

ORIGINAL ARTICLE

Phase 1 safety, tolerability, pharmacokinetics and pharmacodynamics results of a long-acting C-type natriuretic peptide prodrug, TransCon CNP

Vibeke Miller Breinholt¹ | Per Hølse Mygind¹ | Eva Dam Christoffersen¹ |
Ying Zhang² | Sho Ota²  | R. Will Charlton² | Dorthe Viuff¹

¹Ascendis Pharma A/S, Hellerup, Denmark

²Ascendis Pharma, Inc., Palo Alto, CA, USA

Correspondence

Vibeke Miller Breinholt, Tuborg Boulevard
12, 2900 Hellerup, Denmark.

Email: vmb@ascendispharma.com

Funding information

Ascendis Pharma Growth Disorders A/S,
Hellerup, Denmark

Aim: TransCon CNP is a novel prodrug designed to provide sustained release of C-type natriuretic peptide (CNP) for once-weekly therapy, addressing the pathology leading to aberrant skeletal development in achondroplasia. This phase 1 trial was initiated to assess the safety, tolerability, pharmacodynamics (PD) and pharmacokinetics (PK) of TransCon CNP.

Methods: This randomized, placebo-controlled, single-ascending dose phase 1 trial was performed at two sites in Australia and enrolled 45 healthy adult males. Subjects received placebo or TransCon CNP (single-ascending dose cohorts [3, 10, 25, 75 or 150 µg CNP/kg]). The primary endpoint was frequency of adverse events and other safety outcomes. Other endpoints included PK and PD measured by cyclic guanosine-monophosphate (cGMP) and amino-terminal propeptide of CNP (NTproCNP).

Results: TransCon CNP provided continuous systemic exposure to CNP over at least 7 days post-dose. Plasma and urine levels of cGMP were significantly increased in subjects administered TransCon CNP at 75-150 µg CNP/kg, indicating target engagement of active CNP at the natriuretic peptide receptor-B (NPR-B) for at least 1 week post-dose. TransCon CNP was well-tolerated, with no serious treatment-emergent adverse events or discontinuations. Extensive cardiac safety assessments did not reveal any clinically relevant effects on electrocardiogram parameters, including heart rate, PR, QRS and QTcF intervals.

Conclusions: Safety and PD data from this phase 1 trial support that TransCon CNP is well tolerated, with a PK profile compatible with a once-weekly dosing regimen. Further studies are ongoing to evaluate the potential of TransCon CNP to positively impact abnormal endochondral ossification in children with achondroplasia.

KEYWORDS

achondroplasia, C-type natriuretic peptide, FGFR3, pharmacodynamics, pharmacokinetics, prodrug, TransCon CNP

REPRINT REQUESTS: Reprint requests should be addressed to the corresponding author.

This is an open access article under the terms of the [Creative Commons Attribution-NonCommercial](https://creativecommons.org/licenses/by-nc/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited and is not used for commercial purposes.

© 2022 The Authors. *British Journal of Clinical Pharmacology* published by John Wiley & Sons Ltd on behalf of British Pharmacological Society.

1 | INTRODUCTION

Achondroplasia (ACH) is caused by a gain-of-function autosomal dominant mutation in the **fibroblast growth factor 3 receptor** (FGFR3) gene with 100% penetrance. ACH is the most common skeletal dysplasia and the most frequent form of short-limbed dwarfism occurring with a frequency of 1 in 10 000 to 30 000 live births, affecting 250 000 men and women worldwide.¹⁻³ Individuals with ACH have a phenotypically distinct appearance, including short stature (but an average size trunk), macrocephaly with frontal bossing, rhizomelic (proximal) limb shortening, short broad fingers, impaired elbow extension, limited hip extension, genu varum deformity, vertebral hyperlordosis and thoracolumbar kyphosis.^{1,2,4-7} Another characteristic feature of individuals with ACH is a narrowed foramen magnum that can result in compression of the spinal cord and neurological complications. The average height of adult men and women with ACH is 131 ± 5.6 and 124 ± 5.9 cm, respectively.^{6,8}

The FGFR3 mutations that cause ACH result in a ligand-independent gain-of-function state of the mutated receptor.⁹ FGFR3 is a tyrosine-kinase receptor-activating signal transducer and activator of transcription 1 (STAT1) and the mitogen-activated protein kinase (MAPK) pathway (RAS-RAF-MEK-ERK), and both have inhibiting effects on endochondral ossification. The impairment of endochondral ossification in ACH is mainly related to chondrocyte dysfunction, manifested as a post-proliferative pre-hypertrophic differentiation block mediated by the MAPK pathway, thereby hampering the formation of fully differentiated hypertrophic chondrocytes. STAT1 activity, on the other hand, mainly inhibits chondrocyte proliferation in the growth plate.¹⁰⁻¹² In FGFR3 mutations relevant for ACH, the MAPK pathway, but not STAT1, has been documented to be overly active,¹³ supporting the theory that the condition mainly originates from excessive FGFR3-induced MAPK signalling.

Observations in several nonclinical models have demonstrated that **C-type natriuretic peptide** (CNP) influences growth via modulation of the intracellular signalling pathways of FGFR3, through activation of the **natriuretic peptide receptor-B** (NPR-B), and is able to restore the FGFR3-induced stunted growth in ACH mouse models towards wild-type growth.¹⁴⁻¹⁹ Activation of NPR-B in chondrocytes mediates an increase in cyclic guanosine monophosphate (cGMP) that induces type II cGMP-dependent protein kinases, resulting in an inhibition of the MAPK pathway.^{18,19} A daily subcutaneous injection of a CNP analogue, vosoritide, has recently received accelerated approval by the US FDA for the treatment of children with ACH who are 5 years or older and have open epiphyses, and marketing authorization in the EU for the treatment of children with ACH who are 2 years or older and have open epiphyses.²⁰

TransCon CNP is an investigational long-acting prodrug of CNP that has demonstrated pharmacokinetic (PK) properties that provide long-acting effects on bone growth in both murine and nonhuman primate models.²¹ Prolonged suppression of the overactive signalling pathways seen in ACH, resulting from continuous exposure to CNP,

What is already known about this subject

- Achondroplasia is a skeletal dysplasia caused by gain-of-function mutation in fibroblast growth factor 3 receptor (FGFR3).
- C-type natriuretic peptide (CNP) modulates the intracellular signalling pathways of FGFR3 and is able to restore FGFR3-induced stunted growth in achondroplasia mouse models.
- CNP positively modulates bone growth in children with achondroplasia.

