max} /Dose (pM/μg/kg)	T _{max} (hr)	AUC _{0-∞} (hr·pM)	AUC _{0-∞} /Dose (hr·pM/μg/kg)	AUC _{0-∞} (hr·pM)	t _{1/2} (hr)	Cl or Cl/F (L/hr/kg)	V _z or V _z /F (L/kg)
1 (10 0.5 μg/ kg IV)	M	33900 0 (5240 0)	3370 (522)	1 (1-1)	12600000 (960000)	126000 (9550)	1220000 0	59.0	0.00200	0.171
	F	23800 0 (1500 00)	2370 (1490)	2 (1-2)	11600000 (6800000)	115000 (67700)	1240000 0	63.8	0.00305	0.279
	M+F	28800 0 (1150 00)	2870 (1140)	1 (1-2)	12100000 (4380000)	120000 (43600)	1230000 0 (735000 0)	62.2 (3.07)	0.00270 (0.00195)	0.243 (0.17 4)
2 (10 2.3 μg/ kg SC)	M	11300 0 (3160 0)	1110 (309)	8 (4-8)	9430000 (2530000)	92100 (24700)	NA	NA	NA	NA
	F	11900 0 (5570)	1160 (54.4)	24 (4-2 4)	11900000 (1360000)	116000 (13300)	1360000 0	61.7	0.00185	0.164
	M+F	11600 0 (2050 0)	1140 (201)	8 (4-2 4)	10700000 (2260000)	104000 (22100)	1360000 0	61.7	0.00185	0.164
3 (20 5.8 μg/ kg SC)	M	11900 0 (4820 0)	579 (234)	12 (8-2 4)	11200000 (5160000)	54500 (25100)	6830000	52.2	0.00730	0.554
	F	19000 0 (8920 0)	921 (434)	12 (12- 24)	18600000 (7160000)	90600 (34800)	2000000 0 (759000 0)	61.5 (3.51)	0.00283 (0.00137)	0.253 (0.12 1)
	M+F	15400 0 (7480 0)	750 (364)	12 (8-2 4)	14900000 (6910000)	72500 (33600)	1670000 0 (905000 0)	59.2 (5.45)	0.00395 (0.00250)	0.328 (0.18 0)
4 (35 2.8 μg/ kg SC)	M	23700 0 (4200 0)	673 (119)	8 (4-8)	18200000 (2040000)	51700 (5770)	2060000 0	63.8	0.00420	0.386
	F	41100 0 (9710 0)	1160 (275)	12 (8-1 2)	38800000 (15000000)	110000 (42500)	4150000 0	58.0	0.00250	0.198
	M+F	32400 0 (1160 00)	919 (329)	8 (4-1 2)	28500000 (14800000)	80800 (41900)	3110000 0 (183000 00)	60.9 (7.52)	0.00335 (0.00129)	0.292 (124)

F, Female; hr, hour; M, Male; NA, not available.

SD are not calculated or not reported when mean concentration equals zero or $N < 3$. AUC_{last} and $AUC_{last}/Dose$ are equivalent to $AUC_{0-240hr}$ and $AUC_{0-240hr}/Dose$; Secondary parameters (Cl or Cl/F , V_z or V_z/F , $t_{1/2}$, and/or AUC_{0-inf}) reported as NA when it could not be calculated due to insufficient plasma concentration-time data in any animal in the group; Median (minimum - maximum).

[0554] Study Title: A 1-Month Study of Lipid-BC-CNPProdrug by Subcutaneous Injection in Monkeys

[0555] The non-GLP study was a repeat-dose pharmacology study of CNP Prodrug conducted in male NHPs that included some assessments of safety/tolerability. The PK properties of CNP Prodrug and released vosoritide were also evaluated.

[0556] Methods

[0557] Twelve male cynomolgus monkeys (Institut Armand-Frappier, Laval, QC, Canada) were approximately 2 years old with body weights ranging from 2.7 to 3.3 kg at study initiation. Animals were assigned to 3 treatment groups (4/group) and were dosed weekly by SC injection of vehicle or CNP Prodrug at 30 or 150 µg/kg QW for a total of 5 doses. Injections were given in the back in 3 injection sites, with Sites 1 and 2 both receiving 2 doses (Study Days 1 and 15 and Study Days 8 and 22, respectively). The final injection on Study Day 29 was given in a naïve site, Site 3. The design of the study is shown in Table and bioanalytical sample collection for pharmacokinetics are presented in Table .

Table 26.20: Design of the Repeat-Dose Study of CNP Prodrug in Cynomolgus Monkeys

Group	Test Material	CNP Dose Level (µg/kg QW)	Dose Volume ^a (mL/kg)	Dose Concentration (µg/mL CNP content)	Number of Animals and Sex
1	Vehicle ^b	0	1	0	4M
2	CNP Prodrug	30	1	30	4M
3	CNP Prodrug	150	1	150	4M

CNP, C-natriuretic peptide; M, Male; QW, weekly (dosing).

^a Based on the most recent body weight measurement.

^b Vehicle solution: 0.005 mol/L citrate buffer solution, pH 5.5, containing 5.25% (w/v) sucrose, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) polysorbate 80 prepared in Sterile Water for Injection, USP.

Pharmacokinetics: A 1-Month Study of CNP Prodrug by Subcutaneous Injection in Monkeys

		Test Article: CNP Prodrug	
Species	Cynomolgus monkey		
Feeding Condition:	Ad libitum		
Sample (Analyte):	Plasma (CNP Prodrug and vosoritide [PK] and ANP), serum (Collagen type X, BAP, Pro-C1, Pro-C2, and T2CM), and urine (cGMP, CTx-I, and CTx-II)		
Assay:	LC-MS/MS (plasma), CLIA or ECLA (serum), and ELISA (serum and urine)		
Vehicle/Formulation:	0.005 mol/L citrate buffer solution, pH 5.5, containing 5.25% (w/v) sucrose, 1.5% (w/v) mannitol, 0.727 mg/mL methionine, and 0.005% (w/v) polysorbate 80 prepared in sterile water for injection, USP		
Method of Administration:	Subcutaneous Injection		
Dose (µg/kg CNP content)	0 (Control)	30	150
Number of Animals/Group ^a :	M	M	M
Main:	4	4	4
<u>vosoritide</u>			
Day 1			
t _{max} (h)	NA	NC	7
C _{max} (pM)	NA	NC	81.9
AUC _{tlast} (hr•pM)	NA	NC	6630
AUC ₀₋₁₆₈ (hr•pM)	NA	NC	6630
AUC _(0-∞) (hr•pM)	NA	NC	9660
t _{1/2} (h)	NA	NC	65.3
Day 22			
t _{max} (h)	NA	16	48
C _{max} (pM)	NA	20.2	160
AUC _{tlast} (hr•pM)	NA	815	17900
AUC ₀₋₁₆₈ (hr•pM)	NA	NC	20200
AUC _(0-∞) (hr•pM)	NA	NC	30500
t _{1/2} (h)	NA	NC	61.2
Accumulation Ratio			
R _(C_{max})	NA	NC	1.88
R _(AUC_{tlast})	NA	NC	2.62

