Trends in
Molecular Medicine

CellPress

Review

Molecular Mechanisms and Treatment Options of Nephropathic Cystinosis

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Nephropathic cystinosis is a severe, monogenic systemic disorder that presents early in life and leads to progressive organ damage, particularly affecting the kidneys. It is caused by mutations in the *CTNS* gene, which encodes the lysosomal transporter cystinosin, resulting in intralysosomal accumulation of cystine. Recent studies demonstrated that the loss of cystinosin is associated with disrupted autophagy dynamics, accumulation of distorted mitochondria, and increased oxidative stress, leading to abnormal proliferation and dysfunction of kidney cells. We discuss these molecular mechanisms driving nephropathic cystinosis. Further, we consider how unravelling molecular mechanisms supports the identification and development of new strategies for cystinosis by the use of small molecules, biologicals, and genetic rescue of the disease *in vitro* and *in vivo*.

Highlights

Nephropathic cystinosis is a severe, monogenic systemic disorder caused by mutations in the lysosomal cystine/proton cotransporter cystinosin and the leading cause of inherited renal Fanconi syndrome.

Cysteamine efficiently depletes lysosomal cystine and improves clinical outcomes; however, it does not reverse established kidney failure.

A multifaceted impact of cystinosin loss of function is observed in cystinosis pathology, involving increased oxidative stress, apoptosis, and impaired autophagy and energy metabolism.

Several small molecules and biologics correcting non-transport functions of cystinosin are emerging and have been shown to be effective either alone or in combination with cysteamine.

Hematopoietic stem cell transplantation and translational read-through drugs pose promising new treatment options.

Cystinosis Results in Loss of Kidney Function Early in Life

Nephropathic cystinosis is a rare, monogenic autosomal-recessive disease (Box 1) belonging to the family of **lysosomal storage disorders** (LSDs; see Glossary). It is caused by mutations in the ***CTNS* gene** encoding cystinosin, a lysosomal proton/cystine cotransporter (Figure 1). Defective cystinosin is unable to export cystine out of the lysosome into the cytoplasm, leading to the formation of crystals. Given that cystinosin is expressed throughout the body, cystinosis is a systemic disease in which multiple organs are affected; however, the kidneys are most vulnerable [1]. Extrarenal manifestations of nephropathic cystinosis include the eyes, thyroid, pancreas, gonads, muscles, bones, and central nervous system.

There are three recognized clinical phenotypes of cystinosis: infantile nephropathic cystinosis, late-onset (juvenile) nephropathic cystinosis, and ocular (adult) cystinosis (see Clinician's Corner). Infantile cystinosis (OMIM 219800) is the most common form with the most severe phenotype (95% of cystinosis patients). Although cystine accumulation starts *in utero*, patients with infantile cystinosis usually are asymptomatic at birth and have normal development during the first 3–6 months of life [2]. However, these patients develop manifestations of **renal Fanconi syndrome** (RFS) and typically progress to **end-stage kidney disease** (ESKD) within the first 12 years of their life when left untreated. By contrast, patients with juvenile cystinosis (OMIM 219900) present with milder manifestations and with late onset as well as a lower rate of progression [3]. These patients are usually diagnosed in their childhood or during adolescence, but can also present as proteinuric chronic kidney disease (CKD) and may maintain renal function until the age of 30–40 [4]. Renal involvement in the non-infantile patient is largely heterogenous, even within the same family. The adult, non-nephropathic ocular form of cystinosis has no systemic involvement and is characterized only by photophobia due to cystine crystal deposition in the cornea.

The mainstay of cystinosis treatment is life-long treatment with cysteamine, a drug that effectively lowers cystine levels [5]. Cysteamine binds to lysosomal cystine and converts it into cysteine–

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Box 1. Epidemiology and Genetics of Cystinosis

Cystinosis has a general incidence rate of 0.5–1 per 100 000 live births [95,96]. Higher local incidence rates of cystinosis have been reported in Brittany (Northwestern France) [96] and Saguenay (Québec, Canada) [97] due to the prevalence of specific *CTNS* mutations in these populations [9]. Since cystinosis is a monogenic autosomal-recessive disease, patients normally have biallelic mutations in the *CTNS* gene (chromosome 17 p13.2), resulting in loss of functional cystinosin (also known as PQLC4) [81]. As a recessive disease, cystinosis incidence may be correlated with consanguinity. However, accurate epidemiologic data from countries with high levels of consanguinity (such as North Africa and the Middle East) are still lacking [98]. In addition, in other countries, underdiagnosis and improper reporting likely contribute to the low reported incidence of cystinosis [99].

Since the cloning and mapping of *CTNS*, more than 140 mutations have been described [1]. The most prevalent mutation leading to loss of *CTNS* within northern Europe involves a 57-kb deletion of the first nine exons, a part of the tenth exon and an entire gene upstream of *CTNS,* namely the sedoheptulose kinase gene (*SHPK*, also known as CARKL) [100]. Deletion of the promotor region and first ten exons completely abolishes gene function in these patients. More recently, this 57-kb deletion was found to extend to the adjacent transient receptor potential vanilloid 1 gene (*TRPV1*), leading to dysregulation of its transcription. However, the exact roles of *SHPK* and *TRPV1* in cystinosis pathology in patients with the 57-kb deletion is still unclear [100]. Remarkably, this mutation has not been observed in any studies from the Middle East or Africa. However, the c.681G>A splicing mutation, which involves the last base pair of exon nine in *CTNS*, is the most prevalent mutation in countries of the Middle East and is a possible founder mutation [101]. In addition, around 15% of cystinosis patients worldwide carry a nonsense mutation [102]. The most common of these nonsense mutations is W138X that causes a premature termination codon in the seventh exon of *CTNS* [77]. It is worth mentioning that the severity of cystinosis disease correlates with specific *CTNS* mutations [103]. Severe truncations or large deletions often lead to infantile cystinosis, while other mutations allowing residual function of the protein are associated with milder forms and/or late onset of the disease [104].

cysteamine disulfide and cysteamine, which are exported out of the lysosome via the lysine/arginine (PQLC2) and cysteine transporters, respectively. Early initiation and adequate adherence to cysteamine therapy is associated with increased life expectancy and fewer long-term complications in comparison with patients who had no, delayed, or inadequate treatment. Cysteamine slows down the progression of kidney deterioration, delays the onset of ESKD as well as the need for kidney transplantation, and improves growth retardation, neuromuscular, and endocrine manifestations associated with cystinosis. After transplantation, the donor kidney is not affected by the underlying disease, and cystinosis patients demonstrate excellent kidney graft survival [6].

