In the September 2009 issue of Blood, Syres et al. [1] report on syngeneic bone marrow cell (BMC) and haematopoietic stem cell (HSC) therapy as a successful treatment in a mouse model of cystinosis, an autosomal recessive metabolic disease caused by a defect in the transport of cystine across the lysosomal membrane. The accumulation of cystine crystals in lysosomes leads to a multi-organ dysfunction including proximal tubulopathy and renal failure, corneal deposits, myopathy and central nervous system defects. By using Ctns knock-out (Ctns(-/-)) mice as a model for cystinosis, Syres et al. show that BMC transplantation leads to a major reduction of cystine content in all tissues tested, reflected by a significant attenuation of the development and progression of kidney injury and reduction in the number of mice with corneal cystine crystals. These changes were correlated with the engraftment of donor BMC producing a functional cystine transporter in the tissues tested. The transplant...
The renoprotective effects of RAAS inhibition depended solely on a more effective control of systemic blood pressure (for review see [14]).

**What is in for the practising nephrologists?**

The major goal to prevent progression of glomerulosclerosis still remains an effective control of systemic blood pressure. Renal autoregulation is impaired in the remaining glomeruli of chronically diseased kidneys, and glomerular hyperfiltration must be prevented to prevent disease progression (Figure 1). Although the pathomechanism is still incompletely understood, proteinuria can serve at least in part as a surrogate marker for glomerular hyperfiltration. Proteinuria is mostly only partially reversible in patients even with optimal therapy including ACE inhibitors or AT1 blockers, and this might be a consequence of the destruction of the glomeruli by sclerosis. The identification of a regenerative mechanism that might replenish podocytes within the glomerulus of adult patients will open the prospect to develop additional and more specific pharmacological strategies beyond ACE inhibitors to slow or even revert glomerular injury.

**References**


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**Cell therapy for cystinosis**

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**Summary**

In the September 2009 issue of Blood, Syres et al. [1] report on syngeneic bone marrow cell (BMC) and hematopoietic stem cell (HSC) therapy as a successful treatment in a mouse model of cystinosis, an autosomal recessive metabolic disease caused by a defect in the transport of cystine across the
Mutations in CTNS → functional loss of cystinosin:
- Defective cystine efflux from lysosomes
- Lysosomal storage disease → cell dysfunction
- Renal proximal tubule cell dysfunction - renal failure
- Multi-organ dysfunction

**Fig. 1.** Role of cystinosin and pathophysiology of cystinosis. Cystinosin is a lysosomal membrane protein (a) acting as a H⁺-driven lysosomal cystine transporter working in parallel with the vacuolar H⁺-ATPase which acidifies the interior of the lysosome. Thus, the influx of H⁺ in the lysosome drives the cystinosin-mediated transport of cystine from the lysosome to the cytosol. Cystinosin is a 367-amino acid protein predicted to have seven transmembrane domains (b). The N-terminal region is oriented towards the lysosomal lumen. Two lysosomal targeting motifs (a classical domain, GYDQL, in the C-terminus of the protein and a 'conformational' domain, YFPQA, in the third cytoplasmic loop) have been identified. Loss of function mutations of CTNS, the gene that encodes cystinosin, result in lysosomal accumulation of cystine and multi-organ cellular dysfunction (c). Cystinosis is the most common familial form of the renal Fanconi syndrome.
lysosomal membrane. The accumulation of cystine crystals in lysosomes leads to a multi-organ dysfunction including proximal tubulopathy and renal failure, corneal deposits, myopathy and central nervous system defects. By using Ctns knock-out (Ctns−/−) mice as a model for cystinosis, Syres et al. show that BMC transplantation leads to a major reduction of cystine content in all tissues tested, reflected by a significant attenuation of the development and progression of kidney injury and reduction in the number of mice with corneal cystine crystals. These changes were correlated with the engraftment of donor BMC producing a functional cystine transporter in the tissues tested. The transplantation of mouse HSC had the same therapeutic effect than whole BMC in this model, which is important as such HSC can readily be isolated from peripheral blood in humans. This work suggests that BMC or HSC transplantation is a potential treatment for cystinosis and other renal tubular disorders.

A brief review of the field

Cystinosis is a rare lysosomal storage disease (LSD) caused by a defect in the transport of cystine across the lysosomal membrane (Figure 1), leading to the intracellular accumulation of cystine crystals in multiple organs including the kidney [2]. The disease is caused by autosomal recessive mutations in the CTNS gene that encodes cystinosin, the lysosomal cystine transporter [3,4]. The most severe and frequent form of cystinosis is characterized by a proximal tubule (PT) dysfunction that appears a few months after birth. In the absence of treatment, the tubulopathy rapidly progresses (within a few years) towards renal failure and end-stage renal disease [2]. Accumulation of cystine crystals in multiple other tissues is reflected by multi-organ dysfunctions including photophobia and visual impairment, myopathy, endocrine disorders, and central nervous system defects. If used early in the course of the disease, treatment with oral cysteamine (which depletes the cysteamine substrate) reduces the intracellular accumulation of cystine, slowing the rate of progression of tissue injury. However, administration of cysteamine has non-negligible side effects and administration constraints, and it does not improve the PT dysfunction which causes significant morbidity in affected children [5].

