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Authors

Rocca, Celine J
Cherqui, Stephanie

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REVIEW

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Potential use of stem cells as a therapy for cystinosis

Celine J. Rocca[1] · Stephanie Cherqui[1]

Abstract

Cystinosis is an autosomal recessive metabolic disease that belongs to the family of lysosomal storage disorders (LSDs). Initial symptoms of cystinosis correspond to the renal Fanconi syndrome. Patients then develop chronic kidney disease and multi-organ failure due to accumulation of cystine in all tissue compartments. LSDs are commonly characterized by a defective activity of lysosomal enzymes. Hematopoietic stem and progenitor cell (HSPC) transplantation is a treatment option for several LSDs based on the premise that their progeny will integrate in the affected tissues and secrete the functional enzyme, which will be recaptured by the surrounding deficient cells and restore physiological activity. However, in the case of cystinosis, the defective protein is a transmembrane lysosomal protein, cystinosin. Thus, cystinosin cannot be secreted, and yet, we showed that HSPC transplantation can rescue disease phenotype in the mouse model of cystinosis. In this review, we are describing a different mechanism by which HSPC-derived cells provide cystinosin to diseased cells within tissues, and how HSPC transplantation could be an effective one-time treatment to treat cystinosis but also other LSDs associated with a lysosomal transmembrane protein dysfunction.

Keywords Cystinosis · Lysosomal storage disorders · Hematopoietic stem and progenitor cells · Gene therapy · Lysosomal transfer · Tunneling nanotubes

Introduction

Lysosomal storage disorders (LSDs) are a group of metabolic diseases characterized by a disruption of a lysosomal function leading to the storage of diverse macromolecules within lysosomes [1]. The progressive accumulation of incompletely degraded substrates in several tissues ultimately leads to multiple organ dysfunction and clinical complications that usually shorten the lifespan of affected children [1]. The majority of LSDs are due to defective activity of lysosomal hydrolases. For years, the only available treatment for LSDs was the enzyme replacement therapy (ERT) based on the discovery of Hasilik and colleagues that wild-type cells could secrete functional enzymes that are captured and directed to lysosomes of deficient cells through the mannose-6-phosphate receptor pathway [2]. Nine US Food and Drug Administration

(FDA)-approved ERT for the treatment of six LSDs (mucopolysaccharidoses, MPS, I, II, and VI, Gaucher, Fabry, Pompe) are currently available and they are all administered by intravenous infusion, usually weekly or every other week, typically for the life of a patient [3]. Although ERT has improved patients' life expectancy, the major limitation, besides a potential immune-response against the injected protein, is that administrated enzymes cannot cross the blood-brain barrier (BBB) which reduces treatment efficacy for LSDs with central nervous system (CNS) manifestations [4].

Among the ∼ 50 known LSDs, 1/5 are caused by lysosomal membrane protein dysfunction [5]. Cystinosis, which belongs to this category, is an autosomal recessive metabolic disorder with an estimated incidence of 1/100,000 to 200,000 live births. The gene involved, *CTNS* gene, encodes for the 7 transmembrane H^+-driven lysosomal cystine transporter, cystinosin [6–8]. Defects in *CTNS* result in accumulation of cystine, the oxidized dimer of the amino acid cysteine, within lysosomes resulting in intracellular crystal formation, cell death, and eventually tissue damage. Because cystinosin is ubiquitously expressed, most of the organs are affected. However, depending on the type of mutation in the *CTNS* gene, there are different forms and severity of the disease [9, 10]. The most common and most severe form is infantile

✉ Stephanie Cherqui
  scherqui@ucsd.edu

[1] Department of Pediatrics, Division of Genetics, University of California, 9500 Gilman Drive, MC 0734, La Jolla, San Diego, CA 92093-0734, USA

cystinosis (MIM 219800). Patients are normal at birth, but develop renal tubular Fanconi syndrome at 6–18 months of age, accompanied by failure to thrive, polyuria and polydipsia, dehydration, and hypophosphatemic rickets. Patients also develop chronic kidney disease that eventually leads to end-stage renal failure requiring renal transplantation. They also present with photophobia and eventually later in life with retinal blindness, hypothyroidism, diabetes mellitus, muscle weakness, and neurological defects [11]. There are two other forms of cystinosis that are rarer and less severe, juvenile cystinosis (MIM 219900) characterized by photophobia and glomerular and tubular alterations leading to proteinuria and eventually end-stage renal disease (ESRD) [12], and the ocular cystinosis (MIM 219750) defined by adult-onset of mild photophobia [13].