While each year of good compliance with cysteamine is estimated to prolong kidney function survival with 1 year, some complications, such as visuomotor impairment and established proximal tubular dysfunction (i.e., RFS), are not restored [5]. This can be attributed to the fact that cysteamine mainly acts by reducing lysosomal levels of cystine and as an antioxidant [7], but does not replace cystinosin. Multiple cellular processes are affected by the lack of the transporter, as shown in detail in Figure 2, including **autophagy**, apoptosis, and energy metabolism, adding to the complexity of nephropathic cystinosis.

Multiple Cellular Processes Affected by the Lack of Cystinosin

The progressive accumulation of cystine in lysosomes leads to crystal formation when concentrations exceed the threshold of approximately 5 mM because of the acidic nature of the lysosome. The rate-limiting step in cystine transport is the number of cystinosin transporters in the lysosomal membrane [8], which may also be predictive of the severity of disease phenotype. Remarkably, crystal formation is not observed in lysosomes of *in vitro*-cultured cystinotic cells, indicating that the process of cystine crystallization is slow [9]. In addition, cystine crystals are absent or rarely found in renal proximal tubule cells (PTCs) of children with cystinosis in whom crystals are predominantly deposited in interstitial macrophages [10]. This implies that cystine crystals are not the main factor in RFS initiation and suggests other important functions of cystinosin beyond cystine transport [9,11].

Glossary

Autophagy: the natural and highly conserved mechanism by which dysfunctional organelles and endogenous long-lived cytoplasmic proteins are catabolized via the lysosomal pathway.
Cystinosis gene (*CTNS*): encodes the lysosomal transporter cystinosin, which is responsible for the lysosomal export of cystine. Two main isoforms with different targeting motifs exist (viz., canonical cystinosin, trafficked to late endosomes and lysosomes, and cystinosin-LKG an alternative splice variant that is less abundant but also resides in the plasma membranes and the Golgi apparatus).
End-stage kidney disease (ESKD): the most advanced stage of chronic kidney disease (CKD) in which patients are dependent on kidney replacement therapies.
Inflammasomes: a group of cellular protein complexes that recognize a diverse set of inflammation-inducing factors and closely control the maturation and production of proinflammatory cytokines.
Lysosomal storage disorders (LSDs): inherited metabolic diseases that are characterized by an abnormal accumulation of metabolites in cells throughout the body as a result of enzyme or transporter deficiencies.
Mechanistic (mammalian) target of rapamycin (mTOR): an evolutionarily conserved serine–threonine kinase that acts as cellular nutrition and energy status sensor. The two mTOR complexes mTORC1 and mTORC2 can be activated by diverse growth factors, mitogens, and nutrients to regulate cellular functions, such as cell growth, proliferation, development, memory, longevity, angiogenesis, autophagy, and innate as well as adaptive immune responses.
Mitophagy: the selective degradation of mitochondria by autophagy, often in response to defective mitochondria following cellular damage or stress.
Renal Fanconi syndrome (RFS): a rare disorder of kidney tubule function that results in excess amounts of glucose, bicarbonate, phosphates (phosphorus salts), uric acid, potassium, and certain amino acids being excreted in the urine.
Swan-neck deformity: characteristic of RFS by progressive proximal tubule cell flattening, eventually leading to kidney failure.

Reduced Cellular Energy Metabolism

Reduced levels of intracellular ATP in cystinotic cells have been widely reported, possibly because of reduced apical reabsorption of inorganic phosphate without affecting sodium–potassium ATP pump (Na^+/K^+-ATPase) activity [7,12–14]. These reports demonstrated intact respiratory chain complex I to V activity and overall energy-secreting capacity. Conversely, another study described fewer mitochondria, abnormal mitochondrial function, abnormal mitochondrial morphology, and a decrease in ATP in cystinotic fibroblasts and PTCs [15]. In addition, significantly lower cAMP levels, complex I and IV activity, and mitochondrial potential have been reported in cystinotic conditionally immortalized proximal tubule epithelial cells (ciPTECs) with a 57-kb deletion as well as in ciPTECs with heterozygous loss of function (mutCtns) [16]. It is possible that activation of AMP-activated protein kinase (AMPK) renders cystinotic cells more susceptible to apoptosis [17]. AMPK is a major regulator of energy homeostasis and highly sensitive to intracellular energy stores. Reduced levels of ATP and activation of AMPK have been reported in a cystinosis knockdown model of rabbit PTCs. This activation of AMPK is associated with increased sensitivity of cystinotic cells to cisplatin, indicating that AMPK might play a role in regulating apoptosis in these cells [17].

Translational read-through-inducing drugs (TRIDs): compounds able to bind to the mammalian ribosome and inhibit translation termination at the premature stop codon by promoting insertion of near-cognate aminoacyl-transfer RNAs, thereby restoring the full-length mRNA and protein.