What this study adds

- TransCon CNP, a novel prodrug that releases native CNP-38, was tested in healthy adults to determine the safety, tolerability, pharmacodynamics and pharmacokinetics of this agent under investigation for the treatment of children with achondroplasia.
- Safety and pharmacokinetic data indicate that TransCon CNP is well tolerated and that CNP remains in systemic circulation for at least 7 days following a single dose.

may provide an even more effective means of alleviating the short stature seen in ACH.

The current paper describes the first-in-human trial with TransCon CNP, an investigational prodrug of CNP designed to provide continuous exposure to CNP on a once-weekly dosing schedule, evaluating the safety (including immunogenicity), tolerability, pharmacodynamics (PD) (including effects on systemic levels of NTproCNP and cGMP) and PK of subcutaneously (SC) administered single-ascending doses (SADs) of TransCon CNP to healthy male subjects.

2 | MATERIALS AND METHODS

2.1 | Introduction to study drug

TransCon CNP consists of a CNP moiety transiently bound to a 40-kDa branched polyethylene glycol carrier molecule (4×10 kDa). The CNP moiety constitutes the C-terminal 38 amino acids of full-length human CNP conjugated to a lysine residue at the polyethylene glycol carrier molecule attachment site. The CNP moiety is essentially inactive when bound to the carrier.²¹ Active CNP released from TransCon CNP is referred to here as CNP-38.

2.2 | Study oversight

The present study is a phase 1, first-in-human, randomized, double-blind, placebo-controlled, SAD trial in healthy adult male subjects designed to evaluate the safety, tolerability, PD and PK of SC administered TransCon CNP (Trial ID: ACTRN126180011 57268).

The trial, protocol amendments, informed consent forms, investigator's brochure, and any other relevant information were reviewed and approved by the appropriate independent ethics committee at the sites before initiation of the trial, and International Council for Harmonisation Guidelines for Good Clinical Practice, E6(R2) were followed.

The trial was conducted from May to November 2018 at two sites in Australia. All participants provided written informed consent.

2.3 | Clinical trial design and study end points

The trial design included five dosing cohorts (up to 10 subjects per cohort, up to eight active and two placebo), with the placebo subjects pooled to create a sixth treatment arm. Study drug was administered SC in the abdomen in single doses to ascending dose cohorts (3 μ g, 10, 25, 75 and 150 μ g CNP/kg). The drug product formulation containing TransCon CNP was a lyophilized powder in single-use vials (3.9 mg CNP-38/vial) to be reconstituted with sterile water for injection. The reference to CNP-38 per vial refers to the mass of CNP peptide moiety present in the TransCon CNP within the vial. For dose volumes higher than 0.85 mL, the dose was given as multiple injections with equal volume per injection. The placebo product was physiological saline. Safety monitoring included vital signs (including orthostatics), physical examinations, electrocardiograms with 12-lead Holter monitors and safety laboratory assessments (haematology, chemistry and urinalysis), local tolerability assessments and antibodies against CNP.

The trial consisted of a screening visit (day -28 to day -2) to assess eligibility, a subsequent check-in period in which screening was completed and eligibility confirmed (day -2 to day -1) and a dosing period (day 1). This was followed by a safety observational period of 4 weeks with 8 days at the site followed by three visits on days 15, 22 and 28. Up to 10 eligible subjects who had successfully completed screening were enrolled into the lowest dose cohort and randomly assigned (double-blind) to receive TransCon CNP or matched placebo (4:1). Each cohort was enrolled and completed in a sequential fashion. Because hypotension has been reported with CNP exposure, a sentinel pair of subjects (one receiving TransCon CNP and one receiving placebo) for each of the five cohorts was dosed first. After a period of approximately 72 hours, and at the discretion of the principal investigator, the remaining eight subjects were randomized 7:1 (TransCon CNP: placebo) to achieve a total ratio of 4:1 for each cohort. At the completion of the inpatient period for each cohort (inclusive of an inpatient period from day -2 to day 8), blinded clinical safety and

laboratory parameters were assessed by a Data and Safety Monitoring Board (DSMB) to provide a recommendation on dose escalation. After the DSMB provided the recommendation, the Safety Data Review Committee made the decision to continue with dose escalation or to pause (or stop) the dose escalation.

The primary objective was to determine the safety and tolerability of single-ascending SC doses of TransCon CNP in healthy adult male subjects. The secondary objective was to evaluate the PK properties of TransCon CNP in healthy adult male subjects. Assessment of systemic target engagement by measuring cyclic guanosine monophosphate (cGMP) levels in both urine and plasma and potential impact on CNP synthesis (measuring plasma CNP amino-terminal propeptide [NTproCNP]) was conducted on an exploratory basis.

2.4 | Pharmacokinetics: CNP-38

Blood samples for assessment of CNP-38 released from the prodrug following autocleavage (ie, free CNP-38) were collected on day 1 (pre-dose) and at 21 time points post-dose from day 1 to day 28. CNP-38 in heparin plasma was quantified using deuterated CNP-38 as an internal standard. Plasma samples were processed using protein precipitation and solid-phase extraction followed by high-performance liquid chromatography with tandem mass spectrometry detection in positive ion mode measuring the intact CNP-38 peptide. The method was validated according to regulatory guidance documents on bioanalytical method validation (EMA Guideline on bioanalytical method validation [EMA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2 and FDA Guidance for Industry, Bioanalytical method validation May 2018]).^{22,23} The lower limit of quantification for CNP-38 in heparin plasma was 1.38 pM.

2.5 | Pharmacokinetic analysis and dose proportionality

PK parameters, peak plasma concentration (C_{max}), time to peak plasma concentration (T_{max}), time of last measurable concentration (T_{last}), area under the curve to the time of the last measurable concentration (AUC_{0-t}), AUC to infinity ($AUC_{0-\infty}$), percentage AUC extrapolated from T_{last} to infinity (% AUC_{extrap}) and the apparent terminal half-life ($t_{1/2}$) were determined from the plasma concentration-time profiles of CNP-38 using non-compartmental analysis (Phoenix WinNonlin Version 6.4, Certara USA, Inc.). AUCs were calculated using the linear trapezoidal method when concentrations were increasing and the logarithmic trapezoidal method when concentrations were decreasing. PK analyses were carried out using actual post-dose times relative to the start time of dose administration. Dose proportionality was assessed by the power model estimating the slope of the log-transformed dose as the independent variable in a linear regression model with the log-transformed PK parameter as the response/dependent variable. The 95% confidence interval (CI) of the slopes was calculated.

2.6 | Anti-CNP antibody assay

Serum samples were collected pre-dose and post-dose on days 15 and 28. Samples were analysed using a validated bridging electrochemiluminescence immunoassay to detect antibodies against CNP. The assay was developed and validated according to current regulatory guidelines,^{24,25} ensuring adequate analytical sensitivity in the presence of both drug and prodrug.

