TransCon Technology for Sustained Delivery of Proteins, Peptides, and Small Molecules

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September 26, 2018
• Introduction to TransCon Technology
  - Design of TransCon Prodrugs
  - TransCon Linkers
  - TransCon Systemic Carrier (soluble)
  - TransCon Localized Carrier (insoluble Hydrogel)

• Applying TransCon to Solve Unmet Medical Need – Case Studies
  - TransCon hGH for Growth Hormone Deficiency (Phase 3)
  - TransCon PTH for Hypoparathyroidism (Phase 1)
  - TransCon CNP for Achondroplasia (Phase 1)
The TransCon Technology Design

- Parent drug is transiently bound to linker-carrier, which minimizes receptor activity and shields it from clearance.
- Following injection, the linker is designed to autohydrolyze at specific rates to predictably release unmodified parent drug (traceless linker).
- Designed to distribute parent drug like the endogenous compound; linker-carrier is cleared renally.
Ascendis Algorithm for Designing TransCon Prodrugs

Today’s Focus

Higher Value, Lower Risk Pipeline

- Unmet Medical Need
- Validated Parent Drug
- TransCon Technology Suitability
- Clearly Differentiated Product
- Established Clinical & Regulatory Pathway
- Large Addressable Market
## Internal Endocrinology Pipeline

<table>
<thead>
<tr>
<th>PRODUCT CANDIDATE</th>
<th>PRECLINICAL</th>
<th>PHASE 1</th>
<th>PHASE 2</th>
<th>PHASE 3</th>
<th>WW COMMERCIAL RIGHTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>TransCon hGH</td>
<td>Pediatric Growth Hormone Deficiency</td>
<td>Adult Growth Hormone Deficiency</td>
<td></td>
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<td>ascendispharma</td>
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<tr>
<td>TransCon PTH</td>
<td>Hypoparathyroidism</td>
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<tr>
<td>TransCon CNP</td>
<td>Achondroplasia</td>
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<td></td>
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<td>ascendispharma</td>
</tr>
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</table>

## Strategic Collaborations

<table>
<thead>
<tr>
<th>PRODUCT CANDIDATE</th>
<th>PRIMARY INDICATION</th>
<th>DEVELOPMENT STAGE</th>
<th>WW COMMERCIAL RIGHTS</th>
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</thead>
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<tr>
<td>TransCon Anti-VEGF</td>
<td>Ophthalmology</td>
<td>Not disclosed</td>
<td>Genentech</td>
</tr>
<tr>
<td>TransCon Peptides</td>
<td>Diabetes</td>
<td>Not disclosed</td>
<td>SANOFI</td>
</tr>
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</table>
Introduction to TransCon Linkers and Carriers
TransCon Linker Families

<table>
<thead>
<tr>
<th>Linker Families</th>
<th>WO Numbers</th>
<th>For drugs with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aromatic Linkers</td>
<td>WO05099768</td>
<td>aliphat. amine, aromat. amine</td>
</tr>
<tr>
<td>Cyclic Imide Linkers</td>
<td>WO09095479 WO13160340</td>
<td>aliphat. amine, aromat. amine hydroxy group</td>
</tr>
<tr>
<td>DKP Linkers</td>
<td>WO11089216</td>
<td>aliphat. amine</td>
</tr>
<tr>
<td>Carbamate Linkers</td>
<td>WO11089214</td>
<td>phenolic group</td>
</tr>
<tr>
<td>Bicin Linkers</td>
<td>WO06136586</td>
<td>aliphat. amine, aromat. amine</td>
</tr>
<tr>
<td>AEG Linkers</td>
<td>WO16020373</td>
<td>amine group hydroxy group</td>
</tr>
<tr>
<td>Pyroglutamate Linkers</td>
<td>WO16196124</td>
<td>aliphat. amine, aromat. amine</td>
</tr>
</tbody>
</table>

- Linker families designed for drugs containing different functional groups
- All linkers are tunable, enzyme independent and designed to remain covalently bound to the carrier molecule
- TransCon Prodrugs eligible for new composition of matter IP
Cyclic Imide Linkers – Design and Mechanism