The current care for patients affected by cystinosis, beyond supportive therapy (dietary recommendations, indomethacin, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, growth hormones, etc.) [14], is the oral drug cysteamine (mercapto-ethylamine), which allows lysosomal cystine clearance. Cysteamine has certainly improved the life expectancy of cystinotic patients and improved the disease outcome, especially if started before the age of 5 years [15]. However, this treatment delays disease progression, but is not a cure. Indeed, cysteamine fails to correct the Fanconi syndrome and only delays ESRD; dialysis and renal transplantation remain mandatory for most of the cystinotic patients [15–17]. Between supportive therapy and cysteamine, daily medication for a cystinotic patient can reach up to 60 pills. In addition, severe gastric side effects are often associated with cysteamine therapy. Therefore, there is a pressing need for a new therapy for cystinosis.

Hematopoietic stem and progenitor cell transplantation for non-hematopoietic diseases

In most cases, HSPC transplantation is used to treat blood-related diseases (e.g., blood or bone marrow malignancies, non-malignant blood disease, immunodeficiency disorders). However, HSPC transplantation can also treat non-hematopoietic disorders. Exploiting the property that HSPC can home to damage tissues, differentiate in hematopoietic cells, and have a paracrine effect on neighboring cells, HSPC transplantation proved to be effective to treat LSDs that are due to defective lysosomal enzymes. Indeed, with a one-time intervention, bone marrow engrafted cells become a permanent source of healthy cells that can deliver functional lysosomal enzymes to neighbor diseased cells after integrating within tissues including the CNS [18]. Treatment of Hurler patients with HSPC transplantation has been the most gratifying. Indeed, bone marrow transplantation for severe MPS-I,

performed before the age of two, prolongs survival and allows normal or near normal cognitive development and myocardial function [19]. Same results have been obtained for patients affected with Krabbe disease, a demyelinating disorder caused by a deficiency of galactosylceramidase if early transplantation is performed [20–22]. Nonetheless, for some LSDs (Sanfilippo or GM1 gangliosidosis), HSPC transplantation did not show clinical benefit [23, 24]. The reason for unsuccessful HSPC transplant is unclear. In the case of Sanfilippo, it is suggested that the donor-derived microglia cells secrete insufficient amounts of enzymes for cross-correction of the neuronal tissue [24].

Cystinosis belongs to a different class of LSD as the protein involved is a transmembrane lysosomal protein. However, the idea of HSPC transplantation for the multisystemic disorder, cystinosis, emerged from an attempt to try to find an optimal vehicle to bring the functional protein to damaged tissues. Studies have been performed in the mouse model of cystinosis, the Ctns$^{-/-}$ mice [25, 26]. Ctns$^{-/-}$ mice accumulate cystine and cystine crystals in all tissues and develop similar symptoms to those observed in patients, proximal tubulopathy, and ESRD by 15 months of age, ocular anomalies, bone and muscular defects, behavioral anomalies, and hypothyroidism [26–30]. We first hypothesized that mesenchymal stem cells (MSCs) would be the best candidate since they are multipotent stromal cells that can be mobilized from the bone marrow and differentiate into a variety of cell type of tissue cells including osteoblasts, chondrocytes, myocytes, and adipocytes [31]. Moreover, several studies showed that, in the setting of renal injury, transplanted MSCs could generate mesangial and tubular epithelial cells [32] and restore renal structure and function [33, 34]. In our model, MSC transplantation only led to some short-term improvement in tissue cystine content and we observed by confocal microscopy that green florescent protein (GFP)-expressing MSCs did not integrate efficiently within tissues [35]. In parallel, we also performed syngeneic whole bone marrow cell (BMC) transplantation in lethally irradiated Ctns$^{-/-}$ mice, which, surprisingly, led to the dramatic reduction of cystine content in all tissues tested [35]. Therefore, we also investigated the impact of purified HSPCs. Transplantation of Sca1$^+$ HSPCs, Sca1 being the murine homolog marker for CD34 in humans, in lethally irradiated Ctns$^{-/-}$ mice, was as efficient as BMC transplantation leading to significant tissue cystine decrease [35].

