Structural Optimization of TransCon CNP – Development of a Sustained-Release Prodrug of CNP for Achondroplasia

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Background

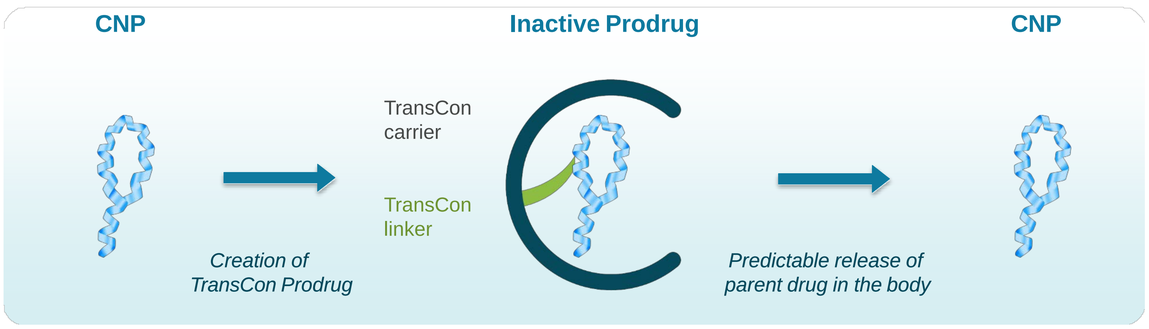
Achondroplasia (ACH), the most common form of human dwarfism, is caused by a gain-of-function mutation in the fibroblast growth receptor 3 (FGFR3) gene, a key negative regulator of chondrocyte proliferation and terminal differentiation.

Via activation of natriuretic peptide receptor B (NPR-B), C-type natriuretic peptide (CNP) inhibits the effects of the FGFR3 signaling pathway and has demonstrated efficacy in longitudinal bone growth.[1,2]

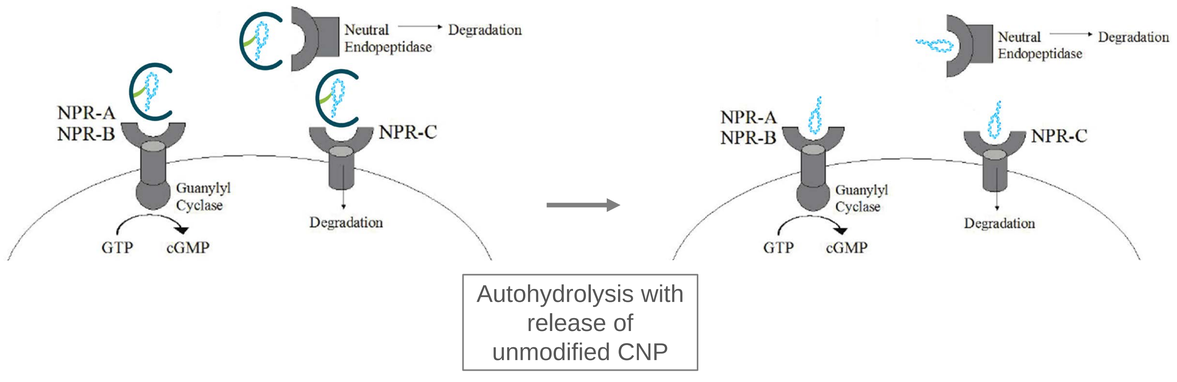
CNP is rapidly cleared and degraded by natriuretic peptide receptor C (NPR-C) and neutral endopeptidase (NEP), respectively, resulting in a 2-minute half-life.

Vosoritide, a mutated CNP analogue with a 20-minute half-life, is in phase 3 development for use in ACH. However, its short half-life leads to high peak-to-trough ratios and limited therapeutic coverage. Humans and animals dosed with vosoritide have also demonstrated hypotension coinciding with maximum blood concentrations (C_{max}), indicating a causal relationship.[1,2]

A potential long-acting CNP drug candidate should provide sustained exposure of CNP at therapeutic levels. Additionally, a safe and efficacious candidate should not cause adverse cardiovascular effects or undergo rapid clearance and degradation of CNP.



TransCon CNP is a prodrug in which CNP is transiently bound to a TransCon carrier via a proprietary linker. The carrier molecule shields CNP from binding to NPR-B or NPR-C and degradation by neutral endopeptidase (NEP). Through autohydrolysis of the linker from the prodrug, fully active, unmodified CNP is sustainably released over 7 days and can diffuse into the growth plate.



The present work describes the structural optimization of TransCon CNP.

Methods

Through a series of in vitro assays (including a potency assay with NIH 3T3 cells expressing murine NPR-B, a binding assay with HEK cells expressing NPR-C, and a NEP incubation assay), the EC_{50}, IC_{50}, and degradation time, respectively, of multiple TransCon CNP candidates was compared to unmodified CNP. To avoid release of unmodified CNP in the in vitro assays, a permanently conjugated TransCon CNP molecule, in which the linker does not autohydrolyze, was used.

