Hematopoietic Stem Cell Gene Therapy for Cystinosis: Clinical Translation and Mechanism of Action

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Disclosure

• I am cofounder, shareholder and a member of both the scientific board and board of directors of GenStem Therapeutics Inc.

• I am a Consultant for AVROBIO, Inc.

• I am a member of the Cystinosis Research Foundation Scientific Review Board and Board of Trustees
Cystinosis, a multisystemic lysosomal storage disorder

- Autosomal recessive
- Incidence 1/100,000

Challenging disease model for gene therapy:
How can we deliver a lysosomal transmembrane gene product
to every tissue?

Treatment: cysteamine
- Taken every 6 hours
- Severe side effects
- Only delays the progression of the disease
- 40-60 pills a day

Multisystemic degenerative disease

Cherqui et al., J. Biol. Chem., 2001
Kalatzis et al., Embo J., 2001
Hematopoietic Stem Cell Transplantation for Cystinosis

Can HSCs be engineered as an intelligent delivery system for a genetic disorder?
Adult bone marrow stem cells

- **Adult bone marrow stem cells**
  - Pluripotent
  - Safe
  - Currently used in clinical applications

- **Three types of BMSC:**
  - Whole bone marrow cells (BMC)
  - Hematopoietic stem cells (HSC)
  - Mesenchymal stem cells (MSC)
Hematopoietic stem cell (HSC) transplantation in the Ctns−/− mice

Confocal Microscopy 4 months post-transplantation

GFP transgenic wild-type mouse

GFP-HSC Sca1+ cells

Ctns−/− mice

Cystine content at 2 and 4 months post-transplant

Syres et al., Blood, 2009
Impact of HSC transplantation on the kidney pathology in Ctns-/- mice

Kidney histology in 15-17 month old mice after over 1 year post-transplantation

Wild-type

Treated Ctns-/-

The higher the quantity of bone marrow cells expressing Ctns the better the preservation of the kidney

High level of donor-derived blood cell engraftment expressing Ctns (>50%)

Low level of donor-derived blood cell engraftment expressing Ctns (<50%)
Impact of HSC transplant on cystine crystals in the kidney

Yeagy et al., *Kidney Int.*, 2011

![Ctns-/-](image1.png)

![Treated Ctns-/- (low engraftment)](image2.png)

![Bar graph](image3.png)

KO

BMSC/HSC

*p<0.00001*
Ocular Pathology in Cystinosis

Slit-lamp photographs: In vivo confocal microscopy: Long-term complication:

Antoine Labbe et al., 2009

Slit-lamp photographs: In vivo confocal microscopy: Long-term complication:

Jennifer L. Simpson et al., 2011

Current treatment: Cysteamine (eye drops)
- Hourly applied
- Adverse effects: stinging, burning and blurred vision at instillation
- Poor compliance
Impact of HSC transplant on the eye defects in Ctns⁻/⁻ mice

Eye study after over 1 year post-transplantation

Rocca et al., IOVS., 2016
Thyroid pathology in Ctns<sup>−/−</sup> mice and impact of HSC transplantation

Most frequent and earliest endocrine complication of cystinosis

Cystine measurement in the thyroid

Mesure of Thyroid Stimulating Hormone (TSH) in serum

Drs X.H. Liao & S. Refetoff, UChicago

Drs H.P. Gaide Chevrornnay & P.J. Courtoy, UCL-Brussels, BE

Gaide Chevrornnay et al., Endocrinology, 2016
Mechanism of Action

How do transplanted HSCs mediate tissue repair in cystinosis?
Transplanted HSCs differentiate into macrophages within tissues in Ctns^{-/-} mice

**Hypothesis for HSC-derived macrophages mediating tissue preservation:**

Cross-correction

i.e. transfer of cystinosin from the transplanted cells to the adjacent Ctns^{-/-} cells
Mechanism of Action: *in vitro* studies

Cystinosin transfer via cell-cell contact

**Nanotubular Highways for Intercellular Organelle Transport**
Amin Rustom, Rainer Saffrich, Ivanka Markovic, Paul Walther, Hans-Hermann Gerdes
*Science*, 2004

Cystinosin-GFP fusion protein

Naphade et al., *Stem Cells*, 2015
Mechanism of Action: *in vivo* studies

**Kidney**

Drs H.P. Gaide Chevronnay & P.J. Courtoy, UCL-Brussels, BE

**Thyroid**

10µm

*Mechanism of Action: in vivo studies*

**Kidney**

**Cornea sclera margin**

**Thyroid**

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Drs H.P. Gaide Chevronnay & P.J. Courtoy, UCL-Brussels, BE
In vivo evidence of lysosomal cross-correction

Naphade et al., Stem Cells, 2015

cystinosinGFP - Green
HSC-derived macrophages – Red
Dapi - Blue
Other lysosomal disease applications

- **Danon disease** – Dr. Eric Adler (UCSD)
  - lysosomal disorder due to a transmembrane lysosomal protein, LAMP-2
  - Patients develop Cardiomyopathy and require heart transplant
  - Autophagy impairment

- **Dent disease** - Dr. Oliver Devuyst (University of Zurich)

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**Basic research**

Bone marrow transplantation improves proximal tubule dysfunction in a mouse model of Dent disease

Sarah S. Gabriel, Hendrica Beige, Alkay Gassama, Huguette Debaix, Alessandro Luciani, Thomas Fehr, and Olivier Devuyst