			Test Article: CNP Prodrug
$R_{(AUC0-\infty)}$	NA	NC	4.05
$R_{(C_{168})}$	NA	NC	2.92
CNP Prodrug			
Day 1			
t_{max} (h)	NA	22	38
C_{max} (pM)	NA	8300	86800
AUC_{last} (hr*pM)	NA	813000	8230000
AUC_{0-168} (hr*pM)	NA	813000	8230000
$AUC_{(0-\infty)}$ (hr*pM)	NA	491000	9670000
$t_{1/2}$ (h)	NA	60	65.3
Day 22			
t_{max} (h)	NA	14.5	28
C_{max} (pM)	NA	15500	105000
AUC_{last} (hr*pM)	NA	1670000	12500000
AUC_{0-168} (hr*pM)	NA	1670000	12500000
$AUC_{(0-\infty)}$ (hr*pM)	NA	NC	12000000
$t_{1/2}$ (h)	NA	NC	63.8
Accumulation Ratio			
$R_{(C_{max})}$	NA	2.09	1.24
$R_{(AUC_{last})}$	NA	2.36	1.50
$R_{(AUC0-\infty)}$	NA	3.37	1.42
$R_{(C_{168})}$	NA	3.15	1.67
Normalized cGMP/Creatinine			
Day 9	559	700	819
Day 13	715	727	748
Day 16	537	732	848
Day 20	663	668	723
Day 23	557	568	730
Day 27	641	646	660
Day 30	589	658	755

Test Article: CNP Prodrug			
Change from baseline Normalized cGMP/Creatinine			
Day 9	-40.5	96.1	223
Day 13	103	123	152
Day 16	-105	83.9	252
Day 20	76	63.9	127
Day 23	-48	-80.2	134
Day 27	54.2	42.5	63.9
Day 30	-10	53.8	159
Change from baseline BAP (ng/mL)			
Day 29	-88.5	-34.4	-40.7
Day 31	-71.6	-39.9	-64.2
Additional Information:	Results of ANP in plasma and Collagen type X in serum were generally or completely BLQ and CTx-II normalized to urine creatinine concentrations at most timepoints were above the ULQ.		

ANP, Atrial natriuretic peptide; AUC₀₋₁₆₈, the area under the concentration time curve from time zero to 168 hours following dosing; AUC_(0-∞), area under the concentration-time curve from time zero extrapolated to infinity; AUC_{tlast}, the area under the concentration versus time curve from the start of dose administration to the last observed quantifiable concentration; cGMP, cyclic guanosine monophosphate; BAP, Bone-specific Alkaline Phosphatase; BLQ, Below the limit of quantitation; cGMP, cyclic guanosine monophosphate; C_{max}, maximum observed concentration measured after dosing; CNP, C-type natriuretic peptide (39 amino acid); CTx-I, Telopeptide of Type I collagen; CTx-II, Telopeptide of Type II collagen; M, male; NA, Not applicable; NC, Not calculated; Pro-C1, N terminal Pro-peptide of Type I collagen; Pro-C2, Released N-terminal Pro-peptide of Type II collagen; R_(AUC_{0-∞}), the area under the curve from time zero extrapolated to infinity after repeat doses divided by the area under the curve from time zero extrapolated to infinity during the initial dosing interval; R_(AUC_{tlast}), the area under the curve from 0 to T₂ after repeat doses divided by the area under the curve from 0 to T₂ during the initial dosing interval; R_(C₁₆₈), the trough concentration at 168 hours after repeat doses divided by the trough concentration at 168 hours during the initial dosing interval; R_(C_{max}), the C_{max} after repeat doses divided by the C_{max} during the initial dosing interval; t_{max}, the time after dosing at which the maximum concentration was observed; t_{1/2}, terminal half-life; T2CM, Neo-epitope of MMP-1 and MMP-13 mediated degradation of Type II collagen; ULQ, Upper limit of quantitation; ^a The n was between 0 and 4 for each parameter/group

Table 3: Bioanalytical Sample Collection for Toxicokinetics

Groups	Time Post-Dose on Days 1 and 22									
	0 hr ^a	30 min	1 hr	2 hr	4 hr	8 hr	24 hr	48 hr	72 hr	120 hr
1 to 3	X	X	X	X	X	X	X	X	X	X
Time Post-Dose on Days 8 and 15										
1 to 3	X	-	-	-	-	-	X	-	-	-
Time Post-Dose on Day 29										
1 to 3	X	-	-	-	-	-	X	X	-	-

X, Sample to be collected; -, Not applicable; hr, hour; min, minute.

^a Sample was collected pre-dose.

[0558] Plasma concentrations of vosoritide, vosoritide (ox), CNP Prodrug (Lipid-BC-BMN 111), and CNP Prodrug (ox) concentrations were analyzed using LC-MS/MS (method: CNP Prodrug-24-040). The lower limit of quantitation (LLOQ) of the assay was 12.5 pM, 12.5 pM, 500 pM, and 500 pM for vosoritide, vosoritide (ox), CNP Prodrug and CNP Prodrug (ox), respectively. For PK analysis, non-oxidized and oxidized forms of CNP Prodrug and released vosoritide were combined at each time point.

[0559] A NCA approach consistent with the subcutaneous route of administration was used for parameter estimation. All parameters were generated from CNP Prodrug and released vosoritide (including oxidized forms) individual concentrations in plasma after single dose (from Day 1 and day 22). Nominal doses (pmol) and times were used for NCA analysis and calculating dose-normalized concentrations. For the PK analyses, the dose levels were converted from mass to molar units using the molecular weight (MW) of vosoritide (4102.78 g/mol)

Results

[0560] The mean vosoritide and CNP Prodrug plasma concentration vs time profiles and dose normalized plasma concentration vs time profiles after SC dose are presented in Figure 30-32. The mean vosoritide and CNP Prodrug plasma concentration vs time profiles after single SC dose on Day 1 and after three weekly doses on Day 22 are presented in . Plasma concentrations of released vosoritide were not quantifiable for the majority of animals treated with 30 µg/kg QW on Study Days 1 and 22 but were quantifiable for animals treated with 150 µg/kg QW on Study Days 1 and 22.

[0561] The summary of PK parameters of released vosoritide and CNP Prodrug are presented in Table 26.22, and the summary of accumulation ratio are presented in Table 26.23.

[0562] Neither CNP Prodrug nor vosoritide were detected in the plasma of vehicle-treated animals. After SC administration, CNP Prodrug was slowly absorbed with a median T_{max} of 16 hrs after both the first dose of 30 µg/kg QW on Day 1 and after multiple weekly doses on Day 22 and a median T_{max} of 48 hrs after the first dose of 150 µg/kg QW on Study Day 1 and 28 hrs after multiple weekly doses on Study Day 22. The CNP Prodrug mean $t_{1/2}$ was 60 to 65.3 hrs on Day 1, which was consistent for both 30 and 150 µg/kg QW dose levels.

[0563] After SC administration of 30 µg/kg QW, CNP Prodrug mean exposure (AUC_{tlast}) increased by weekly dose from 813000 h*pM to 1670000 h*pM as well as C_{max} from 8300 pM to 15500 pM, respectively from Day 1 to Day 22. After SC administration of 30 µg/kg QW, CNP Prodrug mean exposure (C_{max} , AUC_{tlast} and $AUC_{0-\infty}$) increased of single dose from Day 1 (86800 pM, 8230000 h*pM and 9670000 h*pM, respectively) to weekly dose on Day 22 (105000 pM, 12500000 h*pM and 12000000 h*pM, respectively). After 3 weeks of 150 µg/kg QW dosing, 1.24-, 1.5-, 1.42-, and 1.67-fold accumulation in C_{max} , AUC_{tlast} , $AUC_{0-\infty}$, and C_{trough} at 168 hrs, respectively, of CNP Prodrug was observed. The apparent clearance of drug, apparent volume of distribution based upon the terminal phase, and $t_{1/2}$ values were similar on Day 1 and Day 22 indicating that CNP Prodrug PK remains consistent after repeat dosing in NHPs. Overlapping dose normalized plasma Concentration-Time profile on day 1 and day 22 supports CNP Prodrug exposure increased proportionally with dose over a range of 30 to 150 µg/kg QW.

