



THINKSTOCK

TARGETS & MECHANISMS

ALL-PURPOSE HSCs

By Selina Koch, Associate Editor

While most eyes are on hematopoietic stem cells for their ability to treat blood cancers, a UCSD group has built a body of work showing the immune cell precursors can treat diseases with no relation to the immune system, and has spun out GenStem Therapeutics Inc. to develop the technology.

The company was formed last year based on work from the lab of Stephanie Cherqui, an associate professor in the Department of Pediatrics at the University of California San Diego. Its funding is undisclosed.

In the last month, the researchers have signed a deal with gene and cell therapy company AvroBio Inc. to co-develop a program in cystinosis, and published a preclinical study in *Science Translational Medicine* showing the cells can be used to treat the neurological disorder Friedreich's ataxia.

Bone marrow transplants have long been used in oncology, sickle cell disease and a handful of other indications in which immune cells are cancerous or defective. The procedure replaces the diseased cells with hematopoietic stem cells (HSCs) from a disease-free donor that differentiate into mature cells with the relevant myeloid or lymphoid phenotypes.

Cherqui told BioCentury that although it was unclear whether or how HSCs could treat non-immune indications, the stem cells had been shown to secrete paracrine factors and vesicles carrying molecules that are “good for cells.” She tested the idea

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Stephanie Cherqui, UCSD

on the lysosomal storage disorder cystinosis, a multisystem disease involving kidney and eye pathology that is caused by a mutation in the lysosomal cystine transporter gene CTNS.

In a series of studies conducted between 2009 and 2016, her group showed transplantation of HSCs expressing a normal copy of CTNS successfully treated symptoms in mice. The key feature, according to Cherqui, was the mechanism her team uncovered. Instead of secreting factors, the group demonstrated the cells used a previously described cellular structure — tunneling nanotubes — to rescue the genetic disorder.

HSCs are destined to give rise to immune and blood cells, and therefore don't regenerate kidney or eye tissue. Cherqui's team showed that instead, transplanted HSCs homed to damaged tissues and differentiated into macrophages that formed nanotube passageways to the diseased cells. The macrophages

then passed healthy lysosomes carrying wild-type CTNS protein from themselves through the nanotubes to treat the diseased cells (see “HSC Highways”).

In *Sci. Transl. Med.*, Cherqui and colleagues showed the approach can be extended to a neurological disorder that involves dysfunctional mitochondria rather than lysosomes. In a mouse model of Friedreich’s ataxia, a progressive movement disorder, HSC-derived immune cells transmitted their mitochondrial proteins to neurons both inside and outside the CNS and restored motor function.

Cherqui suspects HSCs can deliver a wide range of therapeutic RNAs, proteins and organelles, opening up a slew of translational opportunities for the cells. “I think this is a premise for many other disorders; the mechanism of action is very efficient.”

ATTACKING ATAXIA

Like cystinosis, Friedreich’s ataxia is caused by loss-of-function mutations; in this case the defect is in the gene encoding the mitochondria-associated protein FXN.

The disease involves loss of neurons in the dorsal root ganglia (DRG) and cerebellum, with primary symptoms being muscle weakness and loss of coordination in the arms and legs. However, according to Cherqui, patients most commonly die from heart malfunction.

In the *Sci. Transl. Med.* study, her group showed transplantation of wild-type HSCs into a mouse model of Friedreich’s ataxia restored activity levels, muscle coordination, and grip strength to levels indistinguishable from those in wild-type littermates.

Mechanistic studies showed the HSCs migrated into DRG, spinal cord, brain and heart tissue. Inside the CNS, the HSCs differentiated into microglial cells; outside the CNS they developed into macrophages, consistent with the group’s observations in cystinosis.

When the HSCs were engineered to express GFP-labeled FXN or another mitochondrial protein, the labeled proteins ended up in neurons or heart cells directly contacted by the HSC-derived microglia or macrophages, respectively. About 50% of neurons in spinal cord sections contained the labeled proteins, an efficiency difficult to achieve with gene therapy, said Cherqui.

The transfer of multiple mitochondrial proteins raised the possibility that the cells transmitted whole mitochondria to neurons, similar to the lysosome transfer the group reported in the past.

However, the paper stopped short of answering that question, nor did it confirm the presence of tunneling nanotubes between the cells.