1 Institute of Physiology, University of Zürich, Zürich, Switzerland; 2 Division of Nephrology, University Hospital Zürich, Zürich, Switzerland; and 3 Department of Internal Medicine, Cantonal Hospital Graubünden, Chur, Switzerland
From Bench to Bedside

Towards a Phase I/II Clinical Trial
Clinical Case Report: allogeneic HSC transplantation

- Patient was a 16-year-old Caucasian male diagnosed with cystinosis
- Allogeneic HSCT from a full HLA-matched unrelated donor
- Acute graft-versus-host disease (GVHD)
- Graft failure and 2\textsuperscript{nd} transplant with same donor cells 15 months after the first HSCT
- Developed chronic GVHD developed
- Patient died 35 months after transplantation

- Kidney function stabilized and polyuria decreased
- Patient’s photophobia score improved from grade 5 to no photophobia
- Stomach biopsies showed a significant decrease in cystine crystal accumulation
- In patient’s tubular epithelial cells collected from urine and liver biopsy revealed that CTNS expressed in 22% and 40% of cells, respectively
Clinical translation: autologous gene-modified HSC transplantation

- Safety
- Gene frequency
- Risk of integration mutagenesis

pCCL-EFS-CTNS-WPRE

Lentivirus vector (safe version of HIV)

Provided by Dr. Donald Kohn (UCLA)

Adapted from Leboulch, Nature 2013
Preclinical studies for gene-modified HSC transplant using a lentivirus vector

Cystine crystals quantification

8 months post-transplant

Kidney cystine content

Renal function

Table 1. Serum and urine analyses for renal function

<table>
<thead>
<tr>
<th></th>
<th>Wildtype</th>
<th>Control Ctns-/- HSC</th>
<th>pCCL-CTNS Treated Ctns-/- HSC</th>
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</thead>
<tbody>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.27 ± 0.03</td>
<td>0.31 ± 0.08</td>
<td>6.22 ± 0.11*</td>
</tr>
<tr>
<td>Creatinine clearance (mL/min)</td>
<td>4.44 ± 0.39</td>
<td>3.08 ± 1.42</td>
<td>4.89 ± 5.86</td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>14.56 ± 1.67</td>
<td>20.29 ± 16.11*</td>
<td>24.10 ± 7.93</td>
</tr>
<tr>
<td>Phosphate (mg/dL)</td>
<td>12.29 ± 2.38</td>
<td>13.23 ± 2.99</td>
<td>15.40 ± 2.51</td>
</tr>
<tr>
<td>serum</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>urine</td>
<td></td>
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<tr>
<td>Phosphate (mmol/L)</td>
<td>6.62 ± 2.90</td>
<td>8.84 ± 4.60</td>
<td>4.78 ± 3.87*</td>
</tr>
<tr>
<td>Volume (mL)</td>
<td>1.05 ± 0.51</td>
<td>1.26 ± 0.54</td>
<td>0.70 ± 0.60*</td>
</tr>
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*P<0.05 compared to wildtype mice

Harisson et al., *Mol. Ther.*, 2013
Towards a clinical trial for autologous gene-modified HSC transplantation using a lentivirus vector for cystinosis

**Food and Drug Administration (FDA)**

**Target Product:** CD$_{34}^+$ HSCs from patients *ex vivo* gene-modified using pCCL-CTNS

**Lentivirus vector pCCL-CTNS:**

- Submitted pre-Investigational New Drug (IND) in March 2013
  - Teleconference with FDA in April 4, 2013
  - No objection to the design for the Pharmacology/Toxicology Studies

- Good Manufacturing Practice-comparable (GMPc) lentivirus preparation of pCCL-CTNS (Dr. Kenneth Cornetta, Indiana University) and established Good Laboratory Practice (GLP)-like facilities at UCSD (lab, LBR vivarium, ACP biochemistry lab)
Investigational New Drug application

A Phase 1/2 Study to Determine the Safety and Efficacy of Transplantation with Autologous Human CD34+ Hematopoietic Stem Cells (HSC) from Mobilized Peripheral Blood Stem Cells (PBSC) of Patients with Cystinosis Modified by Ex Vivo Transduction using the pCCL-CTNS Lentiviral Vector

Toxicology/Pharmacology studies
- In Vitro Immortalization assays
- Serial transplantation in Primary and Secondary Ctns-/- Recipient mice

Manufacturing Development
- Protocol development using human CD34+ cells from healthy donors and cystinosis patients at UCSD
- GMP virus preparation (IUVPF) and characterization in 2017
- Transfer Technology to UCLA (Dr. Donald Kohn)
- Small Scale Runs using cystinosis patient cells at UCLA
- Large Scale Run using healthy donor

Clinical
- Cystinosis Stem Cell and Gene Therapy consortium – 14 members
  - Clinical Design
  - Clinical Protocol
  - Consent forms
- IRB and IBC approvals in July 2018 (upon IND approval)

IND approved on December 19, 2018

Primary recipients Ctns-/- mice
Secondary recipients Ctns-/- mice

Comprehensive clinical, histological, biochemical and molecular evaluations

Sca1+ Ctns-/- HSCs
CTN S

Ctns-/- mice
6 months
Rady’s Children’s Hospital, Cystine Determination Lab

Dr. Bruce Barshop, Dr. Nadine Benador, Dr. Rajan Dohil, Dr. Robert Mak

Shiley Eye Institute

Dr. Eric Nudleman

Koman Outpatient Pavilion

Dr. Susan Philips and Dr. Doris Trauner

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