- Linker cleavage by intramolecular cyclization under physiological conditions
- Cleavage independent of enzymes – dependent on pH and temperature
- Traceless linker – drug is released unmodified
- Linker designed to stay on carrier after drug release
- Simple aliphatic structures
- No reactive cleavage products formed

\[ X = \text{CH}_2, \text{NH} \]
Linker Cleavage: Tunable by varying structure

<table>
<thead>
<tr>
<th>TransCon PTH Variant</th>
<th>R¹</th>
<th>R²</th>
<th>TransCon linker t₁/₂ (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>-CH₃</td>
<td>-CH₃</td>
<td>3</td>
</tr>
<tr>
<td>2</td>
<td>-CH₃</td>
<td>-H</td>
<td>9</td>
</tr>
<tr>
<td>3</td>
<td>-CH₂OH</td>
<td>-H</td>
<td>11</td>
</tr>
<tr>
<td>4</td>
<td>-CH₂CH(CH₃)₂</td>
<td>-H</td>
<td>25</td>
</tr>
</tbody>
</table>

Linker half-lives from hours to months can be engineered supporting daily to half-yearly administration frequencies.
Linker Cleavage: pH Dependency

- Linker hydrolysis pH dependent with higher stability at lower pH (4-5.5) and lower temperature (not shown).
- pH dependence of in vitro linker hydrolysis at 37°C and varying pH:

\[
\text{released peptide} / \% \\
\begin{array}{cccc}
\text{pH} & \text{8.5} & \text{7.4} & \text{7.0} & \text{6.5} & \text{5.0} \\
\text{t / d} & \\
0 & 0 & 0 & 0 & 0 & 0 \\
10 & 0 & 0 & 0 & 0 & 0 \\
20 & 0 & 0 & 0 & 0 & 0 \\
30 & 0 & 0 & 0 & 0 & 0 \\
\end{array}
\]

- Linker temperature and pH dependence enables formulation as ready to use formulation with long shelf-life.
In Vitro Release Kinetics

• TransCon PTH incubated *in vitro* under physiological conditions (pH 7.4 and 37°C); released PTH analyzed by RP-HPLC

• Complete release of PTH from PEG carrier
Release of Unmodified Drug – hGH

MS analysis and in vitro biopotency determination

Test Compound | In vitro bioactivity
---|---
Reference hGH | 100%
hGH released from TransCon hGH | 100%

**ESI Mass spectra of reference hGH and hGH released from TransCon hGH.** Identical mass spectra demonstrate that hGH released from the prodrug is unmodified.

**In vitro potency determination.** The potency of a reference hGH and hGH released from the prodrug were compared in the Nb2-11 assay. The hGH released form the prodrug retains full hGH activity.

Unmodified and in cellular assay fully active hGH released from TransCon hGH
In Vitro / In Vivo Correlation

- Correlation of TransCon PTH phase 1 interim PK data (124 µg dose) and in vitro release kinetics in phosphate buffer at pH 7.4 and 37°C

- Excellent correlation of in vitro and in vivo release rates

- High predictability of PK profile from in vitro data enables optimization of PK profile of lead candidates in vitro prior to initiating in vivo development
TransCon Technology Systemic Carrier

• Topography of the systemic carrier can be optimized to meet the requirements for each indication

• Shielding of parent drug is optimized for example by:
  - Branching degree of polymer
  - Shortening of length from drug to first branching point
  - Size of carrier
TransCon Technology for Localized Delivery

Polymerization generally precedes drug loading

Drug conjugation and release governed by TransCon linker

Sequential drug release and degradation

Following injection, hydrogel resides at the injection site and releases drug with predictable and reliable rate

High Loading capacity (100 mg/mL) and injectable through fine gauge needles (30G)
Tuneable Release / Degradation Kinetics

Cross-linker type I / Linker I

In vitro drug release and hydrogel degradation kinetics determined at 37°C and pH 7.4 for TransCon Paliperidone

Cross-linker type II / Linker II

Paliperidone content in hydrogel [%]