2.7 | cGMP assay

Both 24-hour urine and plasma samples were collected on day 1 (pre-dose), and on days 2, 8 and 28 post-dose. Following sample extraction, a radioimmunoassay (RIA) was applied to measure cGMP in both plasma and urine.^{26,27}

In urine samples, results were adjusted according to 24-hour urine volumes and levels of urine creatinine. To minimize the impact of intra-individual variation in absolute levels of cGMP in both matrices, all post-dose samples (days 2, 8 and 28) were analysed in the same analytical run and data were baseline-adjusted using individual pre-dose levels.

For results obtained in both plasma and urine samples, testing for differences was performed by means of sequential *t*-tests comparing changes in cGMP levels in TransCon CNP-treated cohorts relative to the placebo group.

2.8 | NTproCNP

Plasma samples were collected pre-dose (day -1) and on post-dose days 2, 8 and 28. Following sample extraction, an RIA was applied to measure NTproCNP.^{27,28} To minimize the impact of intra-individual variations in absolute levels of NTproCNP, all post-dose samples (days 2, 8 and 28) were analysed in the same assay run and baseline-adjusted using individual pre-dose levels.

Testing for differences was performed by means of sequential *t*-tests comparing changes in NTproCNP levels in TransCon CNP-treated cohorts relative to the placebo group.

2.9 | Electrocardiography

All subjects were fitted with a continuous 12-lead electrocardiography (ECG) Holter recorder (M12R, Global Instrumentation, LLC, Manlius, New York, USA) from 25 hours prior to dosing to 72 hours after dosing. ECGs were evaluated at 45, 30 and 15 minutes prior to dosing and at 2, 4, 8, 12, 15, 24, 30, 36, 48, 54, 60 and 72 hours after dosing, with subjects resting in the supine position from 10 minutes before to 5 minutes after each scheduled time point. ECGs were extracted and analysed at a central ECG laboratory (ERT, Rochester, New York, USA). At each timepoint, up to 10 ECG replicates were extracted using TQT Plus,

a computer-assisted algorithm based on quality and heart rate (HR) stability criteria. All readable cardiac cycles from these ECG replicates were assessed for multiple quality parameters, including beat stability, HR changes and noise, and were categorized into high- and low-confidence beats. All low-confidence beats were fully reviewed and adjudicated manually by ECG technicians using pass-fail criteria. The beats found acceptable by manual review were included together with the high-confidence beats in the analysis. With lead II as the primary analysis lead, ECG interval measurements were made using the high-precision QT measurement technique. Categorical T-wave morphology analysis and measurement of PR and QRS intervals were performed manually in three of the 10 ECG replicates at each timepoint. Additionally, safety ECGs were obtained at screening, check-in, pre-dose (within 1 hour) and at approximately 2, 4, 8, 12, 24 and 48 hours post-dose and on days 15, 22 and 28.

2.10 | Blood pressure and HR

Blood pressure (BP) and HR were recorded in the supine position after at least 5 minutes of rest. BP and HR were assessed at the same time points as the ECG parameters. In addition, subjects were examined for signs of orthostatic intolerance, defined as a decrease of ≥ 20 mm Hg systolic BP or >20 bpm increase in HR (considered clinically significant or resulting in >120 bpm) at 3 minutes of standing, or symptoms of lightheadedness, dizziness or fainting on standing. Safety evaluation of adverse events (AEs) focused on treatment-emergent adverse events (TEAEs).

3 | RESULTS

3.1 | Subjects

A total of 49 healthy male subjects were randomized to the study in a 4:1 ratio (active:placebo). Four subjects (two subjects in the 75 μ g CNP/kg cohort, one in the 25 μ g CNP/kg cohort and one in the 10 μ g CNP/kg) cohort were excluded after enrolment but prior to receiving study drug due to (1) HR < 50 bpm on pre-dose ECG, (2) orthostatic lightheadedness, (3) significant increase in orthostatic HR and (4) HR < 50 bpm on pre-dose ECG. One subject randomized to placebo in cohort 3 was dosed but withdrew due to personal reasons before completing the trial. Of the 44 subjects who completed the trial, 36 received TransCon CNP and eight received placebo. In cohort 1, six subjects received 3 μ g CNP/kg (plus one subject received placebo). In cohort 2, six subjects received 10 μ g CNP/kg (plus two subjects received placebo). In cohort 3, eight subjects received 25 μ g CNP/kg (plus one subject received placebo). In cohort 4, eight subjects received 75 μ g CNP/kg (plus two subjects received placebo). In cohort 5, eight subjects received 150 μ g CNP/kg (plus two subjects received placebo).

Baseline characteristics are presented in Table 1.

3.2 | Pharmacokinetics

All subjects were included in the PK analysis set, but PK samples for all subjects from the 3 µg CNP/kg cohort and one subject from the

TABLE 1 Summary of baseline demographics for subjects who received study treatment (CNP or Placebo)

Group	n	Age (years)	Body weight (kg)	BMI (kg/m ²)
3 µg CNP/kg	6	32.2 (25-50)	73.3 (68-84)	23.1 (21-26)
10 µg CNP/kg	6	29.2 (25-34)	70.5 (60-86)	23.0 (20-26)
25 µg CNP/kg	8	33.4 (26-54)	74.3 (59-88)	23.2 (20-27)
75 µg CNP/kg	8	33.8 (25-56)	80.0 (59-95)	24.6 (20-30)
150 µg CNP/kg	8	35.4 (27-60)	87.1 (75-97)	26.1 (23-28)
Placebo	9	32.8 (25-45)	77.9 (69-93)	24.1 (21-27)

Note: Mean (range). Subjects in the two highest dose cohorts received two to five injections to receive the assigned dose. In the placebo group, five subjects received one injection, two subjects received two injections and the remaining two subjects received four or five injections, respectively.

10 µg CNP/kg cohort were not analysed for CNP-38 due to a sampling error.

3.3 | CNP-38

The mean PK profiles of CNP-38 following SC administration of TransCon CNP are presented in Figure 1. The CNP-38 PK profile following release from the prodrug was characterized by a slow rise to peak plasma concentrations with median T_{max} ranging from 45 to 66 hours post-dose. After reaching C_{max} , CNP-38 concentrations declined slowly in an apparent monophasic manner. The apparent $t_{1/2}$ was estimated to be approximately 120 hours (mean $t_{1/2}$ ranged from 112 to 128 hours over the 25-150 µg CNP/kg dose range). No marked dose-dependent trend for T_{max} or $t_{1/2}$ was observed. The compiled PK parameters are provided in Table 2. The between-subject variability in exposure ranged from 25% to 46% for C_{max} and AUC_{0-t} . The concentration of CNP-38 in systemic circulation returned to levels below the lower limit of quantification in all subjects, with median T_{last} ranging from 96 to 338 hours.