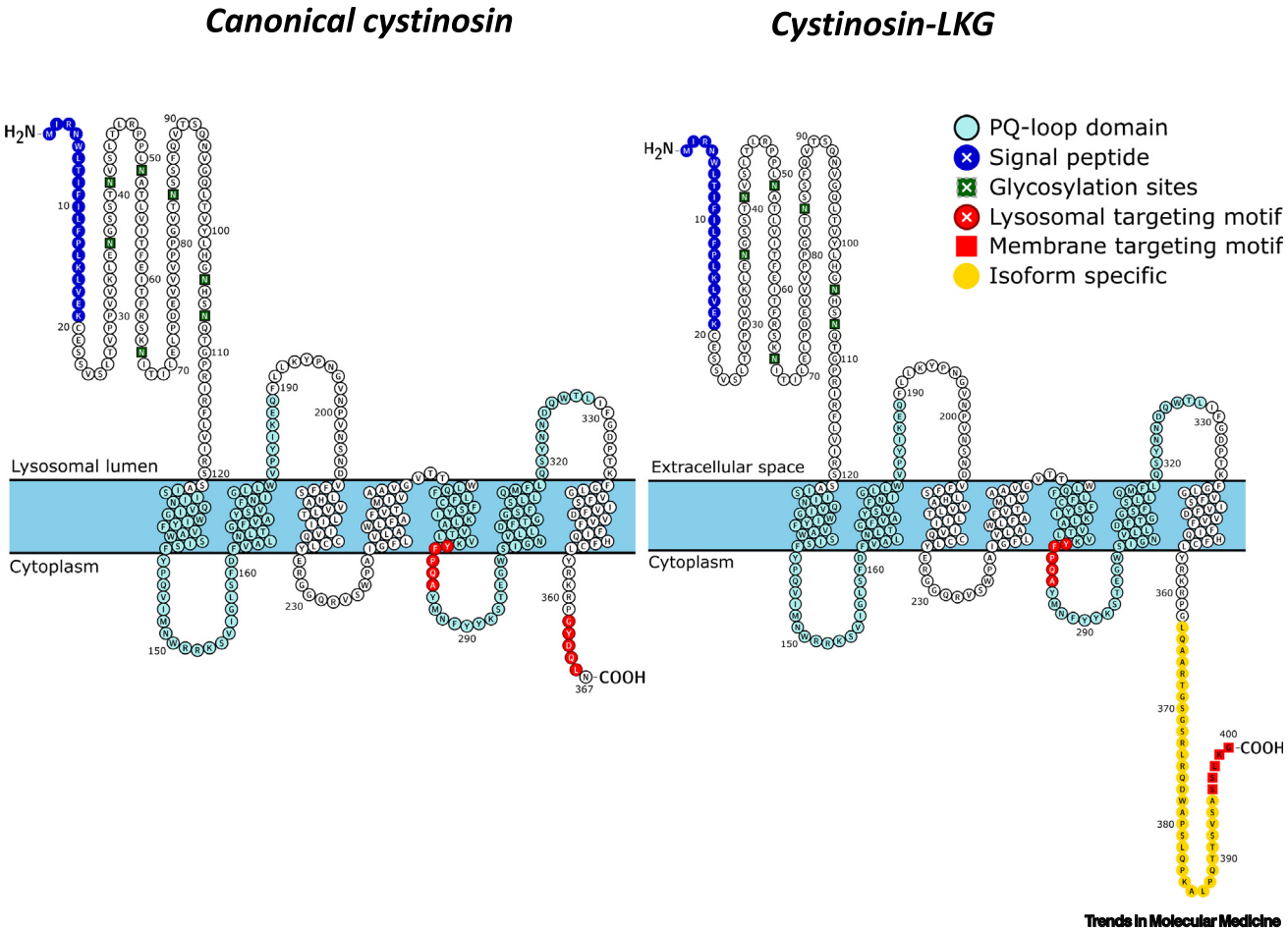

Figure 1. Schematic Representation of the Two Cystinosin Isoforms. Cystinosin has two protein-coding isoforms, the canonical isoform (left) and cystinosin-LKG (right). The latter is produced by alternative splicing of exon 12. Both isoforms have the lysosomal-targeting motif YFPQA; however, instead of the additional lysosomal-targeting motif GYDQL in the canonical isoform, cystinosin-LKG is composed of a plasma membrane-targeting peptide (SSLKG). This results in a different cellular distribution of the two isoforms, where the canonical isoform is confined to the lysosome, while cystinosin-LKG is also present at the plasma membrane as well as the Golgi apparatus. Information presented is based on Uniprot CTNS_HUMAN (O60931-1 and O60931-2). More splice variants have been predicted, but for these molecular and functional data they are not available. PROTTER, an open-source tool, was used to visualize the two isoforms of cystinosin [107].