[0564] Demonstrating a slow release of vosoritide, at 150 µg/kg QW, plasma concentrations of released vosoritide had a median T_{max} of 8 hrs after the first dose and 48 hrs after 3 weekly doses of 150 µg/kg QW. After 3 weeks of 150 µg/kg QW dosing, 1.88-, 2.62-, 4.05-, and 2.92-fold accumulation of released vosoritide in C_{max} , AUC_{0-t} , $AUC_{0-\infty}$, and C_{trough} at 168 hrs, respectively, was observed. Due to the slow-release rate relative to the clearance of released vosoritide, $t_{1/2}$ of vosoritide was similar to CNP Prodrug (65.3 hrs on Study Day 1 and 63.8 hrs on Study Day 22 at 150 µg/kg QW). The ratio of released vosoritide to CNP Prodrug for AUC_{0-t} was 0.1% after a single dose on Day 1 and 0.14% after three weekly doses on Day 22.

[0565] In conclusion, in NHPs after weekly SC administration of CNP Prodrug, no time-dependent changes in PK parameters were observed in CNP Prodrug or released vosoritide through Day 22. CNP Prodrug was slowly absorbed with median T_{max} of 16-48 h after single dose and 16-28 h after the third weekly dose on Day 22. A similarly long T_{max} was observed for released vosoritide, which confirmed there was no unintended dose dumping. Compared to rodents, CNP Prodrug had a longer mean $t_{1/2}$ of 60 to 65.3 hrs in NHP, which was consistent for both 30 µg/kg QW and 150 µg/kg QW dose levels, and consistent with albumin binding. As expected with flip-flop kinetics, released vosoritide had a similar plasma

concentration vs time curve to CNP Prodrug, but with consistently lower plasma concentrations. The mean terminal $t_{1/2}$ released vosoritide was similar to CNP Prodrug (~65 hrs) and was consistent between Day 1 and after weekly dosing on Day 22. Steady state based on $t_{1/2}$ was expected to be reached by 2 weeks. After weekly dosing of 150 µg/kg on Day 22, < 2-fold accumulation in CNP Prodrug was observed. Plasma exposure of CNP Prodrug increased proportionally with dose over a range of 30 to 150 µg/kg QW. The vosoritide to CNP Prodrug ratios in C_{max} and AUC_{0-24} were less than 1% across the evaluated dose range. These data confirm the intended mechanism of half-life extension in NHP is concordant with albumin binding, and the slow continuous release of vosoritide by CNP Prodrug hydrolysis leads to sustained exposure across multiple doses.

Table 26.22: Summary Pharmacokinetic Parameters for Released Vosoritide and CNP Prodrug on Day 1 and 22

Analyte	Dose (µg/kg)	Study Day	T _{max} (hr)	C _{max} (pM)	AUC _{0-12h} (hr·pM)	AUC _{0-168h} (hr·pM)	AUC _{0-∞} (hr·pM)	t _{1/2} (hr)	CL/F (L/hr/kg)	V _d /F (L/kg)
Vosoritide	30	Day 1	NA	NA	NA	NA	NA	NA	NA	NA
		Day 22	24 (0,24)	20.2 (4.8)	815 (45.9)	NA	NA	NA	NA	NA
	150	Day 1	8 (4,8)	81.9 (20.3)	6630 (1840)	6630 (1840)	9660 (954)	65.3 (2.87)	NA	NA
		Day 22	48 (48,48)	160 (66.6)	17900 (8440)	20200 (8630)	30500	61.2	NA	NA
CNP Prodrug	30	Day 1	16 (8,48)	8300 (4610)	813000 (472000)	813000 (472000)	491000 (217000)	60 (2.7)	0.0165 (0.00731)	1.44 (0.697)
		Day 22	16 (2,24)	15500 (6250)	167000 (702000)	167000 (702000)	NA	NA	NA	NA
	150	Day 1	48 (8,48)	86800 (32700)	823000 (243000)	823000 (243000)	967000 (342000)	65.3 (1.91)	0.00414 (0.00155)	0.388 (0.134)
		Day 22	28 (8,48)	10500 (35800)	125000 (471000)	125000 (471000)	120000	63.8	0.00305	0.281

Hr, hour; NA, not available.

Data are presented as Mean (SD), T_{max} is presented as Median (Min, Max). SD are not calculated or not reported when mean concentration equals zero or N < 3.

Table 26.23: Accumulation Ratio of Vosoritide and Prodrug CNP Prodrug on Day 22 after Once Weekly Dosing of CNP Prodrug

Dose (µg/kg)	Animal	Analyte							
		Vosoritide				CNP Prodrug			
		R _(C_{max})	R _(AUC_{0-168h})	R _(AUC_{0-∞})	R _(C_{168h})	R _(C_{max})	R _(AUC_{0-168h})	R _(AUC_{0-∞})	R _(C_{168h})
30	Mean	NA	NA	NA	NA	2.09	2.36	3.37	3.15
	SD	NA	NA	NA	NA	0.626	0.866	0.945	1.1
150	Mean	1.88	2.62	4.05	2.92	1.24	1.5	1.42	1.67
	SD	0.405	0.74	1.55	2.43	0.173	0.248	0.155	0.398

C_{168h}, Concentration at 168h; NA, not applicable; R: ratio of exposure (C_{max}, C_{168h} and AUC) on Day 22 divided by exposure on Day 1.

[0566] Table 26.24: Summary of Pharmacokinetic Parameters of Vosoritide, CNP Prodrug-Released Vosoritide, and CNP Prodrug After a Single Subcutaneous Dose

Analyte		Species	Dose (µg/kg) ^a	C _{max} ^b (pM)	C _{max} /Dose ^b (pM/(µg/kg))
Vosoritide after SC dose of vosoritide		Rat	150	3209	21.4
			540 Male	20011	37.1
			540 Female	9335	17.3
		NHP	300	21987 (7870)	73.3 (26.2)
			90	4820 (2036)	53.6 (22.6)
Released vosoritide after SC dose of CNP Prodrug		Mouse	627	331	0.528
			1891	1170	0.619
		Rat	150	159 (44.9)	1.06 (0.29)
			280	255 (83.9)	0.91 (0.29)
			560	240 (85)	0.429 (0.152)
			1680	614 (359)	0.365(0.213)
		NHP	102.3	54.9 (3.84)	0.537 (0.038)
			205.8	79.6 (37.0)	0.386 (0.18)
			352.8	195 (67.8)	0.552 (0.192)
			30	NC	NC
			150	81.9 (20.3)	0.546 (0.135)

Table 26.24 Continued

Analyte		Species	AUC _{0-∞} ^b (hr* μ M)	AUC _{0-∞} ^b / Dose ^b (hr* μ M)/(μ g/kg)	T _{max} ^c (hr)	t _{1/2} ^b (hr)
Vosoritide after SC dose of vosoritide		Rat	1331	8.87	0.08	0.20
			10684	19.8	0.25	0.28
			5240	9.70	0.08	0.25
		NHP	19523 (12808)	65.1 (42.7)	0.25 (0.083-0.25)	0.56 (0.21)
			4347 (4370)	48.3(48.6)	0.15 (0.09)	0.48 (0.16)
Released vosoritide after SC dose of CNP Prodrug		Mouse	7980	12.7	4	9.6
			30900	16.3	12	9.91
		Rat	2790 (938)	18.6(6.25)	12 (8-12)	7.88 (0.28)
			NC	NC	10 (4-12)	7.6
			6350 (1540)	11.3 (2.75)	10 (4-12)	9.87 (1.21)
			18300 (6960)	10.9 (4.14)	12 (4-12)	12.8 (3.63)
		NHP	NC	NC	16 (4-24)	NC
			NC	NC	10 (4-12)	NC
			NC	NC	8 (4-8)	NC
			NC	NC	NC	NC
			1331	64.4(6.36)	8 (4-8)	65.3 (2.87)