Statistical assessment concluded dose proportionality in exposure to CNP-38 over the 10-150 µg CNP/kg dose range for C_{max} and AUC_{0-t} based on regression slope estimates of 0.973 and 1.09 with

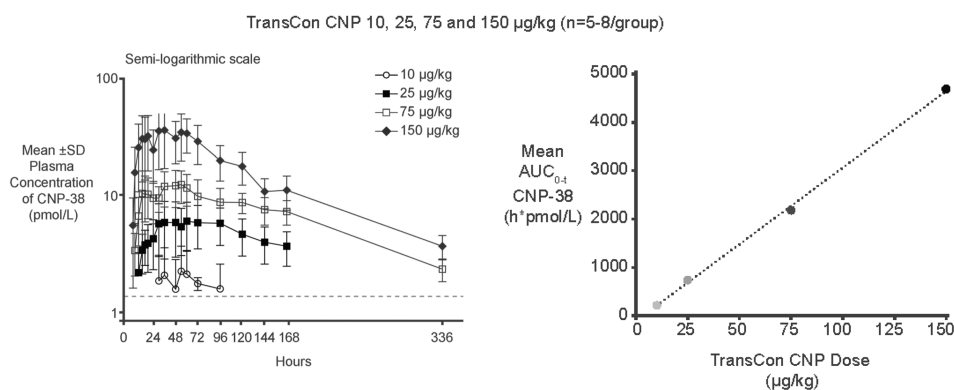


FIGURE 1 CNP-38 Plasma concentration profiles and dose proportionality between TransCon CNP dose and AUC of CNP-38. Left panel: CNP-38 plasma concentration (pmol/L) profile (arithmetic means (\pm SD) and semilogarithmic scale), following single-dose administration of 10, 25, 75 and 150 µg/kg CNP to adult male subjects. Right panel: CNP-38 AUC_{0-t} ($h \cdot pmol/L$) (geometric means and linear scale), as a function of TransCon CNP dose level. AUC_{0-t} , area under the curve to the time of the last measurable concentration

TABLE 2 Summary of CNP-38 pharmacokinetic parameters

Dose (µg CNP/kg)	n	T_{max} (hours)	C_{max} (pmol/L)	AUC_{0-t} (pmol \cdot h/L)	$AUC_{0-\infty}$ (pmol \cdot h/L)	% AUC_{extrap}	$t_{1/2}$ (hours)	T_{last} (hours)
10	5	60 (54-72)	2.47 (37.7)	218 (40.1)	NC	NC	NC	96 (72-144)
25	8	66 (37-96)	6.33 (45.5)	741 (43.8)	NC	NC	128 (59.8) ⁿ⁼⁴	168 (168-168)
75	8	51 (12-72)	13.7 (36.9)	2180 (24.6)	2570 (22.5)	14.9 (4.48)	112 (20.3) ⁿ⁼⁸	337 (315-340)
150	8	45 (8-72)	39.1 (44.6)	4680 (37.3)	5230 (33.8)	10.5 (3.72)	114 (15.3) ⁿ⁼⁸	338 (335-509)

Note: C_{max} and AUCs are presented as geometric mean (CV%). T_{max} and T_{last} are presented as median (range). $t_{1/2}$ and % AUC_{extrap} are presented as mean (SD) and includes the number of subjects (n) with an estimated half-life.

Abbreviation: NC, not calculated.

the 95% CIs ranging from 0.811 to 1.131 and 0.951 to 1.24, respectively, and thereby encompassing 1. Dose proportionality was not assessed for $AUC_{0-\infty}$ as data were only available for two dose cohorts (75 and 150 μg CNP/kg) (Figure 1).

3.4 | cGMP

cGMP was measured in both urine and plasma samples. The average (\pm SD) baseline level of cGMP in plasma was 4.1 nM (\pm 1.6 nM). No statistically significant increase in cGMP levels on exposure to CNP was observed at lower dose levels (3, 10 and 25 μg CNP/kg). Subjects exposed to TransCon CNP in the highest dose cohorts (75 and 150 μg CNP/kg) showed statistically significant increases from baseline in both urine and plasma levels of cGMP (days 2 and 8 post-dose) (Figure 2). The effect of TransCon CNP on cGMP levels was generally considered dose-dependent.

3.5 | NTproCNP

The average (\pm SD) level of NTproCNP at pre-dose was estimated at 24.2 pM (\pm 4.8 pM). Levels of endogenous NTproCNP measured in post-dose samples collected on days 2 and 8 did not show any statistically significant impact of treatment irrespective of the time point of sampling or dosing cohort.

3.6 | Injection site reactions

Injection site reactions were assessed daily for the first 8 days post-dose and then at days 15, 22 and 28. The assessment of local tolerability at the injection site included redness, bruising, swelling and pain, and overall findings in subjects receiving TransCon CNP were comparable to placebo. Overall, no consistent trends in local tolerability were found with increasing dose level. One severe local tolerability finding of bruising was reported for one subject in the 150 μg CNP/kg treatment group. One TEAE of mild injection site

discomfort (in a subject dosed with 10 μg CNP/kg) and one TEAE of mild injection site pain (in a subject dosed with 75 μg CNP/kg) were reported. Both TEAEs were reported on day 1 and both resolved on the same day with no action required. Additionally, there were no hypersensitivity reactions or anaphylaxis reported.

3.7 | ECG

TransCon CNP did not have an effect on HR or cardiac conduction (PR and QRS intervals) at the studied doses, with mean placebo-corrected change-from-baseline HR ($\Delta\Delta\text{HR}$) ranging from -6.8 to 5.6 bpm across dose cohorts, without indication of dose dependence. Mean placebo-corrected change-from-baseline QTcF ($\Delta\Delta\text{QTcF}$) ranged from -7.7 ms at 8 hours post-dose at the highest dose (150 μg CNP/kg) to 7.9 ms at 54 hours post-dose at the 25 μg CNP/kg dose, without an indication of dose dependence. Thus, TransCon CNP at the studied doses did not have a clinically relevant effect on the studied ECG parameters.

3.8 | BP, HR and orthostatic changes

Due to the previously reported vasodilatory effect of CNP at high concentrations,^{29–32} BP, HR and orthostatic changes were thoroughly investigated. No dose-dependent patterns or changes from baseline in supine BP or HR were observed across treatment groups. In concert with this, no correlation was observed between supine BP or reflex tachycardia and plasma levels of CNP-38 in any dose cohort.