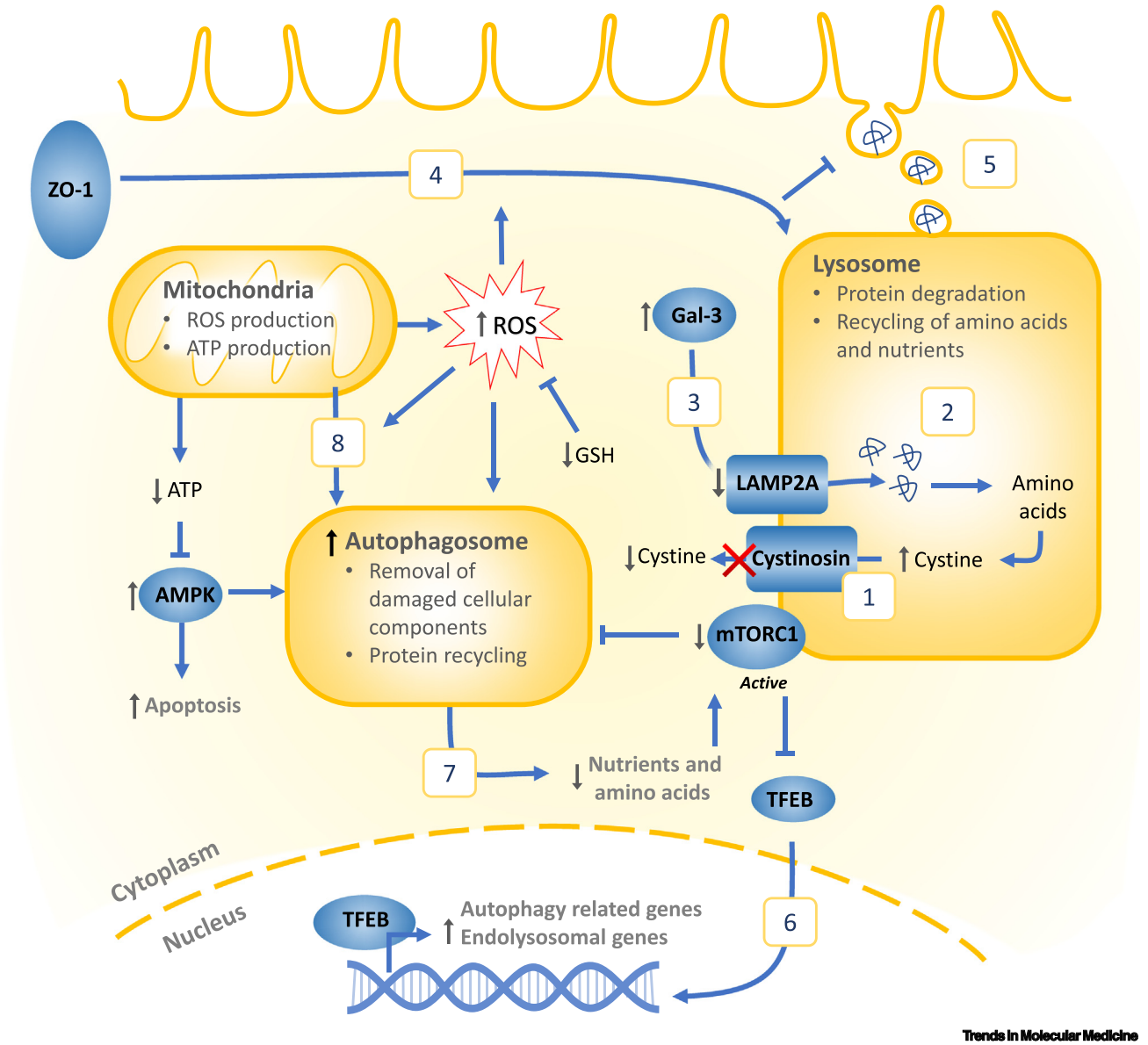

Figure 2. Molecular Mechanisms Underlying Nephropathic Cystinosis. (1) Dysfunctional cystinosin leads to the accumulation of cystine within the lumen of the lysosome. (2) Reduced protein degradation due to defective lysosomal enzyme activation. (3) Reduced chaperone-mediated autophagy leads to galectin-3 overexpression and has been associated with chronic kidney disease progression. (4) Phosphorylation of tight junction adapter protein ZO-1 results in its misrouting to endolysosomal compartments and disruption of tight junction integrity. (5) Disruption of tight junctions leads to epithelial dysfunction and dedifferentiation, repressing apical endocytic receptors and megalin/cubilin-mediated endocytosis. (6) mTORC1 inhibition results in increased TFEB nuclear translocation, which activates transcription of autophagy related genes. (7) Reduced autophagic flux and degradation. (8) Abnormal mitophagy can lead to increased oxidative stress, further promoting epithelial dysfunction, dedifferentiation, and apoptosis. Abbreviations: AMPK, 5' adenosine monophosphate-activated protein kinase; ATP, adenosine triphosphate; GSH, glutathione; Gal-3, galectin-3; LAMP2A, lysosome-associated membrane protein 2A; mTORC1, mammalian target of rapamycin complex 1; ROS, reactive oxygen species; TFEB, transcription factor EB; ZO-1, zonula occludens 1.

Oxidative Stress Leading to Mitophagy

The roles of oxidative stress, reactive oxygen species (ROS), and altered glutathione (GSH) metabolism in cystinosis have also gained attention. GSH is a tripeptide of glutamate, cysteine, and glycine and is synthesized in a process involving two enzymatic reactions, each requiring one ATP molecule [18]. Studies suggest that cystinotic cells are more vulnerable to oxidative

stress as a result of impaired GSH synthesis and a compromised gamma glutamyl cycle, possibly due to altered mitochondrial function and depleted ATP levels [7,19–22]. Reduced levels of GSH result in the inability of the cell to scavenge ROS, which puts the cells under oxidative stress, particularly in the mitochondria where most cellular ROS are produced. This might also explain the increase in **mitophagy**. In another study, cell lines established from urinary-shed PTCs of cystinotic patients exhibited elevated levels of cellular oxidative stress compared with control cell lines, which was observed as increased oxidized GSH, while total GSH remained unchanged [23]. In the same study, it was shown that cysteamine treatment normalized GSH redox status and increased GSH in urinary ciPTECs. Further, in mouse kidneys and primary mouse PTCs, it was demonstrated that oxidative stress resulting from abnormal mitophagy stimulates Gα12/Src-mediated phosphorylation of tight junction protein zonula occludens-1 (ZO-1) and its subsequent misrouting to the endolysosome, leading to disrupted tight junction integrity [24]. As a result, ZO-1-associated Y-box factor ZONAB is released, leading to further epithelial dysfunction and dedifferentiation by promoting abnormal cell proliferation and repressing apical endocytic receptors, such as megalin and cubilin [10,25,26]. The loss of endocytic function and PTC differentiation in *Ctns*$^{-/-}$ mice might temporally precede apoptosis and the development of **swan-neck deformity** [10]. However, blocking the uptake of disulfide-rich plasma proteins through inhibition of megalin-mediated endocytosis efficiently prevented the accumulation of cystine and delayed progression of kidney disease in *Ctns*$^{-/-}$ mice [27].

Increased Apoptosis

Several studies reported an increased rate of apoptosis in cystinotic human cells as well as in CTNS-mutant zebrafish larvae [24,28,29]. The rates of apoptosis also increased in response to proapoptotic stimuli, such as UV light and tumor necrosis factor-α, in cystinotic fibroblasts and PTCs [30]. In the kidneys of patients with cystinosis, this increased rate of apoptosis is particularly evident as swan-neck lesions, whereby apoptotic cell death and shedding of tubule cells leads to tubular atrophy [31,32]. Cysteamine was able to reduce apoptosis in cystinotic cells and zebrafish larvae, which may be linked to its effects on the redox status in cystinosis [33]. Administration of mitochondrial-targeted antioxidants, such as mitoquinone, was shown to delay swan-neck lesions in *Ctns*$^{-/-}$ mice, further signifying a link between apoptosis and oxidative stress [32].

The beneficial effect of cysteamine on apoptosis could also be explained by a reduction in lysosomal cystine load [30]. During early apoptosis, lysosomes are permeabilized, and it is suggested that the excess lysosomal cystine released during this phase leads to increased activation of proapoptotic proteins, such as protein kinase C-delta type (PKC-δ), by cysteinylation and contributes to increased apoptosis observed in cystinotic cells [30,34]. However, a recent report using iPSC-derived kidney organoids could not find a reduction in apoptosis after cysteamine treatment, but instead found a beneficial effect of the **mechanistic (mammalian) target of rapamycin** (mTOR) inhibitor everolimus [35]. The cellular processes contributing to apoptosis are likely dependent on the cell type studied and on methodologies used, explaining the reported differences.

Abnormal Autophagy and Defective mTOR Signaling

By degrading and recycling damaged organelles and proteins, autophagy provides new building blocks that are required for the renewal of cellular components and thereby is a crucial process for maintaining homeostasis [36]. Defective cystinosin induces a major alteration in lysosomal autophagy dynamics, coupled with the impaired activity of mTOR signaling and delayed lysosomal cargo degradation [26,37–40].

The mTORC1 and mTORC2 complexes are ubiquitously expressed in the kidneys and play a central role in homeostasis, metabolism, and proliferation as well as in kidney diseases [41,42].

mTORC1 is a nutrient sensor that can be activated by amino acids and nutrients [43]. The pentameric Ragulator complex and a group of small GTPases known as Ras-related GTP-binding proteins (Rag) are fundamental for mTORC1 activation as they coordinate recruiting mTORC1 to the lysosomal membrane. The lysosomal vacuolar ATPase (v-ATPase) proton pump is also necessary for amino acid activation of mTORC1 by interacting with the Rag–Ragulator complex [44]. In the presence of amino acids, these regulatory complexes activate mTORC1, triggering protein synthesis and inhibiting autophagy. However, amino acid starvation inactivates mTORC1, thereby activating autophagy to supply new building blocks, nutrients, and energy [45]. Recently, the v-ATPase, Rag-GTPase, and Ragulator complex have been demonstrated to interact with cystinosin, suggesting that the absence of cystinosin itself may affect mTOR recruitment and activation, which was not rescued by cysteamine [37]. Although baseline activity of mTOR (as seen by S6K1P) does not seem to be altered in cystinotic cells [46], several studies report a change in mTOR reactivation and subcellular localization after starvation [37,38].

