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THEMED ISSUE REVIEW

Therapeutic RNA interference: A novel approach to the treatment of primary hyperoxaluria

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RNA interference (RNAi) is a natural biological pathway that inhibits gene expression by targeted degradation or translational inhibition of cytoplasmic mRNA by the RNA induced silencing complex. RNAi has long been exploited in laboratory research to study the biological consequences of the reduced expression of a gene of interest. More recently RNAi has been demonstrated as a therapeutic avenue for rare metabolic diseases. This review presents an overview of the cellular RNAi machinery as well as therapeutic RNAi design and delivery. As a clinical example we present primary hyperoxaluria, an ultrarare inherited disease of increased hepatic oxalate production which leads to recurrent calcium oxalate kidney stones. In the most common form of the disease (Type 1), end-stage kidney disease occurs in childhood or young adulthood, often necessitating combined kidney and liver transplantation. In this context we discuss nedosiran (Dicerna Pharmaceuticals, Inc.) and lumasiran (Alnylam Pharmaceuticals), which are both novel RNAi therapies for primary hyperoxaluria that selectively reduce hepatic expression of lactate dehydrogenase and glycolate oxidase respectively, reducing hepatic oxalate production and urinary oxalate levels. Finally, we consider future optimizations advances in RNAi therapies.

KEYWORDS
calcium oxalate, end-stage renal disease, glycolate oxidase, hyperoxaluria, kidney stones, lactate dehydrogenase, micro-RNA, primary hyperoxaluria, RNA interference, small interfering RNAs

1 | BACKGROUND

There is a paucity of disease-modifying therapies for rare and ultrarare diseases. This has been attributable to a poor understanding of the molecular disease mechanisms and the challenge of recruiting sufficient numbers of patients to perform adequately powered trials of novel therapies. Advances in genomic sequencing have increased the rate of discovery for novel, disease-associated genotypes and this increases the understanding of disease pathobiology, opening avenues to novel therapeutic targets and mechanisms.

RNA interference (RNAi) describes an innate biological pathway found in almost all eukaryotic cells. This pathway enables homology-dependent, post-transcriptional gene silencing by a micro-RNA (miRNA) or small interfering RNA (siRNA) oligonucleotide complexed with a group of proteins called the RNA-induced silencing complex (RISC). RNAi has established roles in development,[1] differentiation,[2,3] cancer biology[4,5] and cellular antiviral defences.[6,7]

RNAi represents an attractive biological pathway to exploit in human disease therapeutics where the protein product of the gene cannot be easily targeted by small molecules. Indeed, synthetic siRNA molecules have been available for *in vitro* and *in vivo* animal model research for many years, reproducibly demonstrating potency for knockdown of gene expression.

The siRNA sequence and structural design critically influence engagement of the administered compound with the endogenous RNAi machinery. Another major challenge of translating RNAi into a clinically viable therapeutic product lies in the targeted delivery of oligonucleotides to the desired cell type, tissue or organ relevant to the disease in question, in doing so minimising off-target effects. Despite these challenges, several RNAi-based therapies for rare inherited diseases are now Food and Drug Administration (FDA) approved and/or in later clinical trials.[8]

This review serves as a beginner's guide to RNAi therapeutics. We provide an overview of the biology of RNAi as well as the principles of therapeutic siRNA design and pharmacokinetics. We present a summary of primary hyperoxaluria, an ultrarare, inherited enzyme deficiency that can lead to overwhelming oxalate production, recurrent kidney stones, early end-stage kidney disease (ESKD) and fulminant systemic oxalosis. In this context we discuss two novel RNAi therapies that selectively reduce hepatic expression of key enzymes involved in oxalate metabolism. RNAi therapies stand to significantly alter clinical outcomes in this debilitating disease.

2 | BIOLOGY OF RNA SILENCING

First described in 1998,[9] RNAi is an endogenous, highly conserved cellular pathway whereby short strands of RNA guide the RISC to downregulate targeted gene expression, either by direct translational repression, by homology-dependent mRNA cleavage or deadenylation.[8,10–12]

There are two types of double-stranded (ds)RNA molecules that serve as a guide for the RISC (depicted in Figure 1): miRNA or siRNA, which are broadly classified according to their precursor source and mechanism of transcriptional silencing.[13] Primary miRNA transcripts are transcribed from miRNA genes and form large single-strand RNA molecules with multiple hairpin loops housing the *bone fide* miRNA within their double-strand stems.[13] Within the nucleus, a heterotrimeric microprocessor composed of DROSHA (a class 2 ribonuclease III enzyme) and 2 cofactor DGCR8 (also known as *Pasha*) proteins, binds and cleaves short hairpin loops (called pre-miRNA).[14–16]

Cytoplasmic transfer of pre-miRNA occurs by binding to EXPORTIN-5, a RanGTP dependent dsRNA-binding protein.[17] Within the cytoplasm, pre-miRNA binds to TAR RNA binding protein (TRBP) and the terminal loop of the hairpin is cleaved by riboendonuclease DICER leaving approximately 21–23 base pairs of double stranded miRNA. DROSHA processing optimises pre-miRNA molecules for efficient interaction with DICER's PAZ domain by leaving a 2-nucleotide 3′overhang and a mono-phosphorylated 5′ at the end of the hairpin stem.[18–20]

An alternative pathway of RNAi more relevant to the field of therapeutic development is initiated when extracellular dsRNA is taken up by endocytosis or from complementary strands of transcribed endogenous mRNA (Figure 1). This dsRNA is cleaved by cytosolic DICER/TRBP in a DROSHA-independent pathway.