No clinically relevant dose-dependent effects in mean orthostatic BP or HR changes were observed across treatment groups. As an illustration of this, Figure 3 depicts the mean CNP-38 concentration and mean changes in orthostatic BP and HR from baseline relative to the exposure to CNP-38 in the 150 μg CNP/kg treatment group.

No correlations between BP or HR and CNP exposure were observed, although TEAEs associated with orthostatic changes were reported. Orthostatic hypotension was reported in four of the 36 subjects treated with TransCon CNP (one in the 3 μg CNP/kg cohort, two in the 25 μg CNP/kg cohort and one in the 75 μg CNP/kg cohort), of

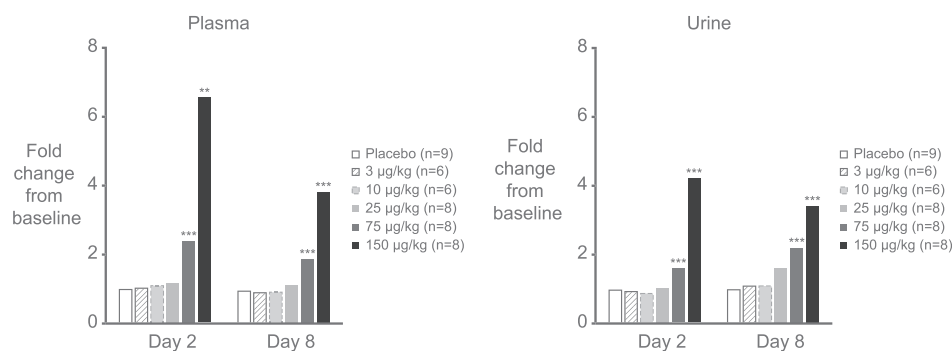


FIGURE 2 Fold change from baseline in plasma (A) and urine (B) cGMP. Normalized levels of cGMP (fold change from baseline) measured in both plasma (left panel) and urine samples collected post-dose at days 2 and 8. ** $P < .01$, *** $P < .001$. Lower dose cohorts showed no significant effects of CNP exposure on cGMP levels ($P > .05$)

FIGURE 3 Mean orthostatic changes in BP and HR and associated mean plasma concentrations of CNP-38 over time in healthy male subjects dosed at 150 µg/kg

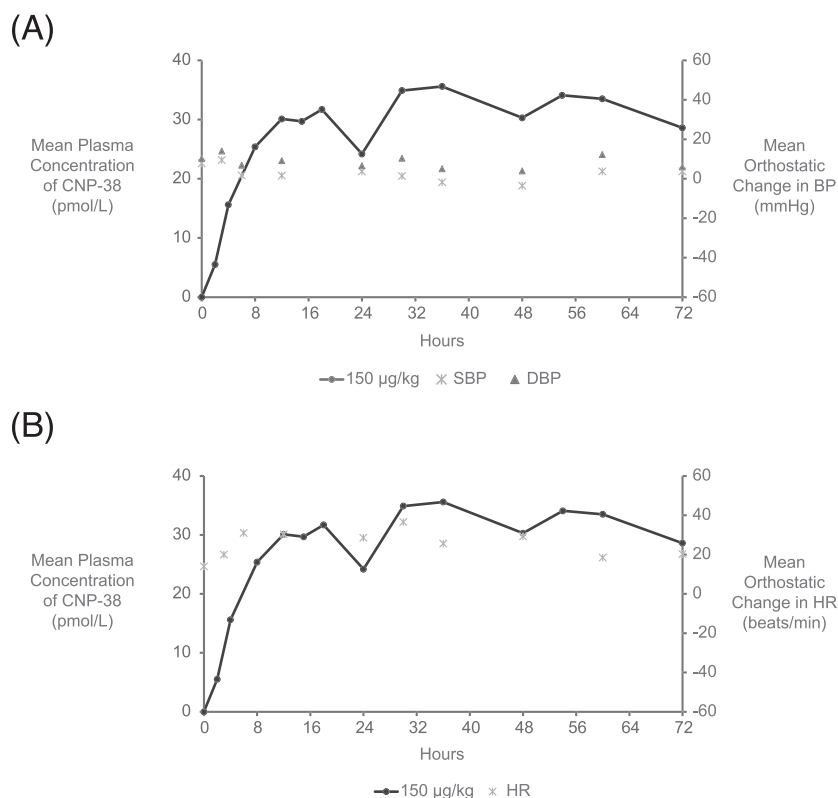


TABLE 3 Summary of treatment-emergent adverse events

AE, n (%)	Dose cohort						Total TransCon CNP subjects (N = 36)
	3 µg CNP/kg (N = 6)	10 µg CNP/kg (N = 6)	25 µg CNP/kg (N = 8)	75 µg CNP/kg (N = 8)	150 µg CNP/kg (N = 8)	Placebo ^a (N = 9)	
Subject experienced any AE	5 (83.3)	4 (66.7)	5 (62.5)	8 (100)	6 (75.0)	7 (77.8)	28 (77.8)
Subject experienced any drug-related AE ^b	3 (50.0)	3 (50.0)	2 (25.0)	6 (75.0)	6 (75.0)	2 (22.2)	20 (55.6)
Discontinued trial participation due to an AE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Subject experienced any moderate/severe AE	2 (33.3)	1 (16.7)	0 (0)	6 (75.0)	3 (37.5)	2 (22.2)	12 (33.3)
Subject experienced any SAE	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Subject death	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Note: Only TEAEs are presented (defined as an event with the date of AE onset being later than or equal to the date/time of the first injection of the dose).

^aConsists of pooled dosage placebo groups.

^bDefined as the causal relationship being definite, probable or possible as reported by the investigator.

which only one subject was symptomatic with lightheadedness on standing after a blood draw. Orthostatic tachycardia was reported in six subjects, 1/9 (11%) placebo subjects and 5/36 (14%) TransCon CNP-treated subjects (two in the 75 µg CNP/kg cohort and three in the 150 µg CNP/kg cohort), but no associated decrease in systolic BP (≥ 20 mm Hg) on standing was observed in any of the subjects. The events were symptomatic in five subjects; reported symptoms included mild postural dizziness, feeling lightheaded on standing, feelings of “heart racing” and palpitations. Orthostatic tachycardia in all

six subjects resolved without treatment prior to attainment of C_{max} and thus did not correlate with CNP exposure.

3.9 | Immunogenicity

A total of 135 serum samples were analysed for anti-CNP binding antibodies. All samples were confirmed negative for anti-CNP antibodies.