Transcription factor EB (TFEB) is a major transcription factor regulating lysosomal biogenesis and autophagy, which physically interacts with mTOR. Lysosomal-dissipated mTOR fails to phosphorylate TFEB, leading to TFEB nuclear translocation and the induction of lysosomal and autophagic genes [47]. Although increased TFEB nuclear translocation has been found in cystinotic cells, levels of endogenous TFEB were found to be reduced [48]. TFEB overexpression or activation of endogenous TFEB using genistein was able to reduce lysosomal cystine levels and facilitated autophagic clearance of abnormal aggregates. This suggests that TFEB activation promotes lysosomal exocytosis, which is not seen with cysteamine treatment alone [48]. Furthermore, mTOR pathway inhibition using everolimus reduced the large lysosomes and activated autophagic flux and, in combination with cysteamine, normalized the cystine load [35]. Accumulation of the autophagy substrate SQSTM1 and decreased lysosomal cargo processing postulate the lack of autophagy completion in cystinosis, in agreement with other LSDs [49,50]. While a block in degradation could explain the increased levels of autophagic markers under basal conditions, multiple reports also indicate that the autophagic flux (as seen in conditions with bafilomycin) was in fact increased in cystinotic cells [38,46].

Contrary to macroautophagy, where the autophagosome nonselectively sequesters bulk cytoplasmic organelles and components, the process of chaperone-mediated autophagy (CMA) is a highly selective process through which single proteins are tagged by a specific pentapeptide recognition motif to be degraded. These tagged proteins then bind a cytoplasmic chaperone complex, which delivers them to lysosomal receptors to be translocated into the lysosome allowing their degradation [51]. To date, lysosome-associated membrane protein 2A (LAMP2A) is the only known lysosomal receptor for CMA function. Interestingly, it has been reported that neonatal cystinotic mouse fibroblasts demonstrated abnormal CMA and decreased LAMP2A expression and LAMP2A mislocalization while maintaining normal macroautophagic flux as well as normal mTOR activity by starvation [46]. Decreased LAMP2A expression was corrected by cystinosin expression but not by cysteamine treatment. It is worth noting that CMA is upregulated during oxidative stress to facilitate the efficient removal of irreversibly modified molecules [52]. Indeed, LAMP2A trafficking and distribution were found to be tightly regulated by cystinosin, Rab-11, and Rab-interacting lysosomal protein (a downstream effector of Rab7), which were found downregulated and defective in cystinotic cells [53]. Upregulation of Rab27a-dependent lysosomal trafficking resulted in reduced cystine accumulation as well as endoplasmic reticulum stress [54]. Taken together, impaired lysosomal autophagy dynamics as a result of cystinosin loss of function are emerging mechanisms contributing to cystinosis pathology.

Inflammasome Activation

Inflammation is generally associated with tissue infection or tissue injury modulated by cytokines. The well-characterized **inflammasome** NLRP3 is activated by diverse stimuli, including endogenous crystals, mitochondrial dysfunction, and the production of ROS. Lysosomal destabilization and potassium efflux have been shown to trigger NLRP3 activation [55]. Accumulation of cystine in human peripheral blood mononuclear cells and in *Ctns*$^{-/-}$ mice can indeed activate inflammasomes and, eventually, trigger inflammation and tissue fibrosis [56]. However, increased activity of plasma chitotriosidase, an enzyme produced by activated macrophages, is a sign of noninflammasome-related inflammation in nephropathic cystinosis [57]. Furthermore, studies have demonstrated a new role for cystinosin in inflammation through its interaction with the lectin and β-galactoside-binding protein family 21 galectin-3 (Gal-3) upregulation, enhancing macrophage infiltration and CKD progression [58]. Accordingly, Gal-3 was found to be markedly upregulated in acute tubular injury [59] and in progressive kidney fibrosis [60]. Gal-3 was also shown to be involved in quality control of endolysosomal organelles and kidney function through the coordination of lysosomal repair and removal by activating autophagy and lysosomal biogenesis during cellular damage [61]. Hence, identification of the specific stimuli that trigger inflammatory responses can facilitate the discovery of potential drug targets in cystinosis.

Overall, our understanding of cystinosis pathology has greatly extended beyond cystine accumulation in the past decades. Recent studies based on *in vitro* and *in vivo* cystinosis models demonstrated that the loss of cystinosin is associated with disrupted autophagy dynamics, accumulation of distorted mitochondria, and increased oxidative stress, leading to abnormal proliferation and dysfunction of kidney cells. Still, the mechanism linking cystinosin loss and epithelial dysfunction remains largely unknown.

New Emerging Therapies

Advances in cysteamine medicine formulations have already significantly improved patients' quality of life, although no curative therapy is yet available for cystinosis. Furthermore, progress has been made in identifying new compounds that are aimed at tackling features of the disease for which cysteamine therapy is not sufficient. The following paragraphs and Table 1 provide an overview of the recently finished and ongoing clinical trials for current and emerging treatments.

New Formulations of Cysteamine

Cystagon, an immediate-release formulation of cysteamine and the first available treatment for cystinosis, was approved by the FDA in 1994. Although Cystagon effectively lowers cystine levels, it is associated with significant gastrointestinal symptoms (due to gastrin release and acid hypersecretion, which is alleviated by treatment with concomitant proton pump inhibitors [62]) and unpleasant sulfuric body and breath odor [63]. These side effects together with a strict dosing schedule (every 6 hours) substantially influence patients' adherence to treatment and can contribute to compromised clinical outcomes [63]. Procysbi, a delayed-release formulation of cysteamine bitartrate, bypasses the stomach and has sustained absorption in the small intestine, resulting in reduced gastrointestinal side effects [62,64]. In addition, it requires only a twice-daily dosing and thereby significantly improves the patient's quality of life by relieving the burdens of interrupted sleep for nighttime dosing [64,65].