BIOCENTURY PRODUCT PROFILE

INNOVATION STAGE	
Product	Autologous hematopoietic stem cells (HSCs) engineered to express a normal copy of FXN, the gene mutated in Friedreich’s ataxia
Concept	Engineered HSCs will differentiate into microglia and macrophages that transmit wild-type FXN to neurons and heart cells impaired in the disease
Disease	Friedreich’s ataxia
Competition	Supportive technologies for neurological deficits; ACE inhibitors for heart symptoms; clinical candidates that increase mitochondrial function or decrease inflammation
Differentiation	Disease modifying; directly addresses the cause of the disease by replacing lost protein
Administration	IV
Risks	Toxicity associated with partial myeloablation preconditioning regimen
Development status	Preclinical
Patents	Patent family covering use of engineered HSCs to treat movement disorders
Company; lead investigator	University of California San Diego; Stephanie Cherqui, GenStem Therapeutics Inc.; Jeffrey Ostrove

“We see that it is transferred but we don’t know if the protein or the whole mitochondria is transferred. And we don’t know if it is transferred by nanotubes, exosomes, both or something else,” said Cherqui. Her lab is currently working on those questions.

But she noted the full mechanism does not need to be fleshed out to advance the program.

GenStem CEO Jeffrey Ostrove said the newco is gearing up for GLP tox and additional efficacy studies in Friedreich’s ataxia. The therapy will comprise autologous HSCs engineered with a lentiviral vector to express a normal copy of FXN. He declined to provide a development timeline or to disclose whether GenStem plans to seek a partner.

HSCS VS. AAVS

Ostrove believes autologous HSC therapies could overcome some of the biggest problems associated with adeno-associated viral (AAV)-mediated gene therapies, such as preexisting antibodies against the vector, limitations on dosing and the difficulties of delivering the therapies to target tissues.

“I think this work is going to open up novel treatments for multiple genetic diseases that, prior to this, really didn’t have a pathway forward,” he said.

Although gene therapies are designed to deliver therapeutic cargo directly to the cells that need it, rendering them capable of producing the therapeutic protein themselves, Cherqui argues HSCs have specific advantages.

“With *in vivo* gene therapy I doubt you would get such great engraftment everywhere and tissue targeting that is so efficient,”

she said. “Dose would be a limitation; it would require a lot of virus.”

By contrast, she said, “HSCs are intelligent. They home to injured tissue and deliver protein where it is needed. In healthy mice these cells don’t go anywhere; they stay in the bone marrow.”

Moreover, she believes the approach will only require a single treatment. “The patient would have a source of healthy cells that

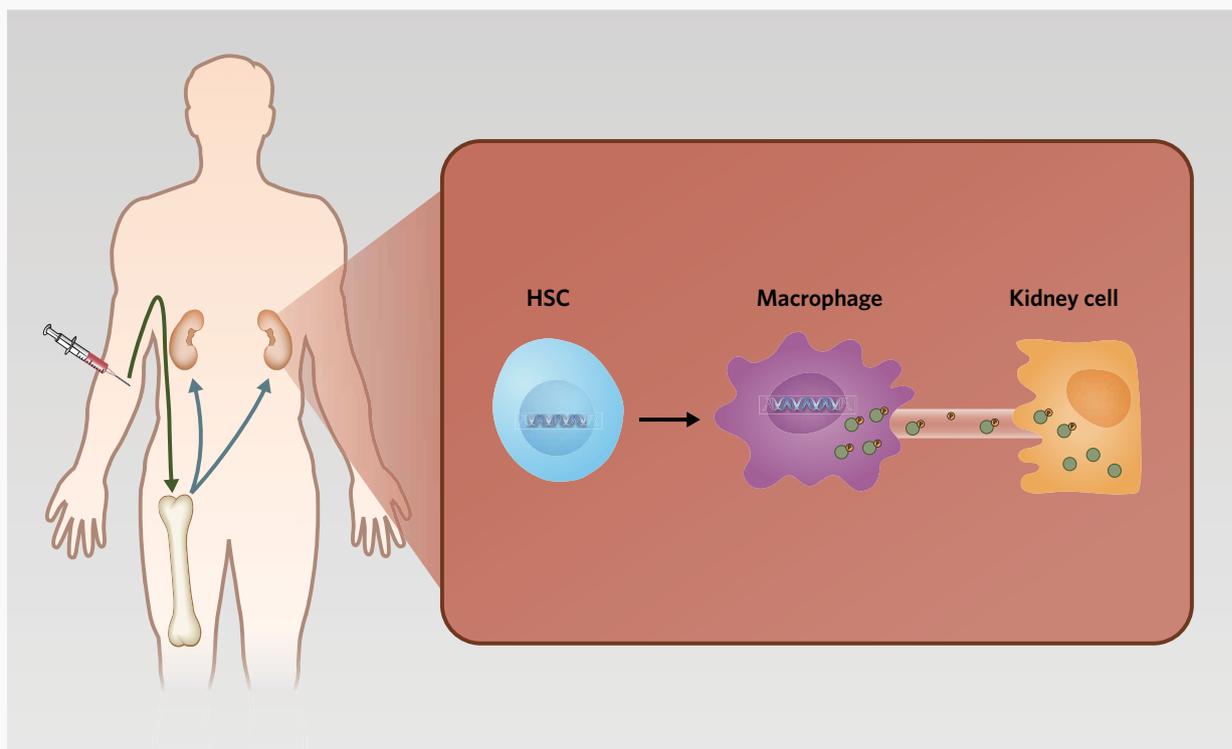
HSC HIGHWAYS

A **University of California San Diego** team has shown hematopoietic stem cells (HSCs) can be used to treat diseases outside of the modality’s standard indications of blood and bone marrow cancers. One such indication is cystinosis, a lysosomal storage disorder caused by mutations in cystinosis lysosomal cystine transporter (CTNS) that result in kidney pathology.