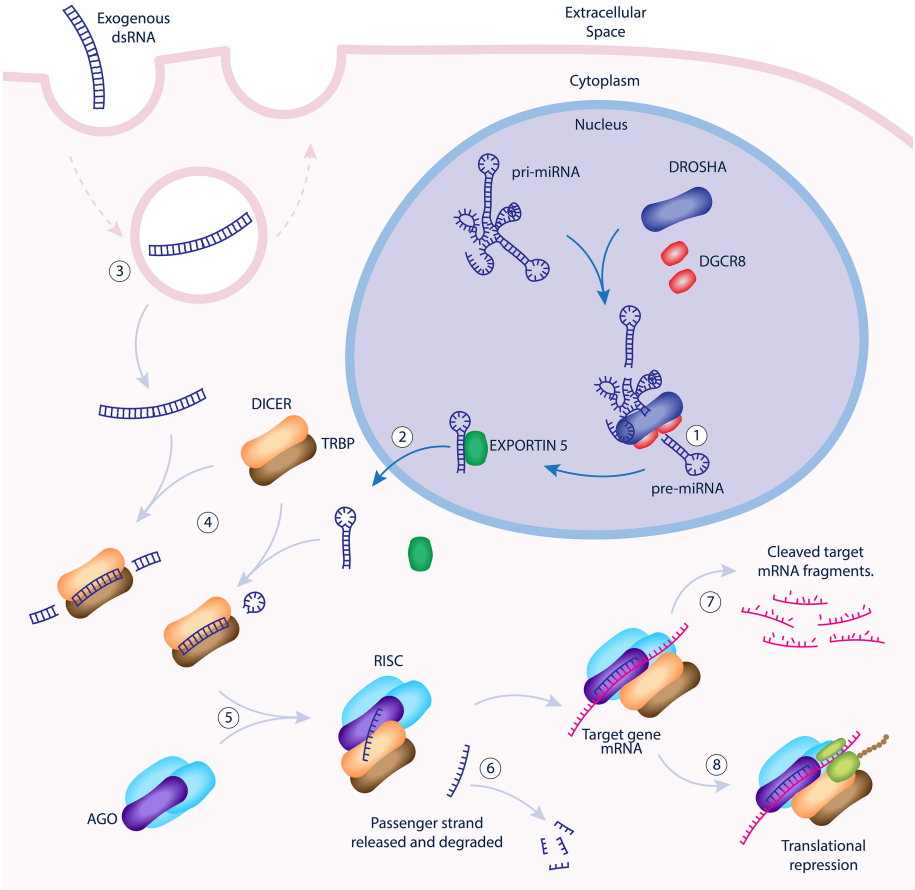

FIGURE 1 Molecular mechanisms of endogenous cellular RNAi. In the nucleus, pre-micro RNA (pre-miRNA) are trimmed from large primary micro-RNA transcripts (pri-miRNA) by DROSHA/DGCR8 complexes (1). Pre-miRNA is exported to the cytoplasm associated with EXPORTIN-5 (2). Exogenous dsRNA molecules enter the cell by endocytosis and cytoplasmic escape (3). dsRNA in the cytoplasm is further processed by DICER/TRBP complexes (4) before recruitment of Argonaut (AGO) and other proteins to form the RISC (5). The RISC unwinds the dsRNA and incorporates the antisense (or guide) strand, releasing the passenger strand for degradation (6). The antisense strand serves as a guide, selecting mRNA targets according to sequence homology, affecting either target mRNA cleavage and degradation (7) or translational repression (8). dsRNA: double-stranded RNA; DGCR8: DiGeorge syndrome chromosomal region 8; TRBP: TAR RNA-binding protein; RISC: RNA-induced silencing complex, AGO: Argonaut protein

Once the miRNA/siRNA has been processed by DICER, it recruits 1 of 4 Argonaut proteins (AGO1–4), which bind the antisense strand of the miRNA, unwind the dsRNA and release the passenger strand for degradation.[8,21] Antisense strand selection is independent of DROSHA and/or DICER cleavage polarity and tends to favour the strand where the 5′ end has a less stable complement pairing.[22,23] Whilst AGO1–4 proteins are all capable of loading miRNA/siRNA, only AGO2 retains endonuclease function capable of cleaving mRNA targets directly.[12] DICER-independent miRNA processing pathways have also been identified for certain miRNA gene products whereby AGO2 performs the cleavage step canonically performed by DICER.[24,25]

AGO proteins also recruit GW182, which enables translational repression and/or deadenylation of mRNA targets with homology to the 3′ untranslated region of the loaded guide RNA strand.[11] MicroRNA-guided RISCs more often affect gene silencing by translational repression or mRNA degradation and siRNA-guided RISCs more often affect mRNA cleavage, although crossover is observed. Whilst the efficiency of gene silencing is related to the degree of homology between the guide antisense miRNA/siRNA strand and target mRNA,[26] there may be as few as 7 complementary base pairs with the guide to affect silencing.[27]

As more is learned about the components of the RNAi pathways, a more diverse range of functions is being discovered including roles in chromatin modification, chromosome arrangement during the cell cycle and DNA damage responses. These mechanisms are beyond the scope of this review but are discussed in detail in previous reviews.[28,29]

3 | THERAPEUTIC RNAI

The ability of the cell to endocytose RNA from the extracellular environment offers the opportunity to use RNAi as a therapeutic avenue to treat human disease, through the use of specifically designed RNA oligonucleotides targeting genes implicated in human disease pathogenesis. The major design challenges to overcome have included gene-target specificity and methods for targeting organ- or tissue-specific delivery.