HSPC transplantation leads to multi-organ rescue in the Ctns$^{-/-}$ mice

Kidney preservation

Kidney is the primary tissue compartment impacted by cystinosis with the development of the renal Fanconi

syndrome at 6–18 months of age [36]. In addition, the glomerular filtration rate progressively starts to deteriorate after 2 years of age which leads to ESRD at the end of the first decade [37]. In the appropriate genetic background, Ctns$^{-/-}$ mice develop kidney pathology, in particular a mild renal Fanconi syndrome, and eventually end-stage renal failure but late in life (~ 18 months of age) as opposed to cystinosis patients [29]. Characteristic histological anomalies of the disease could be observed such as swan neck deformities at the glomerulo-tubular junction, thick basal membrane and perivascular mononuclear infiltrates, and cystine crystals are present within interstitial and proximal tubular cells. HSPC transplantation in Ctns$^{-/-}$ mice prevented the progression of the kidney disease in cystinosis. Indeed, this treatment led to long-term preservation of the kidney function and structure including the Fanconi syndrome, despite lack of HSPC reprogramming into proximal tubular cells (PTCs) [38]. However, effective therapy depended on achieving at least 50% of donor-derived blood cells engraftment of Ctns-expressing HSPCs within the bone marrow. This means that, at least 50% of the exogenous HSPCs have to express a functional *CTNS* gene in the bone marrow for the treatment to be fully effective in the Ctns$^{-/-}$ mice; the cells will then become a permanent source of circulating blood cells expressing *CTNS*. We also tested the impact of HSPC transplantation in older mice, between 6 and 10 months of age, when the disease is already established, and observed normal kidney function if the blood engraftment was sufficient [38]. This would suggest that if injury is not too advanced, the remaining kidney tissue could be rescued or protected by stem cell therapy. However, we do not know if established kidney injury could be reversed. Of note, since the mice male are fertile, in contrast to men affected with cystinosis, impact of HSPC transplantation on fertility could not be evaluated.

Eye pathology

The second tissue impacted by cystinosis is the eye and the main ocular manifestation is crystal deposition within the cornea. Crystals can be observed by slit lamp examination as early as 1 year of age, increases with age, and gradually leads to photophobia, blepharospasm, keratopathy, and recurrent corneal erosion [39]. In older patients, filamentous keratopathy, band keratopathy, and peripheral corneal neovascularization are also observed [39–41]. The mouse model for cystinosis also develops ocular pathology similar to humans [28, 30]. Cornea is a challenging tissue for stem cell-mediated therapy because it is avascular rendering its access by HSPC-derived cells difficult. However, abundant HSPC-derived cells could be observed in the cornea at 1 year post-transplantation, but also in the retina, lens, and ciliary margin [42]. Ctns expression in the eye was increased and cystine level decreased. Using in vivo confocal microscopy, we showed that, if

engraftment of Ctns-expressing HSPCs was sufficient (more than 50%), cystine build up was almost completely prevented in the Ctns$^{-/-}$ corneas. We also demonstrated that we could restore normal intra-ocular pressure as well as normal corneal thickness and structure.

These results demonstrate the versatility of the HPSCs and their therapeutic potential for corneal disorders. However, the limitation resides in the risk of bone marrow transplantation when the pathology is strictly localized to the eye. Recent studies investigated the feasibility of intravitreal HSPC transplantation in different mouse models of retinal degeneration, macular oedema, or ischemia showing phenotypical improvement [43–45].

Hypothyroidism

Accumulation of cystine crystals also leads to impairment of endocrine tissues, hypothyroidism being the most common endocrine involvement [46]. The team of Dr. Courtoy previously showed that Ctns$^{-/-}$ mice present with impaired thyroid hormone production resulting in subclinical hypothyroidism, thyrocyte hyperplasia/hypertrophy, and accelerated cell turnover [27]. In collaboration with Dr. Courtoy's lab, we showed that wild-type HSPC transplantation into Ctns$^{-/-}$ mice decreased thyroid cystine content, normalized thyroid function (TSH and T4), prevented thyrocyte hyperplasia and hypertrophy, and improved biosynthetic and lysosomal overloaded in Ctns$^{-/-}$ thyroid [47].