However, Procysbi still releases cysteamine in the gastrointestinal tract; hence, it is prone to first-pass metabolism and causes gastric disturbances [66]. Furthermore, the cysteamine metabolites dimethylsulfide and methanethiol result in halitosis and poor sweat odors. These adverse events might be overcome using prodrugs of cysteamine that increase metabolic stability, achieve a sustained cysteamine concentration, and guarantee on-target release [67]. Esterified γ-glutamyl-

Table 1. Summary of the Clinical Trial Registry of Current Treatments in Cystinosis[a,b]

NCT ID	Intervention	Age group	Start date	Completion date	Phase	Status	Study aim	PR
Systemic cysteamine formulations								
01432561	Cystagon	Adult, older adult	September 2011	December 2011	NA	Completed	Food effect on bioavailability of the drug	Yes [105]
02012114	Cystagon Procysbi	Child, adult, older adult	September 2011	NA	NA	Unknown status	Compliance to cysteamine and neurological complications	NA
00872729	Cystagon Procysbi	Child, adult, older adult	May 2009	October 2009	Phase I	Completed	Safety, tolerability, pharmacokinetics, pharmacodynamics	Yes[a]
01000961	Cystagon Procysbi	Child, adult, older adult	June 2010	August 2011	Phase III	Completed	Pharmacokinetics, pharmacodynamics	Yes [66]
01733316	Cystagon Procysbi	Child, adult, older adult	January 2013	July 2017	Phase III	Completed	Safety and superior effectiveness	Yes[a]
01197378	Procysbi	Child, adult, older adult	August 2010	June 2017	Phase III	Completed	Long-term safety follow-up	Yes [64]
01744782	Procysbi	Child	December 2012	December 2016	Phase III	Completed	Safety, effectiveness in cysteamine treatment-naive cystinosis patients	Yes[a]
04246060	Procysbi	Child, adult, older adult	July 2020	NA	NA	Recruiting	Observational study to assess the quality of life	NA
03919981	Cysteamine	Child, adult, older adult	April 2019	NA	NA[c]	Recruiting	Evaluate the action of cysteamine on osteoclastic differentiation and resorption activity of nephropathic cystinosis patients	NA
Topical cysteamine formulations (eye drops)								
00001736	Cysteamine	Child, adult, older adult	May 1998	March 2001	Phase I	Completed	Safety, efficacy by corneal crystals score	No
00001213	Cysteamine	Child, adult, older adult	April 1986	July 2013	Phase II	Completed	Safety, efficacy by corneal crystals score	Yes[a]
00010426	Cystaran	Child, adult	December 1999	February 2001	NA	Completed	Safety, efficacy by corneal crystals score	No
02766855	Cysteamine	Child, adult	January 2004	March 2016	NA[c]	Completed	Efficacy: improvement of photophobia, corneal cystine crystals, and visual acuity	Yes [106]
04125927	Cystadrops	Child	September 2020	NA	Phase III	Recruiting	Safety, efficacy: corneal cystine crystal score, and best corrected visual acuity	NA
N-acetyl cysteine								
01614431	*N*-acetyl cysteine	Child, adult	March 2011	January 2012	Phase IV	Completed	Verify the interference of *N*-acetyl cysteine in the progression of CKD in cystinosis patients	No
Translational read-through drugs								
04069260	ELX-02	Child, adult, older adult	August 2019	December 2019	Phase II	Terminated	Safety, pharmacokinetics, and efficacy	NA
Stem cell gene therapy								
03897361	CTNS-RD-04	Adult, older adult	July 2019	NA	Phase I, Phase II	Recruiting	Safety, tolerability, and efficacy	NA

[a]Data are extracted from the clinical trial registry database of the NIH, United states (https://www.clinicaltrials.gov/).
[b]Abbreviations: CKD, chronic kidney disease; NA, not applicable; NCT ID, National Institute of Health (NIH) clinical trial identifier; PR, published results.
[c]Observational, age (in years): child (birth to 17 years), adult (18 to 64 years), and older adult (65+ years).

cysteamine prodrugs maintain the concentration of cysteamine at above baseline levels for at least 24 hours, indicating the potential for less frequent administration [68]. In addition, folate, glutaric acid, succinic acid, and pegylate derivates of cystamine, the disulfide derivative of cysteamine,

were shown to deplete cystine levels *in vitro* with higher efficiency than cysteamine and with no significant toxicity [69–71]. The potential use of these analogs needs further investigation.

Small Molecules and Biologics for Novel Molecular Targets

As cysteamine treatment cannot correct the phenotype, including autophagy, apoptosis, and energy metabolism that further add to the complexity of RFS, novel potential treatments are being developed for nephropathic cystinosis (summarized in Figure 3). For instance, small molecule CMA activators correct LAMP2A localization and are associated with enhanced cell survival in cystinotic mouse embryo fibroblasts, suggesting their potential use in combination with cysteamine [46,53]. AMPK inhibitors also are potential drug candidates to counteract the