3.1 | Design and development

With current technology, short oligonucleotide complexes (21–27 nucleotides) with specific chemical modifications can be synthesized, delivered and evaluated for their activities and drug properties in both *in vitro* and *in vivo* models.[30–36] Therapeutic drug discovery usually starts with a large-scale screen to identify candidates based on potency of target mRNA or protein inhibition, while computer-based algorithms developed from cumulative and empirical screening data can be applied to effectively predict potency based on the sequence information in an effort to improve screening efficiency.[33,36] Several structural design features have been identified that affect DICER engagement and RNAi potency. Antisense strand selection can be favoured by removing the 2–3-base overhang from the sense strand.[37] Longer dsRNA molecules are more likely to be cleaved by DICER, appropriately select the antisense strand for RISC integration and ultimately provide a more potent downregulation.[38] Shorter molecules can trigger RNAi with less potency via DICER independent pathways[25,39] but this allows greater flexibility in the incorporation of chemical modifications designed to maximize the stability of the molecule.[40]

Off-target effects may include silencing of the target gene in unwanted tissues or silencing of nontarget genes through sequence homology.[41,42] This most commonly occurs due to even partial homology between the 3′ untranslated region of the mRNA transcript and the 5′ end of the siRNA guide.[42,43] In silico homology analysis of candidate siRNA sequences using human transcriptome libraries is a potential method to predict such effects. Interspecies variation in off-target transcriptional effects has been observed between mouse and human primary cell cultures; however, there was greater consistency between *in vivo* mouse liver (delivered by lipid nanoparticles) and *in vitro* mouse liver tumour cells (delivered by transfection).[44] Intuitively, these data advocate the use of *in vitro*, organ-specific human or non-human primate models as screens for off target effects in human disease.

The innate immune system is capable of responding to foreign RNA, often presented the form of RNA viruses. Immune stimulation by either the siRNA itself or the delivery vehicle can lead to proinflammatory cytokine production (interlekin-6, tumour necrosis factor-α) and immune cell activation.[41] Indeed, 1 of the first RNAi-based therapies aimed at solid tumours (MRX34) closed its early human trials due to immune-stimulation related adverse events.[45] The downstream effect of immune stimulation can alter transcriptional activity, potentially confounding the therapeutic objectives of the RNAi treatment. Various studies have demonstrated the effect of siRNA length, structure (i.e. single, double strand or hairpin), composition, uridine richness and delivery vehicle (especially cationic liposomes) as factors associated with immune system stimulation.[46–48]

Chemical modifications have been widely applied to improve the stability needed for RNA duplexes to escape degradation in RNAse-rich extracellular or intracellular environments, while simultaneously reducing immunogenicity, improving potency, target-specificity and enhancing drug-like properties.[49–55] These modifications have been extensively applied to all parts of the RNA duplex molecules, including the backbone, the sugars, and the bases on both sense and antisense strands to understand the structure activity relationship and drug stability.[49–52,56] For example, removing the 2–3-base overhang from both ends of the dsRNA molecule confers increased nuclease resistance and RNAi potency.[57] The 2′ position of the sugar moiety of each nucleoside is often modified from 2′-hydroxyl into 2′-deoxy, 2′-o-methyl, 2′-fluoro, 2′-o-methoxyethyl and locked nucleic acid to protect the oligonucleotides from RNAse degradation. Chemical modification of guide strand of siRNAs, in particular the 2′-OMe substitution of position 2 of guide strand, has been demonstrated to significantly reduce off-target effects.[58] Other backbone modifications include methylphosphonate, phosphorothioate, phosphorodithioate, thioester and other phosphate mimics.[40,59–61] The effect of any modification on enhancement or efficiency of RISC loading and AGO2 slicing activities must be validated. Unfortunately, the precise molecular

mechanism of how siRNA molecules dynamically load and achieve target silencing is not completely understood even with the availability of crystal structures of complex of siRNA, target mRNA and AGO2.[59,62–64] The measurement of *in vivo* activity in rodents and non-human primates remains a gold standard to empirically guide the evaluation of structure and activity relationships.

3.2 | Advantages and disadvantages of siRNAs

A major advantage of siRNA *vs.* other antisense-based approaches for therapeutic application is that its use of an innate cellular machinery allows for highly efficient targeting of complementary transcripts.[9,65–67] This reduces production costs and patient doses of siRNA and excipients while achieving sufficient target suppression and reducing toxicity.[68,69] Theoretically, siRNAs can be designed for targeting any gene based on its target mRNA sequence alone. RNAi is therefore an appealing therapeutic option for diseases mitigated by pathological gain of gene function or where substrate reduction can be safely implemented without redundancy to alternative metabolic pathways. siRNA is also an option for so-called *undruggable* targets, which may not be easily inhibited using conventional small molecule and monoclonal antibody-based approaches. Additionally, the siRNA drug discovery process can be relatively short compared to conventional small molecule drug approaches.[70] Chemical modifications and oligonucleotide compositions, which provide the main pharmacodynamic properties of siRNA therapeutics, can be readily applied to new sequences to achieve similar effects, whilst inhibiting different gene targets. Chemical modules for tissue-specific delivery and modifications for activity and stability can be reutilized and applied to other sequences of interest, readying them for *in vivo* confirmation and subsequent clinical testing.