Overall, these data show that one single systemic injection of HPSC is sufficient to address the serious global effects of cystinosis for the life of the mice. However, cystinosin is a lysosomal transmembrane protein that cannot be secreted and the mechanism by which HSPC participate to the phenotypical rescue of the Ctns$^{-/-}$ mouse model was then unclear.

Mechanism of action of HSPC in the case of cystinosis

Fate of the transplanted HSPCs within tissues

Our first hypothesis to explain the drastic effect of BMC and HSPC transplantation to prevent the development of cystinosis were the following: (1) wild-type HSPCs were integrating within the diseased tissue and differentiating into proficient tissue-specific cells or (2) HSPCs were fusing with the deficient Ctns$^{-/-}$ tissue cells. In order to investigate the fate of HSPCs after transplantation, we generated a new system consisting of a DsRed^{+} Ctns$^{-/-}$ mouse model, constitutively expressing the red fluorescent protein DsRed, and HSPCs isolated from wild-type GFP-transgenic donor mice. This dual fluorescent model allowed us to unequivocally discern fusion events (yellow cells) from differentiation/transdifferentiation

(green). We observed that the majority of the bone marrow-derived cells were strictly GFP$^+$, excluding fusion as the main mechanism for tissue repair. In conjunction with lineage-specific antibody staining, we identified the GFP-expressing cells as macrophages within tissues [48]. Macrophages are among the most plastic of immune cells with a large variety of phenotypes and physiological functions [49, 50]. However, their impact for tissue repair in the context of a transmembrane protein was unknown.

In vitro demonstration of lysosomal cross-correction

Cystinosin transfer from the HSPC-derived cells to the diseased cells was the most plausible explanation to account for the long-term tissue preservation in cystinosis. Cystinosin-containing microvesicles/exosomes shed by cystinosin-expressing cells have been shown as vehicles to decrease cystine in cystinotic cells [51, 52]. To address the mechanism in vitro, we established co-culture models using wild-type macrophages and DsRed$^+$ Ctns$^{-/-}$ fibroblasts. When wild-type macrophages were co-cultured with Ctns$^{-/-}$ fibroblasts, cystine levels decreased by ~ 75% in FACS-sorted fibroblasts, whereas when the two populations were physically separated using transwells porous to exclusively microvesicles, cystine levels decreased only by ~ 20%. These findings showed that direct cell:cell contact was necessary for an optimal cystinosin cross-correction as compared to microvesicle-mediated transfer. A large number of macrophages were extending long protrusions connecting to fibroblasts, also known as tunneling nanotubes (TNTs) [48], which could be a route for cystinosin transfer. TNTs have been first described in vitro in 2004 by Rustom et al. [53] formed de novo between numerous cell types allowing complex connections between distant cells

observed both in vitro [53, 54] and in vivo [55, 56]. They have been implicated in a wide variety of biological processes ranging from to bacterial and prion pathogenesis [57, 58] to calcium-mediated cellular communication [54, 59] but also lysosomal and mitochondrial trafficking [60, 61]. To verify the transfer of lysosomes via TNTs in our in vitro assays, we used macrophages transduced with self-inactivating lentivirus (SIN-LV) carrying the cDNA of the fusion protein cystinosin-GFP. Time-lapse confocal microscopy revealed that lysosomes (stained with LysoTracker) containing cystinosin-GFP migrated along the TNTs toward the fibroblasts [48]. Interestingly, we found that this transfer was bidirectional, cystinosin-deficient lysosomes, marked with Lamp2-DsRed also traveled through TNTs to reach macrophages [48] (Fig. 1). Lysosome fusion in both cell types probably occurs, releasing cystine in both cell types and providing a bidirectional correction, accounting for the efficient tissue cystine decrease.