-, data not applicable; AUC_{0-∞}, area under the time-plasma concentration curve from time 0 to infinity; C_{max}, maximum plasma concentration; CNP, C-type natriuretic peptide; CNP, C-natriuretic peptide; hr, hour; NC, not calculated; NHP, non-human primate; NOAEL, no-observed-adverse-effect level; SC, subcutaneous; SD, standard deviation; t_{1/2}, terminal half-life; T_{max}, time of maximum plasma concentration; ^a μ g/kg of CNP content delivered subcutaneously; ^b C_{max}, AUC_{0-∞}, and t_{1/2} are presented as mean (SD), except where SD was not calculable; ^c T_{max} is presented as median (min-max), except where the median represents all values for animal dosed with CNP Prodrug and mean for animals dosed with vosoritide; ^d Study BMN111-11-019 was a 28-day vosoritide GLP toxicity study in male and female cynomolgus monkeys. Values shown are at the NOAEL dose level of 300 μ g/kg/day on Study Day 2 (second dose) for males and females combined; ^e Study BMN111-11-035 was a 26-week vosoritide GLP toxicity study in male and female juvenile cynomolgus monkeys. Values shown are at the NOAEL dose level of 90 μ g/kg/day on Study Day 1 (first dose) for males and females combined.

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Example 27: First in Human Studies with CNP-prodrug CNP Prodrug

[0572] A Phase 1 study to evaluate the safety and tolerability of single subcutaneous injections of CNP Prodrug compared with placebo is planned. The study will also analyze the PK of CNP Prodrug and released CNP peptide in humans; to inform dose selection and design of the subsequent phases of clinical development of CNP Prodrug. This study will collect data on medical history, physical examinations, vital signs, ECG readings, clinical laboratory parameters, PK parameters, anti-drug antibodies, biomarkers, AEs, and concomitant medications.

[0573] The study will enroll healthy adult male and female volunteers aged ≥ 18 to ≤ 64 years. Based on nonclinical data, CNP Prodrug is expected to be safe in both males and females. Participants will enter the study following a 4 week screening period; they will be admitted to the study site 1 day before their scheduled administration of study drug (Day -1) and will reside there until Day 8. Participants will return to the study site on Days 15, 22, and 29 for PK and/or safety follow up.

[0574] Adult healthy male and female volunteers are divided into 5 sequential ascending dose cohorts (10 participants/cohort), with the option of adding up to 2 further dosing

cohorts. The proposed starting dose for the study is 15 µg/kg. The planned doses of CNP Prodrug to be evaluated in the study are the following: 15, 50, 100, 200 and 350 µg/kg. The 350 µg/kg dose corresponds to the highest dose being tested in the ongoing 6-month chronic pharmacology/toxicology GLP study in Non Human Primates (NHPs). The proposed design for this FIH study allows for dose adaptation based on the emerging safety, tolerability, and PK data. For doses above 50 µg/kg, the dose escalation will not exceed 2-fold increase over the previous dose. Doses above 350 µg/kg will be considered if supported by the safety and tolerability data from the lower dose levels and deemed necessary to provide sufficient exposure to CNP Prodrug and released CNP to establish an appropriate safety margin to support later stage development.

[0575] Within each dosing cohort, a sentinel strategy is applied, wherein the first 2 participants will be dosed first (1:1 ratio of CNP Prodrug:placebo), with the remaining 8 participants (6:2 ratio of CNP Prodrug:placebo) dosed after 72 hours if no significant safety issues are observed. The 72-hour monitoring period after sentinel dosing has been selected because the time to peak concentration (T_{max}) of CNP Prodrug in humans is not expected to be longer than 72 hours based on CNP Prodrug PK in animals. Primary and secondary endpoints are set out in Table 27-1.

Table 27-1: Primary and Secondary Objectives and Endpoints

Objectives	Endpoints
Primary	
To evaluate safety and tolerability of single SC injections of CNP Prodrug compared with placebo in healthy volunteers	Incidence of AEs and serious AEs, DLT, physical examination, vital signs, ECG, clinical laboratory tests
Secondary	
To evaluate the PK profile of CNP Prodrug and released CNP following single SC injections of CNP Prodrug in healthy volunteers	$AUC_{0-\infty}$, AUC_{0-t} , AUC_{0-168h} , C_{max} , T_{max} , $t_{1/2}$, CL/F (only for CNP Prodrug), Vz/F (only for CNP Prodrug)

AE, adverse event; AUC_{0-168h} , area under the plasma concentration time curve from time 0 to 168 hours; $AUC_{0-\infty}$, area under the plasma concentration-time curve from time 0 to infinity; AUC_{0-t} , area under the plasma concentration-time curve from 0 to the time of last measurable concentration; CL/F, apparent clearance of drug; C_{max} , maximum observed plasma concentration; DLT, dose-limiting toxicity; ECG, electrocardiogram; SAE, serious adverse event; $t_{1/2}$, elimination half-life; T_{max} , time to reach maximum observed plasma concentration; Vz/F, apparent volume of distribution based upon the terminal phase.

[0576] Based on emerging safety and PK data from the study, up to 2 additional dose cohorts may be included to evaluate additional doses of CNP Prodrug or doses may be adapted. The proposed design for this FIH study allows for dose adaptation based on the emerging safety, tolerability, and PK data. For doses above 50 µg/kg, the dose escalation

will not exceed 2-fold increase over the previous dose. Doses above 350 µg/kg will be considered if supported by the safety and tolerability data from the lower dose levels and deemed necessary to provide sufficient exposure to CNP Prodrug and released CNP to establish an appropriate safety margin to support later stage development.

[0577] Efforts will be made to assign a similar number of males and females to each dose cohort. Up to 2 additional dose cohorts may be included to evaluate additional doses of CNP Prodrug, based on emerging safety and PK data from the study. In addition, doses may be adapted based on emergent safety and PK data from the study. For doses above 50 mcg/kg, the dose escalation will not exceed 2-fold of the previous dose. Doses above 350 mcg/kg will be considered if supported by the safety and tolerability data from the lower dose levels and if deemed necessary to provide sufficient exposure to CNP Prodrug and released vosoritide to establish an appropriate safety margin to support later stage development. In the event additional dose cohorts are included, the maximum number of participants in the study will be 70 participants. Study drug (CNP Prodrug or placebo) will be administered as an SC injection.

[0578] In each dose cohort, if neither sentinel participant experiences DLT within 3 days of dosing, the remaining 8 participants in their cohort will be randomized to receive a single administration of CNP Prodrug (n=6) or placebo (n=2) at the same dose as the sentinel participants; and

[0579] In each dose cohort, if 1 or both sentinel participants experience DLT within 3 days of dosing, the SRC will determine if dosing will continue. If dosing does continue, the remaining 8 participants will receive a single administration of CNP Prodrug (n=6) or placebo (n=2) at the same dose as the sentinel participants.

[0580] Dose escalation will be conducted as follows:

[0581] In each dose cohort, if ≥ 2 participants experience a DLT, ≥ 2 participants experience a study drug-related Grade 3/4 AE from the same SOC, or ≥ 2 participants experience a Grade 3/4 laboratory abnormality, the SRC will review and determine if dose escalation is appropriate.

[0582] The SRC will assess all 7-day safety data and available PK data from all participants in each cohort, in addition to any emerging safety issues, to determine if dose escalation is acceptable. If dose escalation is not acceptable, the remaining participants may be dosed at a lower dose level based on the SRC's decision. The SRC will consider if CNP Prodrug may have caused or contributed to the above events and, hence, may preclude further dose escalation. The SRC may also adapt the planned doses and/or add up to 2 additional dosing cohorts, dependent upon the emergent safety and PK data from the study.

[0583] STUDY TREATMENTS**[0584] Treatment Groups**

[0585] CNP Prodrug is a lyophilized powder for reconstitution, administered as a single SC injection. Placebo for SC injection is supplied as a solution and contains an identical concentration of excipients as the reconstituted drug product.