3.10 | General adverse events

In general, no trends were observed in TEAE incidence rates across dose cohorts or relative to the placebo group. Overall, 28/36 subjects (77.8%) in the TransCon CNP group and 7/9 subjects (77.8%) in the placebo group experienced at least one TEAE. A summary of the TEAEs is presented in Table 3. The majority of TEAEs reported were assessed to be mild in severity. In the TransCon CNP group, 12/36 subjects (33.3%) experienced at least one moderate or severe TEAE; 2/9 subjects (22.2%) dosed with placebo experienced at least one moderate or severe TEAE. Of these, contact dermatitis (four events/three subjects) and headache (two events/two subjects) were the only moderate/severe TEAEs to be reported for more than one subject in the TransCon CNP cohorts. Three TEAEs of moderate contact dermatitis were assessed to be unrelated to study drug (one event related to “telemetry dots” from ECGs) and all resolved without medication. The only severe TEAE was in a subject receiving 75 µg CNP/kg. On day 11, the subject experienced irritant contact dermatitis. This event was non-serious, assessed as unrelated to study medication and not at the site of injection, required no treatment and was resolved on day 83.

4 | DISCUSSION

The importance of CNP for growth plate activity has been known for more than 20 years, and exogenous CNP has been shown to be effective in promoting endochondral bone growth *in vivo*.¹⁵ The clearance of CNP-22 in human plasma is very rapid, with a calculated half-life of 2.6 minutes, a pharmacological limitation which has been attributed to the susceptibility of CNP-22 to Nephilysin (NEP) degradation.^{15,33} CNP-22 has been shown *in vitro* to be inactivated by neutral endopeptidase, and CNP is internalized and degraded by NPR-C.³⁴ Vosoritide is a CNP analogue peptide consisting of 39 amino acids, including the 37 C-terminal amino acids of the human CNP-53 sequence plus the addition of two amino acids (Pro and Gly) to convey resistance to NEP degradation, resulting in prolonged half-life in comparison to endogenous CNP. The NPR-C clearance receptor binds and eliminates vosoritide (BioMarin EMEA/H/C/005475-Voxzogo). Recently, clinical efficacy data for vosoritide has been reported, validating the important potential of CNP in the treatment of ACH. However, nonclinical data have demonstrated that sustained suppression of the overactive FGFR3 signalling pathways may be needed to optimally alleviate the aberrant inhibition of chondrocyte development seen in ACH.^{15,21}

We have previously reported on the nonclinical data for TransCon CNP, also supporting a once-weekly dosing and in the absence of cardiovascular side effects.²¹

In the current trial, TransCon CNP administration to male human subjects resulted in continuous exposure to CNP over at least 1 week, supporting a once-weekly dosing regimen. The average apparent $t_{1/2}$ of approximately 120 hours is in contrast to that of only 2-3 minutes for

endogenous CNP-22 and 19-46 minutes for vosoritide.^{33,35} The prolongation of CNP exposure following TransCon CNP administration underscores the potential of TransCon technology in supporting sustained release over more than a week of a molecule that is otherwise prone to fast systemic elimination.

The *in vivo* release of CNP was evidenced by a significant increase from baseline cGMP levels in both plasma and urine. The measured changes in absolute levels of cGMP were most pronounced in plasma, with a 6- to 7-fold increase from baseline. Slightly lower increases were observed in urine. However, there was a good correlation between the plasma and urinary measurements. Notably, the effect on cGMP was sustained throughout the week, paralleling the sustained systemic CNP exposure. Overall, the increase in systemic and urinary cGMP levels demonstrates that the CNP released from the TransCon carrier is active and that the level of released CNP affords a sustained activation of NPR-B for at least 7 days post administration. Furthermore, there were no observed changes in circulating levels of NTproCNP, indicating that CNP released from TransCon CNP did not interfere with endogenous CNP biosynthesis. These observations are aligned with unpublished data from a study in which healthy adult males were exposed to a CNP analogue.³⁶

The dose-proportional PK profile of CNP-38 supports the ability to titrate the dose based on the individual subject. The circulatory CNP-38 concentration showed a slow rise to peak, governed by the PK of the prodrug, and suggests a slow systemic uptake of the prodrug from the SC compartment.

Despite the vasodilatory effects of CNP reported in the literature,³⁰⁻³³ no significant injection site reactions were observed despite CNP being released to some extent in the subcutis, which may be explained by the slow release of CNP by the TransCon technology. Even in the higher dose cohorts, no major local tolerability issues were observed. The use of multiple injections, to the extent used in the phase 1 trial, is not planned for in the ongoing and future clinical trials in children with ACH. Because the TransCon CNP drug product formulation is optimized for children, adult participants in the trial required large injection volumes that at higher dose levels could not be administered as a single SC injection. No evidence of an immunogenic response was observed following a single dose of TransCon CNP.

Overall, no major safety or tolerability concerns were observed with administration of TransCon CNP in the trial. Bolus administration of CNP, with C_{max} reaching a systemic concentration of 770 pmol/L, has been reported to result in minor and transient decreases in BP and an associated increase in HR.³⁷ C_{max} of CNP-38 after a single dose of 150 µg TransCon CNP/kg reached a level of only 39.1 pmol/L, an exposure where no effects on cardiorenal function have been observed following infusion of CNP to healthy volunteers.³⁸ The slow-release profile and low C_{max} of CNP-38 with administration of TransCon CNP was anticipated to mitigate clinically meaningful BP changes that may be caused by CNP. Accordingly, the HR changes and associated symptoms observed in the trial do not suggest orthostatic intolerance, as BP remained relatively unchanged during the events. On the contrary, they are consistent with physiological

adaptation to postural changes, particularly in fasted patients.³⁹ Additionally, given the lack of correlation between symptoms and CNP exposure, as well as their relationship to time and number of injections (ie, only occurring shortly after administration in participants who received multiple injections), vagal response must be considered in the assessment of contributing factors. Therefore, no indications of clinically relevant impact on HR or BP or trends on ECG parameters (including QTcF interval) were observed at any dose level of TransCon CNP. The only severe TEAE (irritant contact dermatitis) was assessed as not related to study drug.

As with the majority of studies, the design of the current study is subject to certain limitations. With regard to the cardiovascular safety, the potential vasodilatory effect of CNP should to a major extent be translatable from adults to a paediatric setting, but safety in an ACH population may be confounded by associated comorbidities related to the circulatory system in this population. With regard to assessing cardiovascular safety in adults, a single dose of TransCon CNP should suffice as the hypotensive potential is regarded as an acute effect. Any potential adverse effects related to the growth plate will not be evident from the current study population, as all growth plates are assumed to be closed. Therefore, any potential PD effect mediated via growth plate modulation will not be captured in a study in an adult human population.