In vivo demonstration of lysosomal cross-correction within the Ctns$^{-/-}$ kidney

TNTs have been reported in vivo by others in the cornea [55] and lung [56], but not in a context of tissue repair. One of the primary and earliest sites affected in cystinosis is the proximal tubular cells (PTCs) responsible for the Fanconi syndrome. As mentioned earlier, we found that HSPC transplantation could prevent the kidney disease including the proximal tubulopathy [35, 38, 62]. And yet, PTCs are known to be protected from the interstitium by a thick tubular basement membrane (TBM) that strongly limits the passage of macromolecules [63]. By confocal microscopy, we observed that abundant GFP-expressing bone marrow-derived macrophages were indeed surrounding but never inside the proximal tubules in the
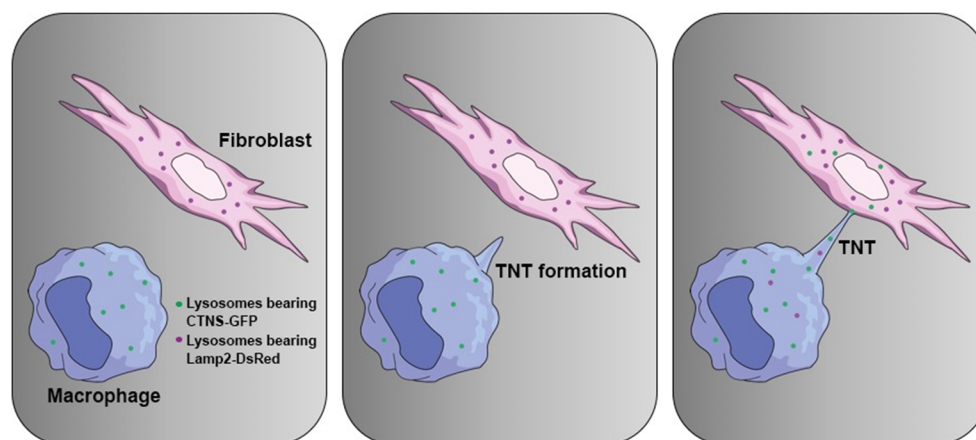

Fig. 1 Mechanism of in vitro lysosomal cross-correction via tunneling nanotubes. Ctns$^{-/-}$ fibroblasts virally transduced to express the fusion protein Lamp2-DsRed are co-cultured with macrophages expressing cystinosin-GFP. The macrophages extend tunneling nanotubes (TNTs) toward the fibroblasts and establish an intercellular connection leading to a bidirectional exchange of lysosomes between both cell types. Fibroblasts are then rescued by the presence of healthy lysosomes carrying the functional cystinosin, while macrophages help discarding cystine-loaded lysosomes

$Ctns^{-/-}$ kidneys. However, long tubular protrusions were observed apposing on and even crossing the TBM [48]. To confirm that those nanotubes were able to deliver cystinosin-containing vesicles to PTCs, we transplanted $Ctns^{-/-}$ mice with $DsRed^+$ HSPCs transduced with SIN-LV-CTNS-GFP, and observed GFP-positive lysosomes in the DsRed-expressing HSPC-derived macrophages surrounding the proximal tubules but also within the PTCs, providing a mechanism for the preservation of the proximal tubules in $Ctns^{-/-}$ mice [48] (Fig. 2). To our knowledge, this is the first evidence of direct transfer of proteins/organelles from interstitial macrophages to epithelial cells via TNTs penetrating the TBM.

Demonstration of bone marrow cell rescue via TNTs in another kidney disease

This novel mechanism of transmembrane lysosomal protein correction, leading to long-term kidney preservation after HSPC transplantation in cystinosis, may be expanded for treatment of other inherited renal disorders. Thus, Dr. Devuyst's group recently tested bone marrow transplantation in a mouse model of Dent disease [64]. Dent disease (MIM #300009) is a rare X-linked tubulopathy caused by mutations in the endosomal chloride-proton exchanger (ClC-5) resulting in defective receptor-mediated endocytosis, severe proximal tubule dysfunction, low molecular weight proteinuria, kidney stones, and renal failure [65]. They demonstrated that transplantation of wild-type bone marrow cells in $Clcn5^{Y/-}$ mice significantly improved proximal tubule dysfunction. Similar to our findings, they observed that

kidney-engrafted cells were mononuclear phagocytes found in the interstitium, surrounding proximal tubules, and also extending TNTs. In vitro experiments showed that cell:cell contact was also mandatory to rescue defective endocytosis suggesting that not only lysosomes but also endosomes could be transferred to diseased cells via TNTs [64].