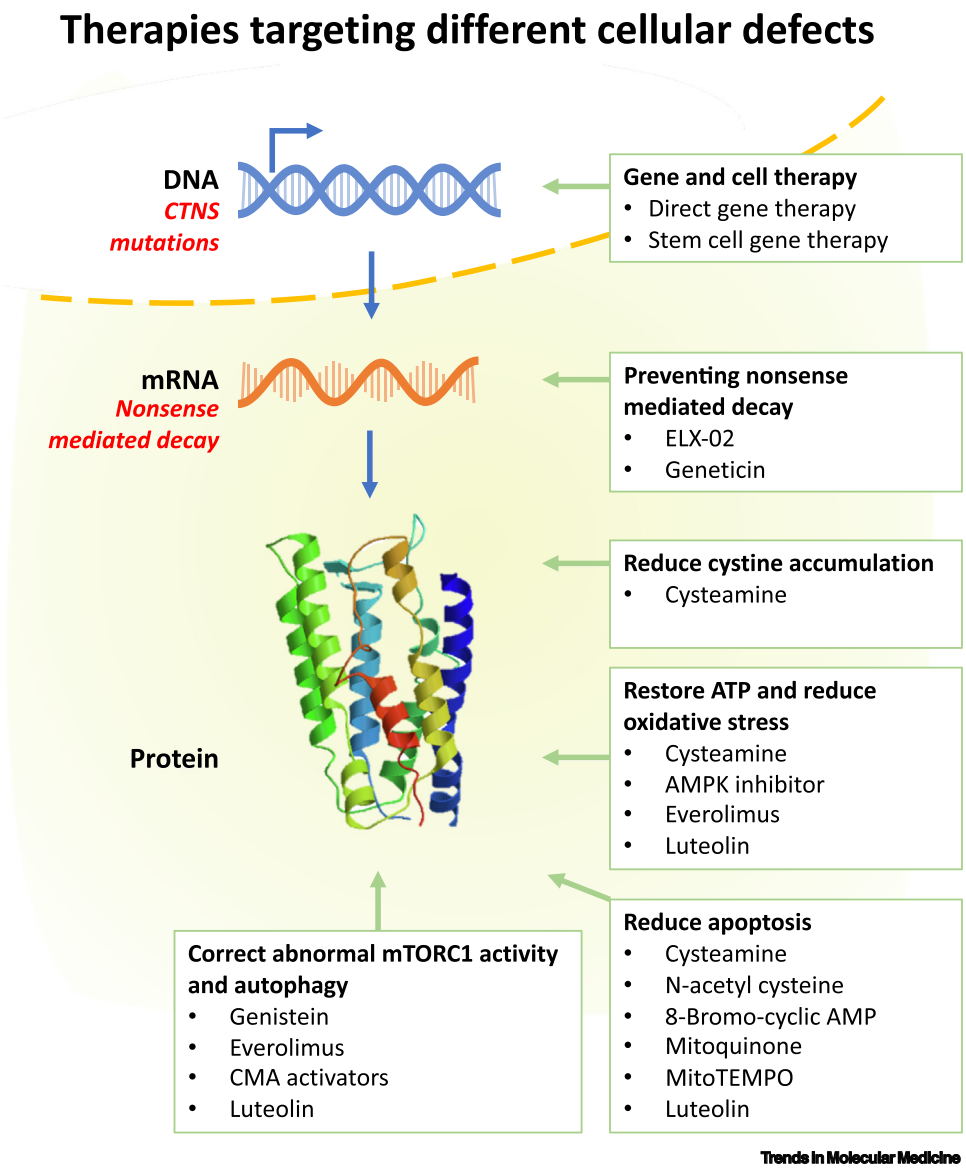

Figure 3. Summary of New Emerging Therapies in Nephropathic Cystinosis. Abbreviations: AMPK, adenosine monophosphate-activated protein kinase; CMA, chaperone-mediated autophagy.

elevated levels of apoptosis observed in cystinotic cells [17], and genistein favorably lowers cystine levels and activates TFEB [72]. Furthermore, luteolin, a natural flavonoid, was found to rescue increased sensitivity to oxidative stress and the impaired lysosomal dynamics in both human and murine cystinotic kidney cells [73]. Luteolin also improved reabsorption of endocytic cargoes.

MitoTEMPO, a mitochondrial-targeted antioxidant, restored integrity, differentiation, and transport function in cystinotic PTCs as well as in *Ctns*$^{-/-}$ mice [24]. Furthermore, the mitochondrial-targeted antioxidant mitoquinone was shown to delay the initiation of swan-neck lesions in *Ctns*$^{-/-}$ mice [32]. Yet, additional safety tests are warranted as mitoquinone was shown to result in mitochondrial swelling and depolarization in opossum kidney cells [74].

Additionally, everolimus (an mTOR inhibitor) was shown to result in a reduction in apoptosis, a reduction in large lysosomes, and activation of autophagy while not affecting cystine load. However, these effects were not observed during cysteamine-only treatment, suggesting a dual therapy of cysteamine as a cystine-lowering agent and everolimus to rescue other cystinosis-related phenotypic abnormalities [35].

Recently, **translational read-through-inducing drugs** (TRIDs) have emerged as novel therapeutics for genetic diseases [75]. Since faulty mRNA with premature stop codons are unstable and liable for nonsense-mediated mRNA decay (NMD), this eventually results in overall little or no mRNA available for TRIDs to perform their read-through capabilities. Therefore, NMD inhibition has been suggested as an adjunctive therapy to TRIDs to improve treatment efficiency and serve as a plausible approach to overcome nonsense mutations in a considerable subpopulation of cystinosis patients [76]. While TRIDS originally aimed at translational read-through, some have been shown to be dual acting and also inhibit NMD decay, warranting their single-therapy use [77–79]. Indeed, geneticin, an aminoglycoside-based TRID, maintains normal *CTNS* mRNA levels, can restore full-length functional cystinosin, and reduces cystine accumulation in patient fibroblasts carrying a W138X mutation [77]. More recently, an aminoglycoside-based TRID, ELX-02 (developed by Eloxx Pharmaceuticals, Israel), was demonstrated to reduce renal cystine accumulation in a CtnsY226X nonsense mutant mouse without overt toxicity [80]. Based on these *in vivo* results, Eloxx Pharmaceuticals launched a Phase II clinical trial for ELX-02 (NCT04069260; Table 1) in patients with cystinosis with nonsense mutations in at least one allele.

Genetic Rescue

Since cystinosis is a multisystem disease, gene transfer with a functional *CTNS* gene has the potential to fully reverse the cystinotic phenotype. Hematopoietic stem cell (HSC) transplantation is mainly applied to treat hematological disorders; however, due to their ability to home to damaged tissues and exert favorable paracrine effects on neighboring cells, HSC transplantation can be used for multisystem non-hematological disorders [81]. To investigate whether stem cell transplantation can sustain functional cystinosin expression and rescue the disease phenotype, syngeneic bone marrow cell (BMC), HSC, and mesenchymal stem cell (MSC) transplantation from wild-type donors in *Ctns*$^{-/-}$ mice was performed [82]. A large quantity (ranging from 5% to 19% of the BMC population) of wild-type BMCs was detected in all organs tested and were mostly phagocytic in nature. In the kidneys, most of these cells were interstitial with no observed transdifferentiation into kidney epithelial cells [83]. Overall, a reduction in organ-specific cystine levels of 57% to 94% was observed in BMC-treated mice, accompanied by prevention of renal dysfunction and corneal cystine deposition. Long-term effects of BMC transplantation were evaluated in *Ctns*$^{-/-}$ mice at 7–15 months after transplantation. The effectiveness of BMC transplantation on kidney dysfunction was found to be dependent on the level of engraftment of cells

Clinician's Corner

Cystinosis is the leading cause of inherited renal Fanconi syndrome (RFS) in young children, accounting for almost 20% of the cases of hereditary tubular disorders. Patients with nephropathic cystinosis develop symptoms of RFS and typically progress to end-stage kidney disease (ESKD) within the first 12 years of life.

There are three recognized clinical phenotypes of cystinosis: infantile nephropathic cystinosis, late-onset (juvenile) nephropathic cystinosis, and ocular (adult) cystinosis. Infantile cystinosis (OMIM 219800) is the most common form with the most severe phenotype (95% of cystinosis patients). Although cystine accumulation starts *in utero*, patients with infantile cystinosis usually are asymptomatic at birth and have normal development during the first 3–6 months of life. However, these patients develop the manifestations of RFS and typically progress to ESKD within the first 12 years of their life when left untreated. Patients with juvenile cystinosis (OMIM 219900) present with milder manifestations and with late onset as well as a lower rate of progression. These patients are usually diagnosed in their childhood or during adolescence but can develop proteinuria and chronic kidney disease (CKD) and may maintain kidney function until the age of 30–40. The renal involvement in the non-infantile patient is largely heterogenous, even within the same family. Adult ocular cystinosis has no systemic involvement and manifests as isolated symptoms of photophobia resulting from cystine crystal deposition in the cornea.

In line with the systemic expression of cystinosin, there are many extrarenal manifestations of nephropathic cystinosis that affect the eyes, thyroid, pancreas, gonads, muscles, bones, and central nervous system. The effects on bone are severe and affect patients at different disease stages, with more than a tenfold increased risk of short stature, bone deformities, and requirement of skeletal surgery compared with other CKD patients.