One major impediment in the development of siRNA therapeutics is focusing the delivery of oligonucleotide duplex to the specific cell types implicated in the disease. The high molecular weight, polyanionic and hydrophobic properties restrict the free diffusion of siRNA across cell membranes to reach cytoplasmic RNAi machinery.[8,71,72] The development of tissue-specific delivery vehicles focusing siRNA delivery to the target organ of interest is one strategy to overcome off-target effects. The development of nano-carriers (lipid nanoparticle, liposome and polymer-based) and conjugate-based delivery has proven fruitful in both preclinical animal models and clinical investigation.[68,73–75] However, clinical validation of RNAi is currently only limited to hepatocytes and cancer cells.[8,72] Conjugate-based delivery of siRNA to hepatocytes, utilizing N-acetylgalactosamine (GalNAc) ligands that bind to the highly abundant hepatocyte cell surface asialoglycoprotein receptor (ASGPR), has revolutionized the therapeutic oligonucleotide field. GalNAc-conjugated oligonucleotides bind to ASGPR and undergo endocytosis.[74,76] By unknown mechanisms, they subsequently escape from endosomes and enter the cytoplasm where they interact with RNAi machinery. In preclinical research, there are reports of delivery using different strategies to hepatic stellate cells, endothelial cells, neurons and other cell types.[77–82]

Also, unlike small molecules which can be crudely identified to act either as agonists or antagonists, siRNA can only inhibit targets of interest with currently available technology, although this may change (see below). Finally, the materials and synthetic processes required for oligonucleotide manufacture is higher than for small molecules, representing a greater end-product cost.

3.3 | Predictable pharmacokinetic properties and tolerability profiles

siRNA therapies are currently delivered by subcutaneous or intravenous delivery, and oral delivery of siRNA is yet to be clinically demonstrated. siRNA's long duration of activity has been established in both preclinical and clinical investigation, which make its characteristic short plasma exposure uninformative for predicting duration of action. Nonetheless, investigators are trying to understand the relationship of maximum plasma concentration and direct pharmacodynamics or surrogate markers.[83–85] This long therapeutic effect is partially due to the stable integration of siRNA within RNAi machinery, forming a durable RISC-capable of catalytic degradation of target mRNA through multiple cycles within targeted cells, even when majority of free siRNA drugs have been removed from metabolism or excretion.[86,87] The use of similar siRNA design principles and fixed delivery modules permits a predictability with respect to the pharmacokinetic properties and adverse effects for oligonucleotide duplexes as a therapeutic class, which stands to streamline the development and approval of new siRNA therapeutics, compared to small molecule therapies.

The long duration of siRNA activity has prompted concern from some investigators as to the long period for recovery should any acute toxicity occur. However, conjugate-based siRNA therapeutics have shown high efficacy and favourable tolerability during clinical development with most common adverse effect as low frequency of mild to moderate injection site reactions, which usually resolve spontaneously.[68,69,88] Furthermore, concerns of off-target effect due to sequence-based complementarity on unrelated genes is yet to be demonstrated in clinical studies.

3.4 | Current clinical application of RNAi therapeutics

The strategies described above have led to the advancement of more than two dozen therapeutics in early or late stages of clinical development in cancer, metabolic and chronic viral disease (summarised in[8,89]). Patisiran (ALN-TTR02 or Onpattro; Alnylam Pharmaeuticals) was the first RNAi therapy to gain FDA approval in August 2018, as a treatment for hereditary transthyretin amyloidosis (OMIM 105210),[85] an autosomal dominant disease where accumulation of misfolded TTR protein throughout the body results in progressive neuropathy, cardiomyopathy and ophthalmic disease among other end-organ effects. The siRNA in patisiran is packaged in liver specific lipid nanoparticles and silences both wild type and

mutant transthyretin genes reducing systemic TTR load.[84,85,90] Givosiran (Givlaari; Alnylam Pharmaceuticals), an RNAi therapy silencing δ-aminolevulinic acid synthase 1 for treatment of acute hepatic porphyria, received FDA approval in 2019.[91] Phase 3 trials of givosiran demonstrated significantly lower rates of porphyria attacks, improved pain scores and lower levels of urinary δ-aminolevulinic acid and porphobilinogen.[92] Many other RNAi based therapeutics are in early phase clinical trials, heralding a new class of therapies with increasing impact on clinical practice for a variety of diseases over the coming decades.

Hypothetically, siRNA therapeutics are well suited for any disease where undesirable disease-causing proteins can be downregulated at a transcriptional level. It is, of course, imperative to ensure that silencing of the of any targeted gene will not be injurious to the recipient, which may restrict the scope of diseases to which RNAi can be applied. Genetic diseases with dominant negative mutations are another class of diseases that are applicable for siRNA approaches. Additionally, for some metabolic and genetic diseases, siRNA approaches can be designed to remove or reduce substrates of toxic metabolites to prevent or alleviate symptoms of those diseases.

4 | PRIMARY HYPEROXALURIA

Primary hyperoxaluria (PH) is an ultra-rare, autosomal recessive, inherited metabolic condition that leads to an accumulation of glyoxylate in the liver which is metabolised to oxalate by lactate dehydrogenase (LDH; Figure 2). Oxalate is readily filtered by the kidneys and is highly insoluble in urine in the presence of calcium, precipitating as calcium oxalate nephrolithiasis and nephrocalcinosis.[93] In severe cases nephrocalcinosis can progress to chronic kidney disease, at which point the reduced oxalate clearance leads to overwhelming systemic oxalosis affecting blood vessels, bones, retina, myocardium[94,95] and skin with a high associated mortality.