Backbone release [%]
Case Studies: Solving Unmet Need

- Application of TransCon to build a pipeline based on known pharmacology
  - Releasing the unmodified parent drug, expected to maintain the properties of parent drug
  - Engineering of release profiles to improve efficacy and safety
  - Protecting the drug molecule from enzymatic degradation and/or rapid clearance
TransCon Growth Hormone: Once-Weekly Replacement Therapy
### Growth Hormone Deficiency: Clinical Manifestations

<table>
<thead>
<tr>
<th>PEDIATRIC¹</th>
<th>ADULT²</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Growth failure</td>
<td>• Truncal fat accumulation and decrease in lean body mass</td>
</tr>
<tr>
<td>• Increased and abnormal fat distribution (especially truncal fat mass)</td>
<td>• Decreased bone mineral density</td>
</tr>
<tr>
<td>• Abnormal metabolic profile</td>
<td>• Dyslipidemia</td>
</tr>
<tr>
<td>• Impaired exercise capacity</td>
<td>• Increased cardiovascular mortality and morbidity</td>
</tr>
<tr>
<td></td>
<td>• Decreased quality of life</td>
</tr>
</tbody>
</table>

Long-acting hGH must fully mimic daily hGH to address totality of disease

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¹ BMC Endocrine Disorders 2012, 12: 26
² J Clin Endocrinol Metab 2006, 91: 1621–1634
Poor adherence with daily hGH therapy is associated with reduced height velocity and impaired quality of life. Reduced frequency of administration is associated with better adherence. In the 1st year, two of three patients miss >1 injection on average per week.
### Optimal Therapeutic Range

<table>
<thead>
<tr>
<th>Low</th>
<th>PHYSIOLOGICAL RANGE</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>Growth Hormone Deficiency</td>
<td>Physiological Range</td>
<td>Therapeutic Range DAILY hGH</td>
</tr>
<tr>
<td>- Short stature</td>
<td>- Tissue overgrowth</td>
<td>- Tissue overgrowth</td>
</tr>
<tr>
<td>- Metabolic abnormalities</td>
<td>- Diabetes</td>
<td>- Diabetes</td>
</tr>
<tr>
<td>- Cardiovascular abnormalities</td>
<td>- Heart disease, stroke</td>
<td>- Heart disease, stroke</td>
</tr>
<tr>
<td>- Cognitive deficiencies</td>
<td>- Risk of colon cancer</td>
<td>- Risk of colon cancer</td>
</tr>
<tr>
<td>- Poor quality of life</td>
<td>- Poor quality of life</td>
<td>- Poor quality of life</td>
</tr>
</tbody>
</table>
TransCon Growth Hormone: Design

Once-weekly prodrug releases unmodified hGH designed to mimic daily hGH:

- Tissue distribution
- Physiological levels
- Therapeutic effects: efficacy, safety and tolerability
Comparable hGH Levels in Phase 2

Maximum hGH concentration comparable between equivalent weekly doses of TransCon hGH and a daily hGH.
Growth Comparable to a Daily hGH in Phase 2\textsuperscript{1,2}

- 26-week treatment period
- Thorough PK/PD assessments at weeks 1 and 13

Annualized height velocity (cm/year, +SD)

<table>
<thead>
<tr>
<th>Dose (GH/kg/week)</th>
<th>0.14 mg</th>
<th>0.21 mg</th>
<th>0.30 mg</th>
<th>0.21 mg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height Velocity</td>
<td>11.9</td>
<td>12.9</td>
<td>13.9</td>
<td>11.6</td>
</tr>
</tbody>
</table>

Intergroup differences not statistically significant

1 J Clin Endocrinol Metab 2017, 102(5): 1673–1682
2 Conducted with a previous lower strength version of TransCon Growth Hormone

TransCon Growth Hormone\textsuperscript{3} Genotropin®

Same weekly dose
Comparative Safety to a Daily hGH in Phase 2¹

- No serious adverse events related to study drug
- Immunogenic profile comparable to a daily hGH
  - No occurrence of neutralizing antibodies
  - Low incidence of low-titer non-neutralizing antibodies
- Injection site tolerability comparable to a daily hGH
  - >1100 TransCon hGH injections administered
  - No reports of lipoatrophy or nodule formation