Allogeneic bone marrow cell (BMC) transplantation is already used to prevent disease progression of several LSDs caused by defective hydrolases, like Hurler disease [6]. Successive BMC transplantation in these LSDs is based on the secretion of the normal functional enzyme by tissue-engrafted cells, allowing recapTURE by deficient cells [7]. However, since cystinosin is a lysosomal transmembrane protein that cannot be secreted [3], the therapeutic efficacy of stem cell transplantation for cystinosis requires the local integration of donor cells with the functional protein which would then reverse the accumulation of cystine in the tissue.

The latter paradigm has been illustrated by the study of Syres et al. [1], based on the use of C57BL/6 Ctns−/− mouse model which presents a clear renal phenotype [8]. Following a single infusion of BMC derived from GFP-transgenic (WT) mice in irradiated Ctns−/− mice, the GFP-positive cells efficiently engrafted in the kidney, whereas the lethally irradiated wild-type mice exhibited few BMC-derived cells in the tissues, proving the need of chronic parenchyma injury for colonization by BMC-derived stem cells. Four months after transplantation, kidneys of Ctns−/− mice transplanted with GFP BMCs display abundant BMC-derived cells. These cells localized mostly as non-lymphoid lineage interstitial cells (which are known to accumulate abundant cystine crystals) but were also found to be associated with proximal, distal, glomerular and endothelial cells. The cell engraftment was paralleled by a 13% Ctns expression level compared to wild-type kidneys, a 70% decrease in cystine content in the kidney and improvement of renal function as assessed by normal serum urea and creatinine levels. In addition to the kidney, BMC-derived cells were detected in the eye, brain, spleen, heart, muscle and liver, with subsequent reduction of cystine content in all organs, demonstrating the potential utility of BMC transplantation for the multi-systemic complications of cystinosis. Furthermore, most of the BMC-derived cells are part of the intrinsic structure of the organ and co-localize with cells that accumulate cystine, like Kupffer cells in the liver, reticuloendothelial cells in the spleen or corneal cells in the eye [1]. The mechanism by which cystinosin-expressing cells replace or fuse with cells that accumulate cystine crystals in each tissue remains to be defined.

A strong point of the study of Syres et al. [1] is that the engraftment of BMC-derived cells in various tissue compartments was detected using three techniques: identification of GFP-positive cells, quantification of cystinosin mRNA by RT-PCR and determination of cystine level by mass spectrometry. This demonstration is of particular importance for the therapeutic potential of adult stem cells for kidney disease [9,10] and the debate about whether glomerular or tubular renal cells may derive from transplanted BMC [11–13]. The effect of the total body irradiation prior to BMC transplantation is also questioned, as this may have a disease-modulating effect in some models [14]. However, this is unlikely to be the case here, since the Ctns−/− mice treated with Ctns−/− BMCs do not present any improvement. Furthermore, Syres et al. [1] showed that haematopoietic stem cell (HSC) transplantation had the same therapeutic effects as BMC transplantation, with effective tissue integration and reduced cystine levels. These findings further support the use of cellular therapy in humans, as HSC can be readily isolated from peripheral blood, potentially manipulated ex vivo, and injected at higher concentration which may yield a lower long-term relapse rate. In contrast, mesenchymal stem cell (MSC), which did not efficiently integrate within the tissue, provided only a mild improvement of renal function, with transient decrease in cystine content.

Animal studies testing intervention protocols require establishment of faithful models of human diseases [15]. The first Ctns−/− mice, obtained on a mixed 129/Sv × C57BL/6 genetic background, showed an accumulation of cystine in the kidney and all organs tested but failed to recapitulate the generalized proximal tubule dysfunction that...
may cause severe episodes of fluid and electrolytes disturbances in children with cystinosis [16]. In contrast, Syres et al. used a recently developed Ctns knockout mouse on a pure C57BL/6 genetic background [1]. These congenic Ctns−/− mice show high renal cystine levels with pronounced histological lesions of the PT, biological manifestations of PT dysfunction such as low-molecular-weight (LMW) proteinuria and development of chronic renal failure within 6 months of age [8]. This model was, thus, particularly appropriate to test the potential effect of cell therapy on the kidney.

How could this affect my clinical work?

Cell therapy is a promising approach to target organ dysfunction, by supplying stem cells that differentiate in situ and become part of the intrinsic structure of the organ. The study of Syres et al. is important as it shows the potential of using BMC transplantation for treating a slowly progressive storage disease due to a defective membrane transporter. Their findings suggest that engraftment of bone marrow-derived cells, including purified HSC that are particularly relevant for future clinical applications, in multiple tissues could provide a continuous source of healthy cells with the functional transporter, resulting in a decreased accumulation of cystine. Of course, the road will be long before extrapolating these results obtained in mouse to humans, especially given the discrepancies between the mouse and human disease. It should be crucial to verify whether these results are maintained in the long term. Also, the mechanism by which this cell engraftment leads to improvement of proximal tubule dysfunction remains mysterious. However, beyond cystinosis, this study is relevant for the treatment of tubular disorders due to mutations of intracellular membrane transporters [17,18] and opens the way for stem cell gene therapy as recently proven successful for adrenoleucodystrophy [19].

Take-home message

This study provides the proof of concept for clinical trials based on BMC or HSC transplantation as a therapy for the progressive kidney and end-organ damage associated with cystinosis and potentially other lysosomal storage diseases due to mutations in specific transmembrane proteins.

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