[0586] Rationale for Dosing

[0587] CNP Prodrug is a prodrug of the CNP analog vosoritide. By binding to the NPR-B in the chondrocytes in epiphyseal growth plates, vosoritide initiates a cascade of intracellular events that antagonize FGFR3 downstream signaling, resulting in chondrocyte proliferation and differentiation and matrix deposition. This leads to a widening of the growth plate followed by longitudinal bone formation.

[0588] CNP Prodrug is a synthetic peptide comprised of vosoritide bound via the Lys27 side chain to a pH-responsive self-immolative linker, a spacer, and C-18 fatty acid albumin binding domain. After SC injection and absorption to plasma, CNP Prodrug binds to albumin and extends the plasma circulation of CNP Prodrug. Upon hydrolysis of the linker at physiologic pH, active vosoritide (unmodified) is released from the albumin-bound CNP Prodrug at a constant rate, allowing for continuous systemic exposure to vosoritide rather than the peaks and troughs observed with daily ("pulsatile") vosoritide treatment. This is expected to result in sustained CNP exposure, persistent NPR-B activation, and an increase in AGV without the vosoritide-related hemodynamic effects associated with high C_{max}. The PK, safety, and efficacy of CNP Prodrug has been tested in the non-clinical setting. In NHPs, CNP Prodrug extended the vosoritide half-life from 0.5 hours to 65 hours. Given the lower C_{max} compared with vosoritide (>10-fold), CNP Prodrug did not have significant effects on heart rate and blood pressure at doses up to 3-fold higher than the effective dose in NHPs (ie, 150 mcg/kg). In NHPs, weekly administration of CNP Prodrug at 150 mcg/kg over a month resulted in growth plate expansion. In addition, treatment with CNP Prodrug in mice resulted in a greater growth response when compared with vosoritide.

[0589] These promising non-clinical results pose the basis for the FIH study of CNP Prodrug. The selected starting dose of 15 mcg/kg provides a 10-fold safety margin based on the NOAEL for local tolerability of 150 mcg/kg observed in the acute toxicity GLP study in NHPs (using a direct body weight scaling approach). The 350 mcg/kg dose corresponds to the highest dose being tested in the ongoing 6-month chronic pharmacology/toxicology GLP study in NHPs, which is below the NOAEL for systemic toxicity and cardiovascular effects in NHPs (500 mcg/kg).

[0590] Formulation and Packaging

[0591] CNP Prodrug is supplied as lyophilized, powder for reconstitution with sterile water for injection (sWFI). The drug product contains 15.7 mg/vial or 47.2 mg/vial of CNP Prodrug substance which is equivalent to 13 mg/vial or 39 mg/vial of CNP, respectively. The reconstituted solution contains 10 mg/mL or 30 mg/mL of CNP equivalent. The target pH of the reconstituted solution is 5.5. The clinical drug product will be supplied in sterile, Type I, single-dose, borosilicate glass vials with a coated stopper and flip-off aluminum cap.

[0592] Matching placebo is supplied as a formulation buffer containing 10 mM histidine buffer, 58 mg/mL trehalose dihydrate, 15 mg/mL D-mannitol, 0.73 mg/mL L-methionine, and 0.05 mg/mL polysorbate 80.

[0593] Endpoints

[0594] The primary endpoint is the incidence of AEs and SAEs, DLTs, physical examinations, vital signs, ECG findings, and clinical laboratory tests. Secondary endpoints include the following PK parameters for CNP prodrug, released vosoritide and lipid linker:

Area under the plasma concentration-time curve (AUC) from time 0 to infinity (AUC_{0-∞});

AUC from time 0 to the time of last measurable concentration (AUC_{0-t});

AUC from time 0 to 168 hours (AUC_{0-168h});

C_{max};

T_{max};

t_{1/2};

Apparent clearance of study drug (CL/F) (only for CNP Prodrug); and

Apparent volume of distribution based upon the terminal phase (V_z/F) (only for CNP Prodrug).

[0595] Exploratory Endpoints

[0596] The study includes exploratory endpoints such as the immunogenicity to CNP Prodrug (anti-drug antibodies) and biomarkers (urine and plasma cyclic guanosine monophosphate [cGMP] biomarkers).

[0597] Blood samples will be collected to evaluate PK. PK analysis will be performed for both vosoritide (active drug), lipid-linker (metabolite) and CNP Prodrug (prodrug).

[0598] *Anti-Drug Antibody, Biomarker and Pharmacokinetics Assessments*

[0599] Total serum anti-CNP Prodrug antibody samples will be drawn pre-dose on Day 1 and during treatment. Plasma PK parameters for CNP Prodrug, released vosoritide, and lipid linker will be determined by non-compartmental analysis, if feasible, from the plasma concentration-time data for participants who have sufficient data for PK analysis. The PK Parameters will be listed and summarized using descriptive statistics by dose group, as appropriate. Dose proportionality will be assessed for CNP Prodrug, released vosoritide across evaluated dose levels.

Example 28: Phase 2 trials in subjects with Achondroplasia

[0600] A 6-month, Phase 2, dose-range finding study of CNP Prodrug administered to pediatric participants with ACH who are treatment naïve is conducted. The proposed Phase 2 study will enroll 30 pre-pubertal participants aged 3 to 10 years for females and 3 to 11 years for males, with ACH confirmed by genetic testing, and naïve to any growth-promoting treatments. A treatment naïve population will allow for a robust determination of dose-response of CNP Prodrug on growth, in the absence of potential carryover effects of other growth-promoting treatments. This study aims to determine the therapeutic dose of CNP Prodrug. Safety, tolerability, and the growth effect of weekly SC injection of CNP Prodrug is evaluated.

[0601] Change from Baseline in annualized growth velocity (AGV), the primary efficacy measure in the Phase 2 study has been accepted as an endpoint for assessment of treatment effect on growth in short stature conditions. AGV is known to rapidly decline in early childhood, before stabilizing by age 3. The minimum age for eligibility of 3 years will ensure a stable baseline on the basis of which the impact of intervention with CNP Prodrug can be assessed. The maximum age for eligibility of 10 years for females and 11 years for males and restriction to Tanner Stage I is chosen to avoid the potential confounding impact of a highly individual and variable pubertal growth spurt on the initial data analyses of this Phase 2 study.

Study Design

[0602] Following 6 months of pre-treatment growth assessments collected prospectively to establish baseline AGV, participants will be randomized in a blinded fashion to 1 of 3 doses of CNP Prodrug (10 participants/dose) and treated for 6 months. An interim analysis will be performed when a minimum of 50% of participant enrolled in each treatment arm have 6 months follow-up to provide a preliminary assessment of CNP Prodrug on growth. Once all participants have completed 6 months of randomized CNP Prodrug treatment, an analysis will be performed to determine the therapeutic dose of CNP Prodrug and all

participants will transition to the selected dose and continue treatment in the long-term phase for xx weeks/months.

[0603] The primary endpoint will be change from baseline in AGV at 6 months. Leveraging the placebo data collected by BioMarin in prior randomized studies of treatment naïve ACH participants, we propose to utilize selected placebo data from based on baseline characteristics of this CNP Prodrug study as a comparator to the CNP Prodrug dose groups, replacing the need for a placebo-control group in the study. Reflecting the current treatment landscape, it is ethically preferred to minimize exposure to placebo, hence this study design ensures that all enrolled participants will have the opportunity to receive active treatment in the study. Additional analysis using natural history data collected by BioMarin, will also be conducted. Individual participants in CNP Prodrug will be matched to participants from natural history data based on baseline characteristics, then the difference between CNP Prodrug and control group on change from baseline at 6-month will be assessed using an appropriate model with appropriate adjustment.