¹ J Clin Endocrinol Metab 2017, 102(5): 1673–1682
TransCon PTH: PTH Replacement Therapy for Hypoparathyroidism
Clinical Manifestations of Hypoparathyroidism

Central nervous system
- Seizures
- Calcifications
- Parkinsonism or dystonia

Cardiovascular system
- Cardiac arrhythmias
- Hypocalcemia-associated dilated cardiomyopathy

Respiratory system
- Laryngospasm

Renal system
- Nephrocalcinosis*
- Kidney stones*
- Chronic kidney disease*

Peripheral nervous system
- Paresthesia
- Muscle cramps
- Tetany

Neuropsychiatric system
- Anxiety and depression

Ophthalmological system
- Cataracts
- Papilloedema

Dental system
- Altered tooth morphology

Dermatological system
- Dry skin
- Onycholysis
- Coarse, thin hair
- Pustular psoriasis

Musculoskeletal system
- Myopathy
- Spondyloarthropathy

Adapted from Nature Reviews 2017, 3: 1-20

* These manifestations are mostly the result of treatment with calcium and activated vitamin D rather than of the disorder itself.
• Natpara QD provides dose-dependent increases in serum calcium for ~24 hours
• Natpara QD effect on urinary calcium excretion is short-lived (10-12 hours) as kidney reabsorption of calcium follows PK profile
TransCon PTH Design

- TransCon PTH is a sustained-release prodrug designed to provide stable PTH levels in the physiological range for 24 hours/day
- TransCon PTH designed to normalize blood and urinary calcium levels, serum phosphate and bone turnover

Diagram:
- TransCon carrier
- TransCon linker
- Unmodified parent drug
- Linker cleavage dependent upon pH and temperature
- Receptor
- Renal clearance
Single Dose PK Data Support Infusion-Like Profile with Daily Administration

TransCon PTH phase 1 data reproduced PK profile from preclinical studies and showed t$_{1/2}$ of ~60 hours (versus Natpara t$_{1/2}$ ~3 hours)

* Free PTH measured as PTH (1-34) and PTH (1-33)
PK Mimics Physiological PTH Levels over 24 Hours

PK After 10 Days of TransCon PTH 12 µg/day (n=8)

TransCon PTH daily dosing for 10 days provided a flat infusion-like profile with low PTH peak-to-trough ratio at day 10

* Free PTH measured as PTH (1-34) and PTH (1-33)
TransCon PTH: Summary

- TransCon PTH based on parent drug PTH(1-34) with clinical proof-of-principle in HP and validated TransCon technology

- TransCon PTH is designed to address all aspects of the disease by restoring physiological levels of PTH throughout the day

- Phase 1 demonstrates that infusion-like kinetics can be achieved, meeting the desired target product profile

- Phase 2 initiation expected Q1 2019
TransCon CNP: Once-Weekly CNP for Achondroplasia
Achondroplasia – Not Only a Skeletal Disease

**Autosomal dominant genetic disorder**

- Most common form of human dwarfism
- Approximately 250,000 patients worldwide\(^1\)
- 80% born to average-sized parents

**Patients suffer numerous comorbidities**

- Back/spine/cord compression
- Cardiovascular complications
- Dental complications
- Ear infections/sleep apnea
- Obesity
- Bowed legs

**No FDA-approved therapy**

- Only option to improve height is surgical limb lengthening

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\(^1\) Lancet 2007, 370: 162-172
Achondroplasia Signaling Defect is Well Understood and CNP Corrects It

- FGFR3 negatively regulates osteogenesis, and hence bone growth
- Achondroplasia results from a mutation in FGFR3 which leaves the receptor constitutively activated
- CNP inhibits the FGFR3 pathway, hereby promoting proliferation and differentiation of chondrocytes, and restoring bone growth

\(^1\) Adapted from Current Opin Pediatrics 2010; 22:516-523
Challenges for Developing Long-acting CNP

- The mutation of FGFR3 leaves the receptor constitutive active – Extended exposure to therapeutic levels of CNP is likely beneficial

- CNP needs to penetrate into growth plate in order to be effective – permanent large polymer conjugation is not an option

- High peak concentration of CNP in circulation can cause hypotension through NPR-B receptor binding