5 | CONCLUSIONS

TransCon CNP administered as single doses to healthy adult subjects up to a dose of 150 µg CNP/kg was well tolerated and the data were not suggestive of clinically relevant effects on any of the studied safety parameters, including cardiovascular endpoints. Data to date do not suggest cardiovascular safety as a major concern. The data from the phase 1 TransCon CNP trial in healthy male subjects support that continuous CNP exposure can be achieved over 7 days with a single dose of TransCon CNP and in the absence of treatment-related adversities. With the recent clinical validation of the importance of the CNP pathway in alleviating the impaired skeletal development seen in ACH, TransCon CNP warrants further exploration in ACH. Two phase 2 dose-escalation trials of TransCon CNP in children with ACH are currently ongoing, in addition to a separate longitudinal natural history study in children with ACH.

FUNDING

This study was sponsored by Ascendis Pharma Growth Disorders A/S, Hellerup, Denmark.

ACKNOWLEDGEMENTS

This work was supported by Ascendis Pharma Growth Disorders A/S Hellerup, Denmark. The authors wish to thank the CNP project team for their contributions to the conduct of the trial as well as our external collaborators, BioAgilytix (North Carolina, USA), Celerion (Zurich, Switzerland) and Tim Prickett (University of Otago, New Zealand).

DISCLOSURE STATEMENT

V.M.B., P.H.M., E.D.C., Y.Z. and D.V. are all employed by Ascendis Pharma A/S or Inc. S.O. and R.W.C. are previous employees of Ascendis Pharma, Inc.

AUTHOR CONTRIBUTION

All authors contributed to the collection and analysis of data, the writing and revision of the manuscript, and reviewed and approved the final version.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

ORCID

Sho Ota  <https://orcid.org/0000-0001-5786-8423>

REFERENCES

- Horton WA, Hall JG, Hecht JT. Achondroplasia. *Lancet (London, England)*. 2007;370(9582):162-172. doi:10.1016/S0140-6736(07)61090-3
- Unger S, Bonafe L, Gouze E. Current care and investigational therapies in achondroplasia. *Curr Osteoporos Rep*. 2017;15(2):53-60. doi:10.1007/s11914-017-0347-2
- Foreman PK, van Kessel F, van Hoorn R, van den Bosch J, Shediach R, Landis S. Birth prevalence of achondroplasia: A systematic literature review and meta-analysis. *Am J Med Genet Part a*. 2020;182(10):2297-2316. doi:10.1002/ajmg.a.61787
- Ho NC, Guarnieri M, Brant LJ, et al. Living with achondroplasia: quality of life evaluation following cervico-medullary decompression. *Am J Med Genet a*. 2004;131(2):163-167.
- Ireland PJ, Pacey V, Zankl A, Edwards P, Johnston LM, Savarirayan R. Optimal management of complications associated with achondroplasia. *Appl Clin Genet*. 2014;7:117-125. doi:10.2147/TACG.S51485
- Merker A, Neumeyer L, Hertel NT, et al. Growth in achondroplasia: Development of height, weight, head circumference, and body mass index in a European cohort. *Am J Med Genet a*. 2018;176(8):1723-1734. doi:10.1002/ajmg.a.38853
- Merker A, Neumeyer L, Hertel NT, Grigelioniene G, Mohnike K, Hagenäs L. Development of body proportions in achondroplasia: Sitting height, leg length, arm span, and foot length. *Am J Med Genet a*. 2018;176(9):1819-1829. doi:10.1002/ajmg.a.40356
- Pauli RM. Achondroplasia: a comprehensive clinical review. *Orphanet J Rare Dis*. 2019;14(1):1. doi:10.1186/s13023-018-0972-6
- Webster MK, Donoghue DJ. Constitutive activation of fibroblast growth factor receptor 3 by the transmembrane domain point mutation found in achondroplasia. *EMBO J*. 1996;15(3):520-527. doi:10.1002/j.1460-2075.1996.tb00384.x
- Zhou ZQ, Ota S, Deng C, Akiyama H, Hurlin PJ. Mutant activated FGFR3 impairs endochondral bone growth by preventing SOX9 downregulation in differentiating chondrocytes. *Hum Mol Genet*. 2015;24(6):1764-1773. doi:10.1093/hmg/ddu594
- Murakami S, Balmes G, McKinney S, Zhang Z, Givol D, de Crombrughe B. Constitutive activation of MEK1 in chondrocytes causes Stat1-independent achondroplasia-like dwarfism and rescues the Fgfr3-deficient mouse phenotype. *Genes Dev*. 2004;18(3):290-305. doi:10.1101/gad.1179104