PH is sub-classified into 3 groups based on genotype, each with a distinct phenotype and prognosis (Table 1, Figure 2). The most common phenotype, PH type 1 (PH1, OMIM 259900), accounts for approximately 80% of disease burden and has a population prevalence between 1–3 per million population. PH1 is associated with recessive mutations in alanine-glyoxylate aminotransferase (AGXT), encoding the enzyme which catalyses the transamination of glyoxylate to glycine within the hepatocyte peroxisome.[96] Tissue expression is specific to the liver. Many PH1 patients will have an elevated urinary glycolate; however, this has also been reported in patients with PH3.[97,98] Whilst ESKD in infancy is not uncommon (around 25% of cases), the median age of ESKD is reported at age 10–24 years.[99,100] Genotype–phenotype correlations have been observed with Gly170Arg variants demonstrating a median age of ESKD at 47 years for homozygotes in a western European/North African cohort.[99] However, there exists wide variation in severity of renal phenotype even between members of the same family, suggesting that genetic modifiers or potentially kidney-specific factors may alter phenotype.[101]

PH2 (OMIM 260000) accounts for roughly 10% of disease burden and is caused by pathogenic variants in the gene encoding glyoxylate reductase and hydroxypyruvate reductase (GRHPR) which metabolises glyoxylate to glycolate and hydroxypyruvate to D-glycerate (Figure 2).[102] GRHPR is expressed throughout the body but is highest in the liver.[103] GRHPR deficiency leave both substrates vulnerable to LDH metabolism, excreted as oxalate and L-glycerate respectively, although presence of the either in the urine is variable[102,104] necessitating genomic sequencing as gold standard for diagnosis. In the largest reported cohort from the OxalEurope

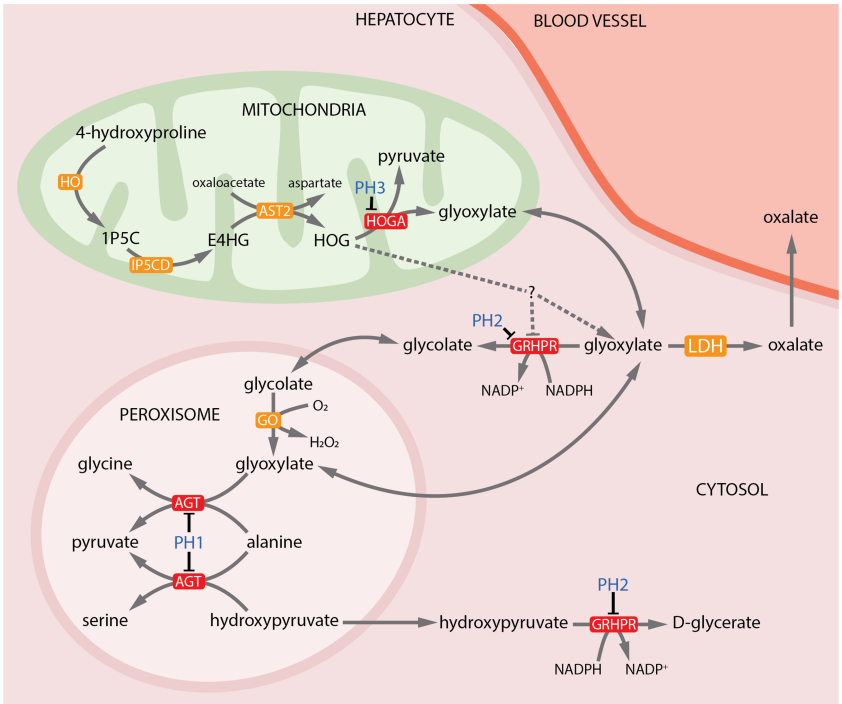

FIGURE 2 Hepatic metabolism of oxalate. Metabolic pathways involved in oxalate metabolism depicting the enzymes defective in the 3 major types of primary hyperoxaluria (red boxes and blue text). LDH catalyses the final step in the production of oxalate from glyoxalate for all PH types. The precise mechanisms by which HOGA mutations lead to increased oxalate production are not fully understood (depicted by dotted lines). Hypotheses include inhibition of GRHPR by accumulated HOG and metabolism of HOG in the cytoplasm to glyoxylate by an unidentified aldolase enzyme. 1P5C: 1-pyrroline-3-hydroxy-5-carboxylate; E4HG: erythrohydroxyglutamate; HOG: 4-hydroxy-2-oxoglutarate; PH: primary hyperoxaluria; AGXT: alanine glyoxylate aminotransferase; GRHPR: glyoxylate reductase/hydroxypyruvate reductase; HOGA1: hydroxyl-oxoglutarate aldolase 1; LDH: lactate dehydrogenase

TABLE 1 Features of the 3 primary hyperoxaluria genotypes

	Type 1	Type 2	Type 3
Gene	AGXT	GRHPR	HOGA1
Chromosome	2q37.3	9p13.2	10q24.2
Tissue expression	Liver	Liver, kidney, brain, skin, cardiac and skeletal muscle	Liver, kidney, brain
Proportion of disease burden	~80%	~10%	~10%
Phenotype	Calcium oxalate nephrolithiasis, nephrocalcinosis, childhood CKD	Calcium oxalate nephrolithiasis, nephrocalcinosis, adult CKD	Calcium oxalate nephrolithiasis, nephrocalcinosis, adult CKD
Biomarker	Urine glycolate (variable)[97]	Urine L-glyceric acid (variable)[104]	Dihydroxyglutamate (all patients)[155]
Treatment	Hydration Urinary alkalinisation Pyridoxine (some variants only) Liver +/− kidney transplant	Hydration Urinary alkalinisation Kidney transplantation (liver transplantation controversial)	Hydration Urinary alkalinisation Transplantation not required
OMIM phenotype number	259 900	260 000	613 616