Dose Selection

[0604] Three dose levels of CNP Prodrug will be evaluated in the Phase 2 study and determined based on results of the Phase 1 study and the 26-week GLP repeat-dose pharmacology and toxicology study in juvenile NHPs.

[0605] The selection of the therapeutic dose will be based on CNP Prodrug effect on efficacy and safety data at the 6-month timepoint in the Phase 2 study. Pharmacokinetic profiles will also be evaluated for each dose and the relationship between PK/plasma concentration and change from baseline in AGV will be explored. A dose of CNP Prodrug that is well tolerated, is associated with the most consistent improvements in efficacy across participants and has the best dose dependent efficacy will be selected for Phase 3.

Example 29: Phase 3 Pivotal Study

[0606] Consistent with the clinical development of CNP in ACH, to confirm the efficacy and safety of weekly SC administration of the CNP Prodrug therapeutic dose in a single, adequate, and well-controlled Phase 3 study of 12 months in pediatric participants with ACH is carried out. The proposed Phase 3 study evaluates the effect of CNP Prodrug versus CNP on change from baseline in AGV at 12 months to support approval in pediatric patients with ACH.

[0607] The planned Phase 3 study will enroll participants aged 3 to 17 years, with ACH confirmed by genetic testing and open epiphyses, who have been previously treated with

CNP. At the time that the Phase 3 study is planned to start, it is anticipated that patients with ACH may have several treatment options available to them; therefore, the feasibility of conducting an adequately powered study in a treatment-naïve population would be challenging. Hence, participants with prior exposure to CNP are proposed for this study, as patients switching from daily CNP to weekly CNP Prodrug are also likely to be representative of the majority of the intended patient population following approval.

[0608] All participants will be required to have at least 6 months of pre-treatment growth assessments collected prospectively in an observational study to determine their baseline AGV. Following a minimum of 6 months in this observational study, participants will be randomized in a blinded fashion to CNP Prodrug or CNP and treated for 12 months. CNP Prodrug will be administered once weekly at the therapeutic dose identified in the Phase 2 study, and CNP as per approved posology. Due to the different administration frequency (weekly for CNP Prodrug versus daily CNP injections), the study will essentially be open-label to the investigator and participants, but with blinded growth assessors utilized, who will have no access to treatment information in order to maintain the integrity of the efficacy endpoints/analyses. After participants have completed the 12 month treatment period, they may be eligible to receive CNP Prodrug in an extension study.

[0609] The Phase 3 study is designed to assess either the superiority or noninferiority of CNP Prodrug versus CNP. The primary efficacy endpoint is change from baseline in AGV at 12 months, an accepted regulatory endpoint for assessment of treatment effect on growth in short stature conditions. This parameter is a key indicator of skeletal growth, is easily and objectively measurable in an accurate, non-invasive manner, and is an accepted primary endpoint to provide evidence of increase in final adult height in proportionate short stature conditions. This endpoint has been demonstrated in the ACH CNP program to be a reliable endpoint to estimate long term gain in growth beyond 12 months. Height Z score at 12 months is assessed as a key secondary efficacy endpoint.

Example 30-Analysis of Clearance of CNP prodrug by NPR-B and NPR-C

[0610] This study was designed to evaluate the affinity of intact CNP Prodrug to the CNP pharmacologic receptor, NPR-B, and primary clearance receptor, NPR-C, using competitive binding assays to confirm that CNP Prodrug is pharmacologically inactive and that vosoritide remains inactive until its release from CNP Prodrug following hydrolysis of the self-immolating linker.

[0611] Methods

[0612] Binding of CNP Prodrug to NPR-B and NPR-C was evaluated in a competitive binding assay and compared to BMN111. Ninety-six well plates were coated with goat anti-human IgG then either NPR B Fc or NPR C Fc. Vosoritide were mixed 1:1 with vosoritide biotin and added to each well, then incubated at room temperature for 2 hours and washed. Streptavidin-horseradish peroxidase was then added to each well to detect the concentration of bound vosoritide biotin, plates were washed, SuperSignal™ enzyme-linked immunosorbent assay pico chemiluminescent substrate was added, and plates were read by a plate reader for signal intensity in relative light units (RLU).

[0613] Results

[0614] While BMN111 was able to out-compete or dissociate bound vosoritide-biotin from both NPR B (50% inhibitory concentration [IC50] = 40 nM) and NPR-C (IC50 = 140 nM), CNP Prodrug was not was able to dissociate vosoritide-biotin from either receptor at concentrations as high as 1 µM (Figure 34).

[0615] Although some free BMN111 was likely released from CNP Prodrug during the 2-hour incubation period, due to its slow formation by a constant rate of hydrolysis of the linker, it was not present in sufficient quantity to detectably out-compete or dissociate bound vosoritide-biotin from either NPR-B or NPR-C. CNP Prodrug was confirmed to be inactive in this assay as binding to NPR-B was not observed. Furthermore, these results confirm that CNP Prodrug will not be cleared in vivo by binding to NPR-C.

Example 31: Other assays

[0616] Binding of the CNP Prodrug to bovine serum albumin (BSA) was confirmed in a non-GLP *in vitro* study using biolayer interferometry. Binding to BSA was compared to vosoritide or phosphate buffered saline. CNP Prodrug showed albumin-specific binding whereas no binding was observed with vosoritide alone. Evaluations of *in vitro* protein binding of CNP Prodrug and lipid-linker in non-human primates and human plasma are ongoing.

[0617] To evaluate genotoxicity, bacterial reverse mutation (Ames) assays of the lipid-linker and *in vitro* micronucleus assay of lipid-linker in human peripheral blood lymphocytes are being conducted.

[0618] It is understood, therefore, that this invention is not limited to the particular embodiments disclosed, but is intended to cover all modifications which are within the spirit and scope of the invention as defined by the appended claims; the above description, and/or shown in the attached drawings. Consequently, only such limitations as appear in the appended claims should be placed on the disclosure.

What is Claimed:

1. A pharmaceutical composition comprising a variant of C-type natriuretic peptide (CNP) PGQEHPNARKYKGANKKGLSKGCFGLKLDRI SMSGLGC (SEQ ID NO: 1), a pharmaceutically acceptable excipient, and a carrier or a diluent, wherein the CNP variant comprises an acid moiety, a spacer, a hydrolysable linker, and is characterized by a structure as shown in Figure 11.
2. A pharmaceutical composition comprising (4R,10S,16S,19S,22S,28S,31S,34S,37S,40S,43S,49S,52R)-52-(2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-1-(L-prolylglycyl- L-glutaminy- L-glutamyl- L-histidyl)pyrrolidine-2-carboxamido)-4-amino-4-oxobutanamido)propanamido)-5-guanidinopentanamido)-6-aminohexanamido)-3-(4-hydroxyphenyl)propanamido)-6-aminohexanamido)acetamido)propanamido)-4-amino-4-oxobutanamido)-6-aminohexanamido)-6-aminohexanamido)acetamido)-4-methylpentanamido)-3-hydroxypropanamido)-6-aminohexanamido)acetamido)-49-benzyl-28-((S)-sec-butyl)-34-(carboxymethyl)-40-((S)-33,51-dicarboxy-8-(2-hydroxyethyl)-6,12,21,30,35-pentaoxo-14,17,23,26-tetraoxa-5,8,11,20,29,34-hexaazahenpentacontyl)-31-(3-guanidinopropyl)-16,22-bis(hydroxymethyl)-10,37,43-trisobutyl-19-(2-(methylthio)ethyl)-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51-hexadeca-1,2-dithia-5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50-hexadecaazacyclotripentacontane-4-carboxylic acid, a pharmaceutically acceptable excipient, and a carrier or a diluent.
3. The pharmaceutical composition of claim 1 or 2, wherein the pharmaceutical composition is lyophilized.
4. The pharmaceutical composition of any one of claims 1-3, wherein the carrier or diluent comprises a buffer.
5. The pharmaceutical composition of claim 4, wherein the buffer comprises a buffer selected from citrate, acetate, phosphate, TRIS, and a combination thereof.
6. The pharmaceutical composition of claim 5, wherein the buffer further comprises histidine, a salt thereof, a solvate thereof, or a solvate of a salt thereof.
7. The pharmaceutical composition of any one of claims 4-6, wherein the buffer is present in a concentration of 5-15 mM.
8. The pharmaceutical composition of any one of claims 4-7, having a pH of 3-9.
9. The pharmaceutical composition of claim 8, having a pH of 4-6.
10. The pharmaceutical composition of claim 9, having a pH of 5-6.
11. The pharmaceutical composition of claim 10, having a pH of 5.2 or 5.5.