Mechanism of action in non-nephropathic tissues

We also studied the mechanism of HSPC-mediated tissue repair in the eye and thyroid. In the cornea, HSPCs also differentiated into macrophages that were also capable of generating TNTs and delivering cystinosin-bearing lysosomes to diseased adjacent corneal cells [42]. Similarly, HSPCs differentiated into macrophages/dendritic cells in the thyroid frequently apposed onto the follicular basement laminae and generating TNTs. However, in addition, some HSPC-derived cells were able to entirely cross the membrane laminae of thyrocytes and fully squeeze into the epithelial monolayer [47].

From bench to bedside: hematopoietic stem cell gene therapy using SIN-lentivirus vectors

Ex vivo HSPC gene therapy as a treatment option for genetic disorders

Allogeneic HSPC transplantation in patients requires a compatible donor and still represents a procedure with high
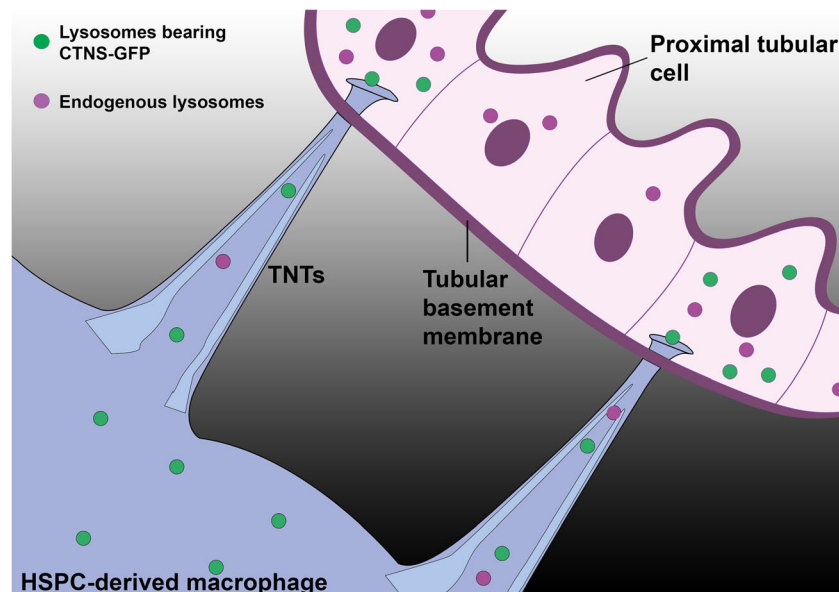

Fig. 2 Mechanism of in vivo lysosomal cross-correction via tunneling nanotubes. Transplanted $Ctns^{-/-}$ HSPCs ex vivo transduced with SIN-LV carrying CTNS-GFP repopulate the bone marrow of $Ctns^{-/-}$ mice, migrate into the kidney where they differentiate into macrophages. Affected proximal tubular cells (PTCs) are protected from the extracellular environment by the tubular basement membrane (TBM). The rescue of PTCs requires that macrophages extend tunneling nanotubes (TNTs) crossing the TBM to deliver functional cystinosin-bearing lysosomes and may be take away the endogenous cystine-loaded lysosomes (never shown in vivo) from the PTCs, accounting for the long-term recue of the proximal tubules in $Ctns^{-/-}$ treated by hematopoietic stem and progenitor cell (HSPC) transplantation