CKD, chronic kidney disease

Consortium, almost 10% of patients presented with normal spot urine oxalate:creatinine ratio.[102] Patients develop recurrent kidney stones and nephrocalcinosis, generally progressing to chronic kidney disease or ESKD in mid-adulthood.[102]

PH3 (OMIM 613616) is due to pathogenic variants in hydroxyl-oxoglutarate aldolase 1 (HOGA1), a mitochondrial enzyme that takes part in hydroxyproline metabolism.[98] PH3 accounts for 10% of PH cases although the population prevalence of the most common variant (c.700 + 5G > T affecting a splice site in intron 5[97]) is much higher than anticipated for the documented disease frequency, suggesting either incomplete penetrance or underdiagnosis.[97] Given the limitations in access to genomic testing in many settings for patients with mild diseases, patients with PH3 suffering from recurrent but infrequent calcium-oxalate stones may well be underdiagnosed. The second most common pathogenic variant is p.E315del effecting a 3-base pair deletion in exon 7, representing 32% of alleles.[97] Patients experience raised urine oxalate excretion with or without recurrent nephrolithiasis and progression to chronic kidney disease is reported but uncommon.[105]

Paradoxically, glyoxylate is a product of the HOGA enzyme rather than a substrate and the biological mechanism of oxalate overproduction in PH3 is not well established.[106] The increased oxalate production in HOGA deficiency may be explained by the accumulation of its precursor 4-hydroxy-2-oxoglutarate (HOG). HOG accumulation may inhibit GRHPR; however, increased urinary L-glycerate is rarely seen, which would argue against this mechanism.[107,108] An alternative explanation is the metabolism of HOG to glyoxylate by an as yet uncharacterized cytosolic aldolase.[109]

4.1 | Current therapies for primary hyperoxaluria are limited in their ability to prevent ESKD

The goals of early conservative therapy for all PH types involves maximizing the solubility of oxalate in the urine in an effort to prevent stone formation and nephrocalcinosis. As for all causes of nephrolithiasis, maintenance of a high intake of water is critical.[110,111] Patients are often prescribed to drink up to 2.5 L/m^2 water daily. Periods of acute dehydration are avoided and managed aggressively with enteral or parenteral fluid supplementation to avoid acute kidney injury from which recovery can be limited.[112,113] Dietary restriction of oxalate rich foods is ineffective[114]; however, patients are advised to avoid vitamin C supplements as ascorbic acid is metabolised to oxalate.[115,116]

Supplementation with citrate and orthophosphate serves to complex with urinary calcium preventing calcium-oxalate crystallization in the urine.[117–120] Although there is no evidence to support its efficacy, magnesium supplementation is proposed to act via a similar mechanism.

For up to 50% of patients with PH1, high-dose pyridoxine supplementation has been established to improve enzyme function and reduce urinary oxalate levels.[119,121–123] Pyridoxine is metabolised to pyridoxal phosphate, which is an essential catalytic cofactor for the AGXT enzyme as well as being a necessary chaperone for peroxisomal localization.[124] Patients on pyridoxine must be monitored for peripheral neuropathy which is reversible if ceased early.[125] Variants known to respond favourably to pyridoxine include Gly170Arg, Phe152Ile, Ile244Thr and Gly41Arg.[99,126,127] In particular, the Gly170Arg variant, present in roughly 30% of PH1 cases, is known to be mislocalised to mitochondria but shows both improved activity and peroxisomal localization with pyridoxine treatment.[124] PH1 patients should be trialled on pyridoxine and continued if at least a 30% reduction in urine oxalate can be achieved over 3 months.[112,113]

4.2 | Dialysis and transplantation

Whilst militant adherence to conservative treatments is associated with improved outcomes, the eventual need for dialysis, kidney transplantation and/or liver transplantation in patients with PH1 is almost

ubiquitous.[128] Renal clearance is the only avenue to adequately remove such substantial amounts of oxalate in PH, and as a result serum oxalate levels increase exponentially with declining glomerular filtration rate. Systemic deposition of oxalate subsequently accumulates in the eyes, heart, bones, skin, nerves and joints, increasing morbidity. Thus, dialysis is essential not only for renal replacement but for oxalate clearance. Whilst haemodialysis is able to very quickly clear oxalate from the circulating bloodstream, the oxalate production in patients with PH1 is sufficiently high and tissue-sequestered that patients remain net oxalate positive, even on intensified peritoneal and haemodialysis.[129] PH1 patients on haemodialysis have 2–3 times the mortality rate of nonhyperoxaluria dialysis patients.[130] In undiagnosed PH1 patients and those with pyridoxine-unresponsive disease, isolated kidney transplantation is associated with high rates of disease recurrence in the graft.[131] Liver transplantation is curative and can be performed in isolation prior to the development of chronic kidney disease or combined with a kidney transplant in ESKD. Sequential liver and kidney transplant can be pursued if there are concerns about immediate risk to the kidney graft[131,132]; however, many patients do well with intraoperative filtration and daily postoperative haemodialysis.[133] Isolated kidney transplant may be considered in pyridoxine-responsive PH1 and remains the most common approach in ESKD due to PH2 and PH3.