12. The pharmaceutical composition of any one of claims 1-11, wherein the pharmaceutically acceptable excipient is selected from a bulking agent, a tonicity agent, an antioxidant, a surfactant, a solubilizing agent, a stabilizing agent, and a combination thereof.
13. The pharmaceutical composition of claim 12, wherein the bulking agent is selected from mannitol, sucrose, dextran, lactose, trehalose, and povidone (PVP K24), and a combination thereof.
14. The pharmaceutical composition of claim 12 or 13, wherein the bulking agent comprises trehalose or a solvate thereof, mannitol, or a combination thereof.
15. The pharmaceutical composition of claim 14, wherein the bulking agent comprises trehalose and mannitol in a weight ratio of 3:1 to 1:1.
16. The pharmaceutical composition of claim 15, wherein the weight ratio of trehalose to mannitol is 3.9:1.
17. The pharmaceutical composition of any one of claims 13-16, wherein trehalose is present in an amount of 3-6 wt% of the composition.
18. The pharmaceutical composition of claim 17, wherein trehalose is present in an amount of 3.5-5.8 wt% of the composition.
19. The pharmaceutical composition of claim 18, wherein trehalose is present in an amount of 3.8-4.8 wt% of the composition.
20. The pharmaceutical composition of any one of claims 12-19, comprising a tonicity agent selected from sodium chloride, dextrose, glucose, glycerin, sorbitol, xylitol, ethanol, and a combination thereof.
21. The pharmaceutical composition of any one of claims 12-20, comprising an antioxidant selected from methionine, ascorbic acid, salt forms of ascorbic acid, thioglycerol, and combinations thereof.
22. The pharmaceutical composition of claim 21, wherein the antioxidant is methionine.
23. The pharmaceutical composition of any one of claims 1-22, comprising a stabilizing agent or surfactant selected from glycine, sorbitol, polysorbate, and a combination thereof.
24. The pharmaceutical composition of claim 23, comprising polysorbate.
25. The pharmaceutical composition of any one of claims 1-24, wherein the pharmaceutical composition comprises L-histidine, histidine monohydrochloride monohydrate, trehalose dihydrate, D-mannitol, L-methionine, and polysorbate.

26. The pharmaceutical composition of any one of claims 1-24, wherein the pharmaceutical composition comprises citrate buffer, trehalose dihydrate, D-mannitol, L-methionine, and polysorbate.

27. A pharmaceutical composition comprising
(4R,10S,16S,19S,22S,28S,31S,34S,37S,40S,43S,49S,52R)-52-(2-((S)-2-((S)-2-((S)-2-(2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-1-(L-prolylglycyl- L-glutaminy- L-glutamyl- L-histidyl)pyrrolidine-2-carboxamido)-4-amino-4-oxobutanamido)propanamido)-5-guanidinopentanamido)-6-aminohexanamido)-3-(4-hydroxyphenyl)propanamido)-6-aminohexanamido)acetamido)propanamido)-4-amino-4-oxobutanamido)-6-aminohexanamido)-6-aminohexanamido)acetamido)-4-methylpentanamido)-3-hydroxypropanamido)-6-aminohexanamido)acetamido)-49-benzyl-28-((S)-sec-butyl)-34-(carboxymethyl)-40-((S)-33,51-dicarboxy-8-(2-hydroxyethyl)-6,12,21,30,35-penta-14,17,23,26-tetra-5,8,11,20,29,34-hexaazapentacontyl)-31-(3-guanidinopropyl)-16,22-bis(hydroxymethyl)-10,37,43-triisobutyl-19-(2-(methylthio)ethyl)-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51-hexadeca-1,2-dithia-5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50-hexadecaazacyclotripentacontane-4-carboxylic acid, a buffer comprising histidine or a salt thereof, and one or more pharmaceutically acceptable excipients.

28. The pharmaceutical composition of claim 27, wherein the buffer comprises L-histidine hydrochloride monohydrate.

29. The pharmaceutical composition of claim 27 or 28, comprising a pharmaceutically acceptable excipient selected from a bulking agent, a stabilizer, an anti-adsorbent, a diluent, and a combination thereof.

30. The pharmaceutical composition of any one of claims 27-29, wherein the pharmaceutically acceptable excipient comprises a bulking agent comprising trehalose or a solvate thereof.

31. The pharmaceutical composition of claim 30, wherein the bulking agent comprises trehalose dihydrate.

32. The pharmaceutical composition of any one of claims 27-31, wherein pharmaceutically acceptable excipient comprises a bulking agent comprising mannitol.

33. The pharmaceutical composition of claim 32, wherein the mannitol is D-mannitol.

34. The pharmaceutical composition of any one of claims 27-33, wherein the pharmaceutically acceptable excipient comprises a stabilizer comprising methionine.

35. The pharmaceutical composition of claim 34, wherein the methionine is L-methionine.

36. The pharmaceutical composition of any one of claims 27-35, wherein the pharmaceutically acceptable excipient comprises an anti-adsorbent comprising polysorbate.

37. The pharmaceutical composition of claim 36, wherein the polysorbate is polysorbate 80.

38. The pharmaceutical composition of any one of claims 27-37, wherein the pharmaceutical composition is substantially free of a citrate buffer.

39. A pharmaceutical composition comprising a C-type natriuretic peptide (CNP) variant that is (4R,10S,16S,19S,22S,28S,31S,34S,37S,40S,43S,49S,52R)-52-(2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-2-((S)-1-(L-prolylglycyl-L-glutamyl-L-glutamyl-L-histidyl)pyrrolidine-2-carboxamido)-4-amino-4-oxobutanamido)propanamido)-5-guanidinopentanamido)-6-aminohexanamido)-3-(4-hydroxyphenyl)propanamido)-6-aminohexanamido)acetamido)propanamido)-4-amino-4-oxobutanamido)-6-aminohexanamido)-6-aminohexanamido)acetamido)-4-methylpentanamido)-3-hydroxypropanamido)-6-aminohexanamido)acetamido)-49-benzyl-28-((S)-sec-butyl)-34-(carboxymethyl)-40-((S)-33,51-dicarboxy-8-(2-hydroxyethyl)-6,12,21,30,35-penta-14,17,23,26-tetra-5,8,11,20,29,34-hexaazapentacontyl)-31-(3-guanidinopropyl)-16,22-bis(hydroxymethyl)-10,37,43-triisobutyl-19-(2-(methylthio)ethyl)-6,9,12,15,18,21,24,27,30,33,36,39,42,45,48,51-hexadeca-1,2-dithia-5,8,11,14,17,20,23,26,29,32,35,38,41,44,47,50-hexadecaazacyclotripentacontane-4-carboxylic acid, a histidine buffer, trehalose dihydrate, D-mannitol, L-methionine, polysorbate 80, and water.