Up until now, there is no curative treatment for cystinosis, and the available drug, cysteamine, aims at preventing and/or delaying

renal and extrarenal manifestations and increasing the patient's life expectancy.

expressing functional *CTNS*. Treated mice with more than 50% donor-derived engraftment had significantly improved kidney function [84]. The authors showed that most BMCs are dendritic cells, suggesting a role in kidney repair by inhibiting the inflammatory response. However, MSCs did not integrate efficiently in any of the organs tested, and only minor initial improvements were observed in MSC-treated mice followed by an increase in cystine accumulation. Initial improvement could be attributed to the protective paracrine effects of MSCs by the secretion of several growth factors rather than cellular transdifferentiation and integration [82,85].

It was argued that the observed improvements due to stem cell transplantation could not be attributed to integration and differentiation of the transplanted cells as the number of engrafted cells did not exceed 15% in most tissues. Instead, it was shown that MSCs or BMCs were found to shed microvesicles containing *CTNS* mRNA as well as wild-type functional cystinosin that could be shuttled to the lysosomal compartment of cystinotic fibroblasts [86]. This hypothesis was confirmed in an *in vivo* mouse study where a self-inactivating lentiviral vector (SIN-LV) was used to modify HSCs to express a functional *CTNS* that could be transferred to *CTNS*-deficient cells [87]. A series of *in vivo* and *in vitro* experiments concluded that direct cell–cell contact is the main pathway for cross-correction of *CTNS* expression. HSCs could differentiate into tissue-resident macrophages that extend tunneling nanotubes, which mediate cross-correction by transferring cystinosin-bearing lysosomes to *CTNS*-deficient cells [88]. This mechanism was confirmed by demonstrating that a single systemic transplantation of wild-type HSCs prevented ocular pathology in $Ctns^{-/-}$ mice, with effects up to 1 year after transplantation [89]. Another potential benefit of genetic rescue by HSC transplantation was observed by engraftment normalizing thyroid function in $Ctns^{-/-}$ mice [90]. These results highlight the potential multisystem benefits of HSC transplantation, improving renal, ocular, and endocrine manifestations of cystinosis [81].

A direct *in vivo* gene therapy strategy was evaluated using an adenoviral-based vector to transduce liver cells of $Ctns^{-/-}$ mice [91]. The advantage of using an *in vivo* approach is that, in principle, various organs and tissues can be directly targeted to restore their function. However, *in vivo* gene therapy also comes with its own limitations that will have to be addressed, including transduction efficiency, tissue specificity of the viral vector, immune response against the vector, or destruction of transduced cells.

Given the promising preclinical results, one study endeavored to clinically validate allogeneic HSC transplantation in a 16-year-old cystinosis patient with infantile cystinosis who was not able to tolerate cysteamine [92]. These allogenic HSCs were collected from a fully human leukocyte antigen (HLA)-matched unrelated donor using mobilized peripheral blood stem cells. After transplantation, the patient showed reduced tissue cystine crystals and signs of clinical improvement of renal and ocular cystinosis, with biopsy-proven successful transfer of wild-type cystinosin protein and RNA to multiple organs. Nevertheless, the patient developed early signs of immunosuppressive toxicity and, following the second HSC donation, suffered from severe graft-versus-host disease (GVHD) and eventually died from severe systemic infection. This underscores the associated risks and mortality of allogenic HSC transplantation that must be considered carefully and weighed against the treatment benefits. An alternative strategy is therefore to genetically modify patient-derived HSCs *ex vivo* followed by autologous HSC transplantation, with potentially less risk of GVHD. To test the feasibility, efficacy, and safety of this approach, a Phase I/II clinical trial (NCT03897361; Table 1) was launched in 2019. Selected patients will undergo HSC mobilization and collection, after which a portion of these HSCs will be gene modified *ex vivo* with a lentiviral vector, pCCL-CTNS, to express the *CTNS* gene. The clinical outcomes are eagerly awaited and may pave the way for future treatment options of cystinosis.

Concluding Remarks

Although advances in medicine formulations have already significantly improved patients' quality of life, no curative therapy is yet available for cystinosis. Over the past decades, our understanding of cystinosis pathology has extended beyond cystine accumulation to include increased oxidative stress, apoptosis, inflammation, and abnormal autophagy. Furthermore, progress has been made in identifying new compounds that are aimed at tackling features of the disease for which cysteamine therapy is not sufficient. The hope is that one or more of these compounds will provide a safe and cost-effective therapy for patients with cystinosis around the world and help them live a longer and healthier life.

The TRIDs that are currently in clinical trials for cystinosis are aimed at restoring full-length cystinosin expression, targeting the disease at its core. It should be noted, however, that only a small subset of cystinosis patients with a specific mutation will be able to benefit from TRIDs. Furthermore, gene therapies with a functional *CTNS* gene transfer have the potential to functionally restore cystinosin. If these therapies prove successful, they may be able to provide a cure for many aspects of the disease that are currently not met, significantly improving the health and quality of life of people living with cystinosis (see Outstanding Questions). Nevertheless, gene therapy, although in theory suitable for all patients, will most likely only reach a small group of cystinosis patients due to availability and costs.

Finally, effective treatment will require early detection and intervention. This is a challenge for all severe diseases and, in particular, for monogenic disorders. For cystinosis, it is expected to fundamentally change once detection of the disease can be included in newborn screening programs. Such screens have long been on the research agenda, but the reliable detection of elevated cystine in blood collected on filter paper for the purposes of newborn screening is technically hampered by oxidation of cytosolic cysteine to cystine. A combination of high-throughput PCR for detecting the three most common mutations followed by next-generation sequencing for identifying mutations on a second allele recently proved successful and allowed for the identification of two patients with cystinosis out of 292 000 newborns [93]. Early treatment immediately after diagnosis may support an improved clinical outcome with respect to RFS development [94].

Outstanding Questions

What determines the development of kidney disease in nephropathic cystinosis, making this organ most vulnerable to cystinosin loss?

Can dual-target therapy, in which both lysosomal cystine accumulation and disturbed autophagy are corrected, restore the cellular defects in nephropathic cystinosis?

Can HSC transplantation prevent the progression of both renal and extrarenal manifestations of cystinosis?

Could detection of cystinosis in newborn screening programs and therapeutic interventions at birth prevent or delay the development of RFS in cystinosis patients in the future?

Acknowledgments

This work was financially supported by a grant from the Dutch Kidney Foundation (grant nr.150KG19).

Author Contributions

A.J., A.O., and M.J.J. performed the literature search and drafted the manuscript. A.J., A.O., and M.J.J. prepared the figures. A.J., A.O., E.L., R.M., and M.J.J. reviewed and edited the manuscript and contributed to the final manuscript.

Declaration of Interests

The authors declare no competing interests.

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