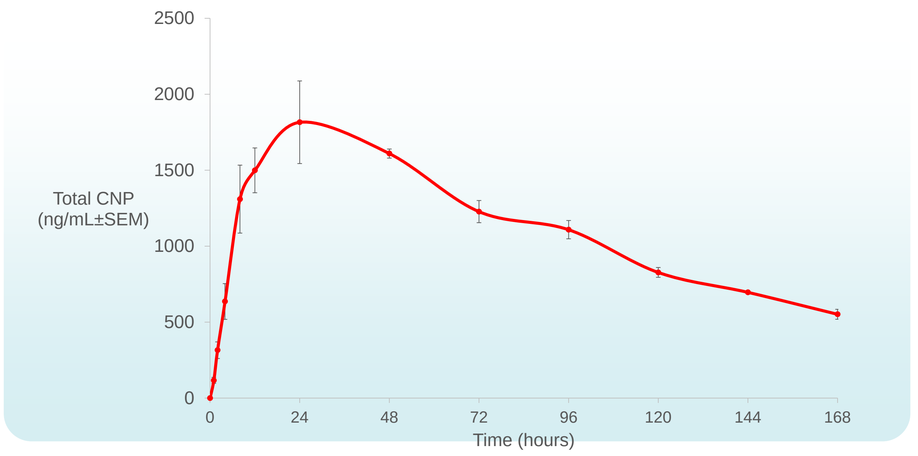
The lead TransCon CNP candidate was administered as a single subcutaneous (SC) dose of 146 µg/kg to male cynomolgus monkeys (n=3) and the pharmacokinetic profile evaluated to confirm that the in vitro results translated into a prolonged in vivo half-life.

Subsequently, a single SC dose of the lead TransCon CNP candidate (at 100 µg/kg) and dose-equivalent vosoritide were administered to telemeterized male cynomolgus monkeys (n=4/group) in which standard cardiovascular endpoints, including the systolic arterial blood pressure (SAP), were evaluated up to 48 hours post-dose.

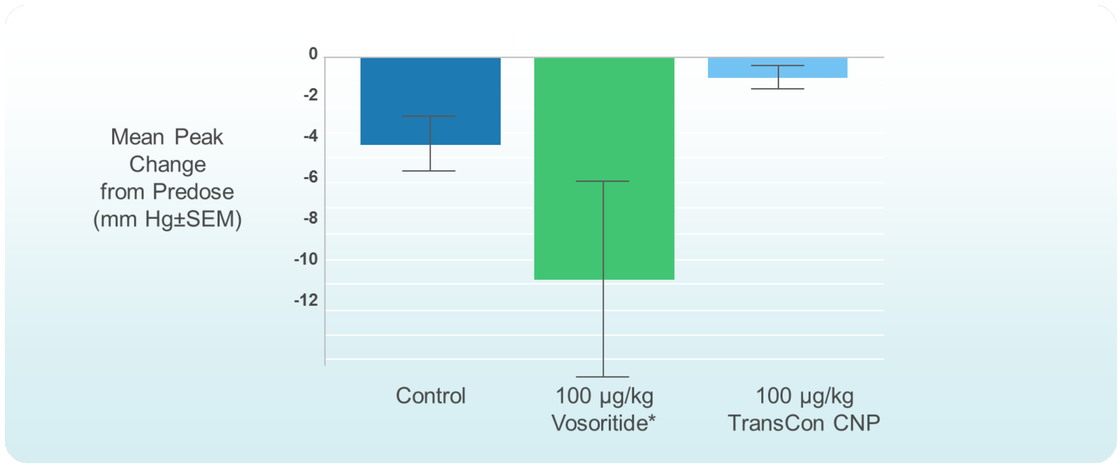
Results

As designed, in the cell-based potency and binding assays, the TransCon prodrug of CNP demonstrated extremely low biological activity against both the NPR-B and NPR-C receptors. Additionally, CNP within the prodrug was protected against NEP degradation. In contrast, liberated, unmodified CNP was fully active.

In monkeys, a TransCon CNP half-life of 79 hours was confirmed:



In monkeys, no decrease in SAP was detected at TransCon CNP doses of up to 100 µg/kg compared to controls during a 48-hour monitoring period. In contrast, an equivalent dose of vosoritide led to a decrease in SAP at 0-1 hours post-dose.



Conclusions

- The TransCon CNP prodrug has minimal binding to the NPR-B and NPR-C receptors and improved NEP stability in vitro compared to unmodified CNP.
- In vivo TransCon CNP demonstrated the desired half-life extension without adverse hemodynamic effects.
- These data support clinical development for weekly dosing and suggest TransCon CNP may be a safe and efficacious option for children with ACH.

Context

Structural optimization of the TransCon CNP molecule provided the background for successful preclinical studies:

- TransCon CNP demonstrated efficacy in healthy juvenile cynomolgus monkeys, increasing long bone growth in a dose-dependent fashion.[1]
- In a murine model of ACH, TransCon CNP improved growth plate architecture and increased naso-anal and femur bone length.[1]

Collectively, these studies support continued development of TransCon CNP.

[1] BioMarin R&D Day 2016 Presentation
[2] Wendt et al. J Pharmacol Exp Ther, 2015; 353, 132–149
[3] Ascendis Pharma IMPE 2017 Presentation

* "Vosoritide" refers to a synthesized molecule with the amino acid sequence prepared by Ascendis Pharma.