5 | NOVEL RNAI THERAPIES FOR PRIMARY HYPEROXALURIA

Whilst the precise mechanisms underpinning the metabolic disturbances giving rise to oxalate overproduction are less well understood in PH3 than for PH1/2, it may be that LDH represents a final enzymatic pathway leading to hyperoxaluria (Figure 2). Hence, an RNAi approach could serve as a potentially effective and safe way to correct the overproduction of oxalate in the liver. In considering a strategy to reduce oxalate production and treat PH, inhibition of glycolate oxidase (GO) is sufficient to reduce the conversion of glycolate to glyoxylate, the proposed main precursor to oxalate[134,135] (Figure 2). Inhibition of expression of LDH, the key enzyme responsible for producing oxalate from glyoxylate, has been demonstrated to be effective in treating PH1 and PH2 mice.[136] Lai *et al.* provided the first *in vivo* evidence in mammals to support the role of LDH for converting glyoxylate to oxalate, where reduction of hepatic LDH by RNAi achieves efficient reduction in serum oxalate and renal calcium oxalate crystal deposition.[136] Notably, when LDH and GO inhibition was compared in PH1 mice, a disproportionate correlation between GO protein suppression and urinary oxalate reduction was observed. The authors further confirmed that suppression of LDH, but not GO, causes the reduction of urinary oxalate levels in PH2 mouse models.[136] Repression of hepatic LDH in mice and non-human primates did not cause any acute elevation of circulating liver enzymes, lactate acidosis, or exertional myopathy, suggesting further evaluation of liver-specific inhibition of LDH as a potential approach for treating PH1 and PH2 was warranted.[136] Subsequently nedosiran (DCR-PHXC, Dicerna Pharmaceuticals Inc, Table 2) was designed to specially inhibit hepatic expression of *LDHA*, which encodes the M subtype of LDH, the major isoform of LDH enzyme in the liver.[136–138] Nedosiran is a double-strand siRNA molecule that is conjugated with GalNAc, which takes advantage of the aforementioned unique ASGPR delivery system in the liver. (Figure 3) Nedosiran is administered by monthly subcutaneous injection. The initial data emerging from a multidose, open-label trial (PHYOX3, Dicerna Pharmaceuticals, Inc; ClinicalTrials.gov Identifier: NCT0402402) demonstrated sustained, long-term reduction in urinary oxalate levels as far as the normal range (less than 0.46 mmol/1.73 m^2 BSA/24 hours) or near-normalization in patients with both PH1 and PH2[139] (abstract only). This study advocated that nedosiran was generally well tolerated, which agrees with the observed absence of any liver-specific adverse effects in natural, systemically *LDHA*-deficient patients.[140–144] This

TABLE 2 Comparison of the 2 RNAi therapies in development for primary hyperoxaluria

	Nedosiran	Lumasiran
Company	Dicerna Pharmaceuticals Inc.	Alnylam Pharmaceuticals
Previous names	DCR-PHXC	ALN-GO1; Oxlumo
Gene product targeted (protein name)	*LDHA* (lactate dehydrogenase)	*HAO1* (Glycolate oxidase)
Molecule	dsRNA covalently linked to GalNAc residues	dsRNA covalently linked to GalNAc residues
Primary hyperoxaluria types targeted	PH1, PH2 (PH3 trials underway)	PH1
Administration	Subcutaneous	Subcutaneous
Dosing	Monthly	Loading: Monthly for 3 mo Maintenance: 3 monthly
Human trials	PHYOX3 early unpublished results: 6 of 7 patients showed consistent normalization or near normalization of urine oxalate levels after 3 doses (≤0.6 mmol/24 h/1.73m^2)[139]	ILLUMINATE-A: 53.5% decrease in 24-h urine oxalate excretion (95% confidence interval 62.3–44.8%) compared to placebo 11.8% reduction.[156]
Approvals		FDA (Nov 2020) EMA (Nov 2020)