After IV injection, HSCs engineered to express wild-type CTNS (**blue DNA**) migrate and home to the bone marrow (**green arrow**), where they engraft. Upon receiving disease-related signals, some of the HSCs migrate to sites of damage in the kidney (**blue arrow**), where they differentiate into macrophages. The

macrophages then form tunneling nanotubes (**pink**) that create passageways to the diseased kidney cells, which contain lysosomes devoid of CTNS. The macrophages pass CTNS-bearing lysosomes (**green circles**) to the kidney cells through the nanotubes, restoring normal kidney function.

In 1H18, UCSD plans to begin a Phase I/II trial of autologous HSCs engineered to express wild-type CTNS to treat cystinosis. Patients will receive a preconditioning regimen to partially ablate their bone marrow to make room for the transplanted HSCs.



are always there in the bone marrow. You wouldn't need to re-transplant."

Avrobio CEO Geoff MacKay added: "Maybe the most impressive feature of this approach is its durability," which he ascribed partly to the use of lentiviral vectors.

Whereas the effects of AAV gene therapies can fade over time, requiring re-treatment, lentiviral-delivered genes permanently integrate into the genome, he said. Although that creates a risk of insertional mutagenesis, hundreds of patients have been treated with lentiviral-modified HSCs "without insertional mutagenesis or serious adverse events," he said.

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Jeffrey Ostrove, GenStem

MacKay added that HSC engraftment will be easier on patients than standard regimens, because it uses preconditioning only to partially ablate the bone marrow. The goal is to create "just enough space for the cells to migrate in and find a home," he said. "This is not full myeloablation," as is used in oncology. "The regimen is far less toxic than that."

LEADING WITH LYOSOMES

Ostrove said UCSD will be the investigator on the upcoming cystinosis trial and noted the university has a clinic dedicated to the rare disease. "They have probably seen the most cystinosis patients in the world; that was very attractive," he said.

MacKay told BioCentury GenStem's cystinosis therapy will become Avrobio's fourth lysosomal storage disorder program and "fits perfectly" into its pipeline. "This is the exact same technology platform we are already using for our other lysosomal storage disorder programs," he said.

Like GenStem, Avrobio modifies HSCs *ex vivo* using lentiviral vectors. "Operationally, it fits like a glove: in manufacturing, CMC and even clinically, the designs of these trials are analogous," said MacKay.

MacKay said the approach has already been validated as a means of replacing lost enzymes in a handful of genetic disorders,

including metachromatic and cerebral leukodystrophies and adenosine deaminase severe combined immunodeficiency (ADA-SCID). "This is not well understood, but there are now four or five really successful clinical trials of this approach with ten-plus years of data."

The only marketed autologous HSC therapy is GlaxoSmithKline plc's strimvelis, in which a retroviral vector is used to deliver ADA *ex vivo* to HSCs. The therapy is approved in Europe for ADA-SCID.

Avrobio's other lysosomal storage disease programs are in Fabry's, Gaucher's and Pompe's diseases. All are enzyme replacement therapies (ERTs) in which HSC-derived immune cells secrete the missing enzyme and target cells take it up.

However, cystinosis results from loss of a membrane transporter rather than an enzyme. And although GenStem's and Avrobio's technologies are "the same on the front end," MacKay said, "the one thing that is distinct is the mechanism on the back end, this concept of nanotubes."

He added: "Stephanie has been *the* pioneer in the world at developing this *ex vivo* approach for cystinosis," and noted Cherqui's program was unusual for an academic project in that "it was really clinic-ready."

Ostrove also said Cherqui's lab has gone the distance with preparing the findings for translation, including providing appropriate documentation and quality controls.

While UCSD is conducting the Phase I/II trial, MacKay said, Avrobio will perform the scale up, the CMC, and the preparations for the pivotal trial. ■

COMPANIES AND INSTITUTIONS MENTIONED

Avrobio Inc., Cambridge, Mass.
 GenStem Therapeutics Inc., San Diego, Calif.
 GlaxoSmithKline plc (LSE:GSK; NYSE:GSK), London, U.K.
 University of California San Diego, La Jolla, Calif.

TARGETS

ADA - Adenosine deaminase
 CTNS - Cystinosis lysosomal cystine transporter
 FXN (FRDA) - Frataxin

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