risk of morbidity and mortality, graft versus host disease being the major complication [66]. In contrast, autologous ex vivo gene-corrected HSPC transplantation represents a safer treatment option because it abrogates the risk of GVHD and immune rejection. However, it requires the use of virus vectors to introduce a normal copy of the gene, which could be a limitation to achieve high enough gene-corrected cell level and could integrate near cancer genes. SIN-LVs are now used for most of the ex vivo gene correction of the cells as they have a great ability to transduce human HSCs, and contain only one internal enhancer/promoter, which reduces the incidence of interactions with nearby cellular genes and thus significantly decreases the risk of oncogenic integration [67]. Clinical trials using SIN-LV to transduce human CD34^{+} HSPCs are being undertaken in the USA and Europe for genetic diseases especially immune deficiency disorders (e.g., X-linked severe combined immunodeficiency, adenosine deaminase deficiency, and Wiskott-Aldrich syndrome) [68–70]. In the context of LSDs, an important example of successful HSPC gene therapy approach using SIN-LV is for metachromatic leukodystrophy, due to the deficiency of the lysosomal enzyme, arylsulfatase A (ARSA). Nine patients have been treated so far with no vector-related toxicity reported. The first three patients have been reported to have extensive and stable ARSA expression in the periphery and in the cerebrospinal fluid, with no manifestation of disease from 7 to 21 months after the predicted age of symptom onset [71]. Autologous CD34^{+} cells transduced with a SIN-LV strategy was also successful for the treatment of X-linked cerebral adrenoleukodystrophy (ALD) [72, 73]. A total of 17 boys have been treated in a phase II/III study and after 29.4-month follow-up; they all presented with significant gene-marking and ALD protein was physiologically expressed [74].

HSPC gene therapy for cystinosis

We developed an ex vivo gene-modified HSPC strategy using a SIN-LV carrying *CTNS* cDNA, pCCL-CTNS, and tested this approach in the mouse model of cystinosis. Pre-clinical studies showed that transduced HSPCs kept their differentiative capabilities, populating all tissue compartments and allowing long-term transgene expression [62]. Cystine content was decreased in all tissues tested and kidney function was improved. We are currently finishing the pharmacological and toxicological studies and assembling the Investigational New Drug (IND) application for a phase I/II clinical trial to assess the safety and efficacy of autologous transplantation of CD34^{+} HSPCs ex vivo modified using pCCL-CTNS in patients affected with infantile cystinosis. If successful, i.e., if we can achieve significant reduction of cystine level and restore normal cellular functions in the majority of diseased cells, this treatment could be a life-long therapy that may eliminate or reduce renal deterioration and the need for kidney

transplantation, as well as the long-term complications associated with cystinosis. However, it is important to note that this treatment will be the first ex vivo HSPC gene therapy for a lysosomal storage disease for which the protein involved is a transmembrane lysosomal protein so cautious optimism is warranted on the efficacy of such a strategy in patients with cystinosis.

Conclusion

Despite the fact that the protein involved in cystinosis is a transmembrane lysosomal protein, HSPC transplantation proved to be efficient to rescue the pathology through their differentiation into macrophages within tissues and the transfer of cystinosin-bearing lysosomes via TNTs to adjacent host cells. This study not only allowed us to reveal for the first time a new potential curative property of the HSPC-derived cells for cystinosis but also demonstrates that HSPCs can act as intelligent vehicles to deliver functional organelle-associated proteins to defective cells in the entire body. These findings open new perspectives to treat diseases for which HSPC transplantation was not considered as a treatment option. Indeed, as mentioned earlier, a new pre-clinical study for Dent disease showed that an endosomal transmembrane protein could be transferred to surrounded the proximal tubular cells through TNTs and prevent the proximal tubulupathy [64]. Most recently, because mitochondria can also be transferred via TNTs [56, 75–77], we also investigated if HSPC transplantation could help to prevent the mitochondrial disorder Friedreich's ataxia, a neuromuscular degenerative disorder, for which there is no treatment. We showed that HSPCs were able to migrate efficiently to all the sites of injury, i.e., the brain, spinal cord, and dorsal root ganglia (DRGs), but also to the heart and skeletal muscle, and differentiate into microglia/macrophages and deliver frataxin to neurons and myocytes [78]. Locomotor deficits and muscle weakness were prevented as well as degeneration of the large sensory neurons in DRGs, and mitochondrial dysfunction was improved in these tissues. Altogether, these findings highlight the potential of HSPC for tissue repair and may expend their use to treat a wider panel of hereditary disorders.

Compliance with ethical standards

Competing interests S.C. is a Scientific Board member and member of the Board of Trustees of the Cystinosis Research Foundation. S.C. is a

cofounder, shareholder, and a member of both the scientific board and board of directors of GenStem Therapeutics Inc. The terms of this arrangement have been reviewed and approved by the University of California San Diego in accordance with its conflict of interest policies.

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