12. Ornitz DM, Marie PJ. Fibroblast growth factor signaling in skeletal development and disease. *Genes Dev.* 2015;29(14):1463-1486. doi:[10.1101/gad.266551.115](https://doi.org/10.1101/gad.266551.115)
13. Krejci P, Salazar L, Kashiwada TA, et al. Analysis of STAT1 activation by six FGFR3 mutants associated with skeletal dysplasia undermines dominant role of STAT1 in FGFR3 signaling in cartilage. *PLoS ONE.* 2008;3(12):e3961. doi:[10.1371/journal.pone.0003961](https://doi.org/10.1371/journal.pone.0003961)
14. Yasoda A, Komatsu Y, Chusho H, et al. Overexpression of CNP in chondrocytes rescues achondroplasia through a MAPK-dependent pathway. *Nat Med.* 2004;10(1):80-86. doi:[10.1038/nm971](https://doi.org/10.1038/nm971)
15. Yasoda A, Kitamura H, Fujii T, et al. Systemic administration of C-type natriuretic peptide as a novel therapeutic strategy for skeletal dysplasias. *Endocrinology.* 2009;150(7):3138-3144. doi:[10.1210/en.2008-1676](https://doi.org/10.1210/en.2008-1676)
16. Wendt DJ, Dvorak-Ewell M, Bullens S, et al. Neutral endopeptidase-resistant C-type natriuretic peptide variant represents a new therapeutic approach for treatment of fibroblast growth factor receptor 3-related dwarfism. *J Pharmacol Exp Ther.* 2015;353(1):132-149. doi:[10.1124/jpet.114.218560](https://doi.org/10.1124/jpet.114.218560)
17. Lorget F, Kaci N, Peng J, et al. Evaluation of the therapeutic potential of a CNP analog in a Fgfr3 mouse model recapitulating achondroplasia. *Am J Hum Genet.* 2012;91(6):1108-1114. doi:[10.1016/j.ajhg.2012.10.014](https://doi.org/10.1016/j.ajhg.2012.10.014)
18. Krejci P, Masri B, Fontaine V, et al. Interaction of fibroblast growth factor and C-natriuretic peptide signaling in regulation of chondrocyte proliferation and extracellular matrix homeostasis. *J Cell Sci.* 2005;118(Pt 21):5089-5100. doi:[10.1242/jcs.02618](https://doi.org/10.1242/jcs.02618)
19. Miyazawa T, Ogawa Y, Chusho H, et al. Cyclic GMP-dependent protein kinase II plays a critical role in C-type natriuretic peptide-mediated endochondral ossification. *Endocrinology.* 2002;143(9):3604-3610. doi:[10.1210/en.2002-220307](https://doi.org/10.1210/en.2002-220307)
20. *Voxzogo (vosoritide) [package insert]*. Novato, CA: Biomarin; 2021.
21. Breinholt VM, Rasmussen CE, Mygind PH, et al. TransCon CNP, a sustained-release C-type natriuretic peptide prodrug, a potentially safe and efficacious new therapeutic modality for the treatment of comorbidities associated with fibroblast growth factor receptor 3-related skeletal dysplasias. *J Pharmacol Exp Ther.* 2019;370(3):459-471. doi:[10.1124/jpet.119.258251](https://doi.org/10.1124/jpet.119.258251)
22. European Medicines Agency. Guideline on bioanalytical method validation. 2011.
23. U.S. Department of Health and Human Services. Bioanalytical method validation – guidance for industry. 2018.
24. Food and Drug Administration. Immunogenicity Testing of Therapeutic Protein Products – Developing and Validating Assays for Anti-Drug Antibody Detection. <https://www.fda.gov/regulatory-information/search-fda-guidance-documents/immunogenicity-testing-therapeutic-protein-products-developing-and-validating-assays-anti-drug>. Published 2019. Accessed 14 March 2022.
25. European Medicines Agency. Immunogenicity Assessment of Therapeutic Proteins. https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-immunogenicity-assessment-therapeutic-proteins-revision-1_en.pdf. Published 2017. Accessed 14 March 2022.
26. Steiner. Radioimmunoassay for Cyclic Nucleotides. *Journal of Biological Chemistry*, 1972;247(4):1106-1113.
27. Prickett TC, Yandle TG, Nicholls MG, Espiner EA, Richards AM. Identification of amino-terminal pro-C-type natriuretic peptide in human plasma. *Biochem Biophys Res Commun.* 2001;286(3):513-517. doi:[10.1006/bbrc.2001.5419](https://doi.org/10.1006/bbrc.2001.5419)
28. Olney RC, Permuy JW, Prickett TC, Han JC, Espiner EA. Amino-terminal propeptide of C-type natriuretic peptide (NTproCNP) predicts height velocity in healthy children. *Clin Endocrinol (Oxf).* 2012;77(3):416-422. doi:[10.1111/j.1365-2265.2012.04392.x](https://doi.org/10.1111/j.1365-2265.2012.04392.x)
29. Clavell AL, Stingo AJ, Wei CM, Heublein DM, Burnett JC Jr. C-type natriuretic peptide: a selective cardiovascular peptide. *Am J Physiol.* 1993;264(2 Pt 2):R290-R295. doi:[10.1152/ajpregu.1993.264.2.R290](https://doi.org/10.1152/ajpregu.1993.264.2.R290)
30. Barr CS, Rhodes P, Struthers AD. C-type natriuretic peptide. *Peptides.* 1996;17(7):1243-1251. doi:[10.1016/S0196-9781\(96\)00110-6](https://doi.org/10.1016/S0196-9781(96)00110-6)
31. Scotland RS, Cohen M, Foster P, et al. C-type natriuretic peptide inhibits leukocyte recruitment and platelet-leukocyte interactions via suppression of P-selectin expression. *Proc Natl Acad Sci U S A.* 2005;102(40):14452-14457. doi:[10.1073/pnas.0504961102](https://doi.org/10.1073/pnas.0504961102)
32. Nakao K, Kuwahara K, Nishikimi T, et al. Endothelium-derived c-type natriuretic peptide contributes to blood pressure regulation by maintaining endothelial integrity. *Hypertension.* 2017;69(2):286-296. doi:[10.1161/HYPERTENSIONAHA.116.08219](https://doi.org/10.1161/HYPERTENSIONAHA.116.08219)
33. Hunt PJ, Richards AM, Espiner EA, Nicholls MG, Yandle TG. Bioactivity and metabolism of C-type natriuretic peptide in normal man. *J Clin Endocrinol Metabol.* 1994;78(6):1428-1435.
34. Potter LR, Yoder AR, Flora DR, Antos LK, Dickey DM. Natriuretic peptides: their structures, receptors, physiologic functions and therapeutic applications. *Handb Exp Pharmacol.* 2009;(191):341-366. doi:[10.1007/978-3-540-68964-5_15](https://doi.org/10.1007/978-3-540-68964-5_15)
35. Savarirayan R, Tofts L, Irving M, et al. Once-daily, subcutaneous vosoritide therapy in children with achondroplasia: a randomised, double-blind, phase 3, placebo-controlled, multicentre trial. *Lancet.* 2020;396(10252):684-692
36. Prickett TCR, Espiner EA, Irving M, et al. Evidence of feedback regulation of C-type natriuretic peptide during vosoritide therapy in achondroplasia. *Sci Rep.* 2021;11(1):24278. doi:[10.1038/s41598-021-03593-1](https://doi.org/10.1038/s41598-021-03593-1)
37. Igaki T, Itoh H, Suga SI, et al. Effects of intravenously administered C-type natriuretic peptide in humans: comparison with atrial natriuretic peptide. *Hypertens Res.* 1998;21(1):7-13. doi:[10.1291/hyres.21.7](https://doi.org/10.1291/hyres.21.7)
38. Barletta G, Lazzeri C, Vecchiarino S, et al. Low-dose C-type natriuretic peptide does not affect cardiac and renal function in humans. *Hypertension.* 1998;31(3):802-808. doi:[10.1161/01.HYP.31.3.802](https://doi.org/10.1161/01.HYP.31.3.802)
39. Hoddy KK, Kroeger CM, Trepanowski JF, Barnosky AR, Bhutani S, Varady KA. Safety of alternate day fasting and effect on disordered eating behaviors. *Nutr J.* 2015;14:44. doi:[10.1186/s12937-015-0029-9](https://doi.org/10.1186/s12937-015-0029-9)

How to cite this article: Breinholt VM, Mygind PH, Christoffersen ED, et al. Phase 1 safety, tolerability, pharmacokinetics and pharmacodynamics results of a long-acting C-type natriuretic peptide prodrug, TransCon CNP. *Br J Clin Pharmacol.* 2022;88(11):4763-4772. doi:[10.1111/bcp.15369](https://doi.org/10.1111/bcp.15369)