40. The pharmaceutical composition of claim 39, wherein the CNP variant is present at a concentration of 10 mg/mL.

41. The pharmaceutical composition of claim 39, wherein the CNP variant is present at a concentration of 30 mg/mL.

42. The pharmaceutical composition of any one of claims 39-41, wherein the histidine buffer comprises L-histidine and histidine monohydrochloride monohydrate.

43. The pharmaceutical composition of claim 42, wherein L-histidine is present at a concentration of 0.35 mg/mL.

44. The pharmaceutical composition of claim 42, wherein L-histidine is present at a concentration of 2.2 mM.

45. The pharmaceutical composition of any one of claims 42-44, wherein L-histidine monohydrochloride monohydrate is present at a concentration of 1.6 mg/mL.

46. The pharmaceutical composition of any one of claims 42-44, wherein L-histidine monohydrochloride monohydrate is present at a concentration of 7.8 mM.

47. The pharmaceutical composition of any one of claims 39-46, wherein the trehalose dihydrate is present at a concentration of 58 mg/mL.

48. The pharmaceutical composition of any one of claims 39-46, wherein the trehalose dihydrate is present at a concentration of about 127 mM to about 153 mM.

49. The pharmaceutical composition of claim 48, wherein the trehalose dihydrate is present at a concentration of 127 mM.

50. The pharmaceutical composition of claim 48, wherein the trehalose dihydrate is present at a concentration of 153.3 mM.

51. The pharmaceutical composition of any one of claims 39-50, wherein the D-mannitol is present at a concentration of 15 mg/mL.

52. The pharmaceutical composition of any one of claims 39-50, wherein the D-mannitol is present at a concentration of about 68 mM to about 82 mM.

53. The pharmaceutical composition of claim 52, wherein the D-mannitol is present at a concentration of 68.1 mM.

54. The pharmaceutical composition of claim 52, wherein the D-mannitol is present at a concentration of 82.3 mM.

55. The pharmaceutical composition of any one of claims 39-54, wherein the L-methionine is present at a concentration of 0.7 mg/mL.

56. The pharmaceutical composition of any one of claims 39-54, wherein the L-methionine is present at a concentration of 4.9 mM.

57. The pharmaceutical composition of any one of claims 39-56, wherein the polysorbate 80 is present at a concentration of 0.05 mg/mL.

58. The pharmaceutical composition of any one of claims 39-56, wherein the polysorbate 80 is present at a concentration of 0.005% (v/v).

59. The pharmaceutical composition of any one of claims 1-58, wherein the composition exhibits lower C_{max} and higher AUC compared to free drug.

60. A pharmaceutical kit comprising the pharmaceutical composition of any one of claims 1-58.

61. A pharmaceutical kit comprising the pharmaceutical composition of any one of claims 39-58, wherein the CNP variant is present in amount of 13 mg.

62. A pharmaceutical kit comprising the pharmaceutical composition of any one of claims 39-58, wherein the CNP variant is present in amount of 39 mg.

63. A method of treating a bone-related disorder or skeletal dysplasia in a subject in need thereof comprising administering to the subject a composition according to any one of claims 1-59.

64. The method of claim 63, wherein the bone-related disorder or skeletal dysplasia is selected from the group consisting of osteoarthritis, hypophosphatemic rickets, achondroplasia, hypochondroplasia, short stature, dwarfism, osteochondrodysplasias, thanatophoric dysplasia, osteogenesis imperfecta, achondrogenesis, chondrodysplasia punctata, homozygous achondroplasia, campomelic dysplasia, congenital lethal hypophosphatasia, perinatal lethal type of osteogenesis imperfecta, short-rib polydactyly syndromes, rhizomelic type of chondrodysplasia punctata, Jansen-type metaphyseal dysplasia, spondyloepiphyseal dysplasia congenita, atelosteogenesis, diastrophic dysplasia, congenital short femur, Langer-type mesomelic dysplasia, Nievergelt-type mesomelic dysplasia, Robinow syndrome, Reinhardt syndrome, acrodysostosis, peripheral dysostosis, Kniest dysplasia, fibrochondrogenesis, Roberts syndrome, acromesomelic dysplasia, micromelia, Morquio syndrome, Kniest syndrome, metatrophic dysplasia, and spondyloepimetaphyseal dysplasia, NPR2 mutation, SHOX mutation (Turner's syndrome/Leri Weill), PTPN11 mutations (Noonan's syndrome), insulin growth factor 1 receptor (IGF1R) mutation, osteoporosis, and idiopathic short stature.

65. A method of elongating a bone or increasing long bone growth in a subject in need thereof, comprising administering to the subject a composition according to any one of claims 1 to 59, and wherein the administering elongates a bone or increases long bone growth.

66. The method of any one of claims 63-64, wherein the composition is administered subcutaneously, intradermally, intraarticularly, orally, or intramuscularly.

67. The method of any one of claims 63-66 wherein the composition provides an extended release composition.

68. The method of any one of claims 63-66, wherein the composition is administered, once every 5 days, once a week, once every two weeks, once every three weeks, once every 4 weeks, once every 6 weeks, once every two months, once every three months or once every six months.

69. A method of treating a CNP-responsive condition or disorder, comprising
-administering to a subject a composition according to any one of claims 1 to 58, and
-monitoring the level of at least one bone- or cartilage-associated biomarker in the subject, wherein an increase in the level of the at least one bone- or cartilage-associated biomarker indicates a therapeutic effect of the CNP variant on the subject or the condition or disorder.

70. The method of claim 69, further comprising adjusting the amount or frequency of administration of the composition, wherein

- i) the amount or frequency of administration of the composition is increased if the level of the at least one bone- or cartilage-associated biomarker is below a target level; or
- ii) the amount or frequency of administration of the composition is decreased if the level of the at least one bone- or cartilage-associated biomarker is above a target level.

71. The method of claim 69 or 70, wherein the at least one bone- or cartilage-associated biomarker is selected from the group consisting of CNP, cGMP, propeptides of collagen type II and fragments thereof, collagen type I and fragments thereof, collagen type I C-telopeptide (CTx), osteocalcin, proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen (PINP) and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, collagen X, alkaline phosphatase proliferating cell nuclear antigen (PCNA), propeptides of type I procollagen and fragments thereof, collagen type I and fragments thereof, aggrecan chondroitin sulfate, collagen X, CXM (noncollagenous 1 (NC1) domain of type X collagen), NTproCNP, and alkaline phosphatase, N-terminal collagen type I pro-peptide, bone-specific alkaline phosphatase, amino-terminal propeptide of type I collagen/procollagen type I N-propeptide (PINP), cross-linked C-telopeptide of type I collagen (CTx), cross-linked N-telopeptide of type I collagen (NTx) tartrate-resistant acid phosphatase 5b (TRAP-5b), transcriptomics readouts, and CNP-variant bioactivity.

72. The method of any one of claims to 63-71, wherein the administration increases the annualized growth velocity (AGV) in the subject at 12 months, optionally compared to baseline or to a normal control.

73. The method of claim 72, wherein the AGV in the subject increases over 1 year or over 2 years, or more.

74. The method of any one of claims 63-73, wherein the administration improves the height Z score at 12 months, optionally compared to baseline or to a normal control.

75. The method of any one of claims to 63-74, wherein the subject is greater than 3 years old.

76. The method of any one of claims to 63-75, wherein the subject is between 3 and 17 years old.

77. The method of any one of claims 63-76, wherein the subject has open epiphyses.

78. The method of any one of claims 63-77, wherein the composition is administered at a dose from about 5 µg/kg to 500 µg/kg or from about 15 µg/kg to 350 µg/kg.

79. The method of any one of claims 63-77, wherein the administration does not result in cardiovascular (CV) side effects.

80. The method of claim 79, wherein the CV side effect is change in systemic blood pressure, mean arterial pressure, systolic and/or diastolic blood pressures, pulse pressure or heart rate.