- CNP is quickly degraded by neutral endopeptidase in subcutaneous tissue and blood compartment

- CNP is cleared quickly from circulation through binding to NPR-C receptor
TransCon CNP as Potential Solution

- TransCon enables effective shielding of CNP:
  - From neutral endopeptidase degradation in subcutaneous tissue and blood compartment
  - Minimize binding of TransCon CNP to the NPR-C receptor to decrease clearance
  - Reduce binding of TransCon CNP to the NPR-B receptor to avoid hypotension

- Unmodified CNP liberated from TransCon CNP maintains small enough size to allow penetration into growth plates
Effective Shielding Achieved

<table>
<thead>
<tr>
<th>Compound</th>
<th>NEP (Neutral endopeptidase) stability</th>
<th>NPR-B Receptor Activity</th>
<th>NPR-C Receptor Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In vitro half life [h]</td>
<td>EC50\text{prodrug}/EC50_{\text{CNP-38}}</td>
<td>IC50\text{prodrug}/IC50_{\text{CNP-38}}</td>
</tr>
<tr>
<td>CNP-22</td>
<td>0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNP-38</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PEG5kDa-CNP-38 (N-terminus)</td>
<td>-</td>
<td>&gt;5</td>
<td></td>
</tr>
<tr>
<td>PEG5kDa-CNP-38 (ring lysine)</td>
<td>63</td>
<td>&gt;100</td>
<td></td>
</tr>
<tr>
<td>PEG 2x20kDa-CNP-38 (ring lysine)</td>
<td>-</td>
<td>&gt;&gt;100</td>
<td>12</td>
</tr>
<tr>
<td>PEG 4x10kDa-CNP-38 (ring lysine)</td>
<td>Essentially Stable</td>
<td>&gt;&gt;100</td>
<td>91</td>
</tr>
</tbody>
</table>

**Increase of molecular weight increases stability of compounds against NEP**

**Increased branching of carrier minimizes affinity to NPR-B and NPR-C receptor**

**Reduction of NPR-C receptor activity was crucial for prolongation of half-life**

NEP: Degradation study by NEP
NPR-B: Functional cGMP stimulation in NIH-3T3 cells
NPR-C: Competitive binding assay using NPR-C expressing HEK-293 cells
TransCon CNP Weekly Profile Confirmed in Primates

Poster presented at ENDO 2017
Effects on Blood Pressure

- No adverse hemodynamic effects (e.g., hypotension) in cynomolgus monkeys or mice at levels exceeding the expected clinical dose
- Lack of adverse hemodynamic effect may widen therapeutic window, thereby enhancing efficacy

*Daily CNP analogue refers to a chemically synthesized molecule formulation with the same amino acid sequence as vosoritide prepared by Ascendis Pharma*
Juvenile Healthy Monkey Growth Study

- Demonstrated dose-proportional tibial linear growth; ulnar growth consistent
- Compared to untreated control, growth increased >70% with highest TransCon CNP dose vs. 35% with a daily CNP analogue* at a higher weekly dose

* Daily CNP analogue refers to a chemically synthesized molecule formulation with the same amino acid sequence as vosoritide prepared by Ascendis Pharma
TransCon CNP: Summary

- TransCon CNP leverages Ascendis technology platform to develop a once-weekly administration, without dose-limiting cardiovascular adverse effects
  - Shields CNP from NPR-C receptor clearance and NPR-B induced-hypotension
  - Prolonged half-life extension and efficacy trend observed in cynomolgus monkeys
  - Reversion of phenotypical traits and comorbidities in mouse model of achondroplasia
- Phase 1 study ongoing; top-line data expected Q4 2018
TransCon Technology enables sustained delivery of small molecules, peptides, and proteins

- Clinically proven across multiple products and indications, including a Phase 3 program for TransCon hGH

TransCon prodrugs release unmodified drug expected to maintain the same mode of action as parent drug (activity, distribution, etc.)

- Daily, weekly, monthly, or longer administration frequencies

- Continuous evolution of TransCon technology to support broad applicability for both systemic and localized delivery

- TransCon is being applied across a pipeline of 3 clinical programs, and is currently being explored in a new therapeutic area