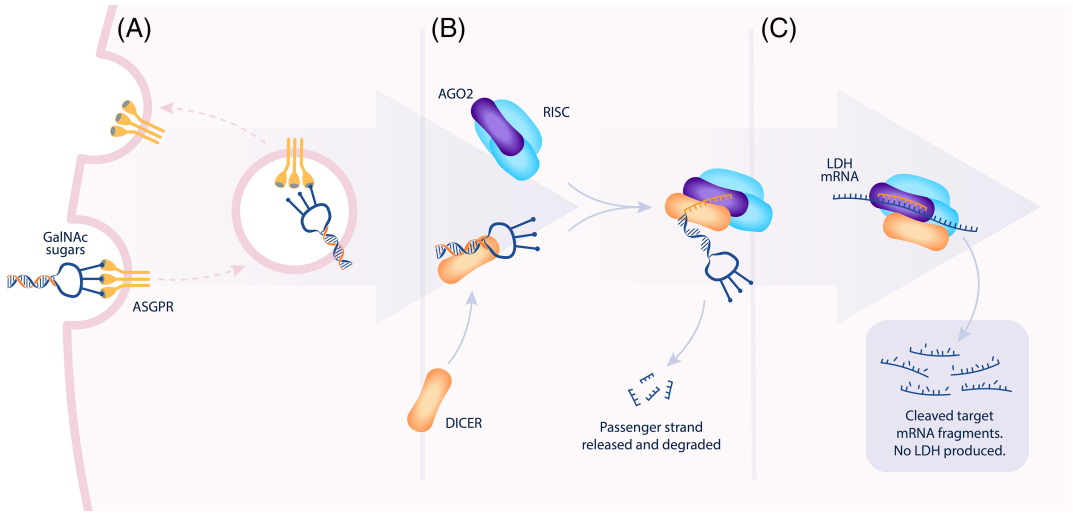

FIGURE 3 Mechanism of action of nedosiran within hepatocytes. Nedosiran is a GalNAc-dsRNA conjugate. (A) Bound GalNac sugar residues bind to ASGPR receptors prompting endocytosis of the dsRNA. Via an incompletely understood mechanism, the compound undergoes endocytic escape and enters the cytoplasm. (B) Within the cytoplasm, the dsRNA first interacts with endonuclease DICER (orange). DICER then passes the antisense RNA strand to AGO2 (purple). The dsRNA compound is designed to favour the loading of the antisense RNA strand into the RISC complex. (C) RISC is guided to the target mRNA by homology to the RNA strand, cleaving the target RNA. GalNAc: N-acetylgalactosamine; ASGPR: asialoglycoprotein receptor; RISC: RNA-induced silencing complex; dsRNA: double-stranded ribonucleic acid

supports preclinical observations in wild type mice where GalNAc-conjugated siRNAs designed for liver-specific knockdown of *LDHA* had no significant effect on gene expression in muscle, skin or uterine tissue, as well as maintained lactate production and exercise performance.[136] Whilst the definitive pathways by which oxalate production occurs in PH3 are less well established, it is conceivable that LDH silencing could also be of benefit in this disease. Accordingly, clinical testing of nedosiran will soon expand to include PH3 patients (PHYOX4: ClinicalTrials.gov Identifier: NCT04555486) and PH1/2 patients with ESKD (PHYOX7: ClinicalTrials.gov Identifier: NCT04580420).

Lumasiran (Oxlumo or ALN-GO1, Alnylam Pharmaceuticals, Table 2) is another RNAi therapy for the treatment of PH1, which gained FDA approval in November 2020. Lumasiran targets GO, reducing the production of glyoxylate, the main oxalate precursor, from glycolate (Figure 2).[145,146] Glycolate is excreted by the kidneys but is highly soluble and not associated with a renal phenotype in GO-deficient (*Hao1* knockout) mouse models.[147] Rare human cases of biallelic loss of function variants in *HAO1* have also been characterized to have high serum and urine glycolate levels without a disease phenotype, endorsing the safety of an RNAi approach to GO.[148,149] In addition, double knockout mice for *Agxt* and *Hao1* demonstrate low levels of urine oxalate excretion.[147] A recently published double-blind, multinational, placebo-controlled, randomized controlled trial recruited 39 adult and paediatric patients with PH1 and an estimated glomerular filtration rate of >30 mL/min/1.73 m^2.[146] Lumasiran effected a 53.5% reduction in 24 hour urine oxalate levels (95% confidence interval 62.3–44.8%) compared to placebo (mean reduction 11.8%) and 85% of patients achieved a urine oxalate excretion within 150% of the upper limit of the normal range.[146] Furthermore, no serious adverse events were reported.

6 | FUTURE PROSPECTS FOR RNAI THERAPIES

Recent regulatory approvals of RNAi-based drugs include a complex lipid nanoparticle formulation of 1 unconjugated siRNA product and far simpler water-for-injection formulations of GalNAc-conjugated siRNAs and many more are in clinical trials, ranging from first-in human studies to registration trials.

Beyond synthetic siRNA, there is potential to manipulate RNAi for therapeutic benefit in other ways. As well as gene silencing, dsRNA molecules designed with homology to sequences near target gene promotor regions have been observed to activate gene expression via a non-endonuclease AGO2-mediated pathway.[150,151] This strategy has been recently applied to the treatment of hepatocellular carcinoma.[152] Anti-mirs (also known as antagomirs), are antisense oligonucleotides targeting miRNA effectively blocking RISC activity on the host cell transcriptome,[153] a strategy that had been applied to the treatment of hepatitis C (Mirversin, Santaris Pharma); however, this has been superseded by highly efficacious and inexpensive small molecule treatments.[154] Finally, block-mirs, which anneal with target mRNA, effectively protecting the target transcript from RISC-mediated degradation, are yet to demonstrate a clinical application.

In principle, good siRNA sequence design to complement the target gene can achieve highly specific target knockdown while avoiding cross-reactivity to functional protein molecules of same family, which is a challenge for small molecule-based approaches. This high

specificity of the RNAi approach may even allow targeting of disease-specific alleles and spare the normal allele even when they differ only by 1 or a few nucleotide substitutions. However, it might not be easy to achieve the balance between the activity and specificity due to the requirement of fixed and short length of oligonucleotide duplexes to trigger RNAi activity. Multiple targets can be inhibited simultaneously without changing the therapeutic principles and fundamental physical composition of RNAi-based therapies, which represents another advantage over small molecule therapeutics.

7 | CONCLUSIONS

RNAi involves the processing of endogenous miRNA or exogenous siRNA molecules into RISCs which are capable of downregulating gene expression by homology directed cleavage of target mRNA or direct transcriptional inhibition.

As a platform, synthetic siRNAs are emerging as well tolerated and effective drug candidates as well as approved drugs with high safety margins. Chemical modifications can specify tissue specific delivery of the compound to the tissue of interest. This bodes well for the use of RNAi to treat primary hyperoxaluria with the potential for adaptation of the system for the treatment of other inherited and metabolic diseases.

COMPETING INTERESTS

C.L. and B.D.B. are employees of Dicerna Pharmaceuticals, which is developing siRNAs as therapeutics, including nedosiran. T.A.F. is the Site Principle Investigator at the Royal Children's Hospital in Melbourne, Australia supervising Dicerna (nedosiran) and Alnylam (lumasiran) clinical trials.

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