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REVIEW ARTICLE

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Development of RNA interference-based therapy for rare genetic diseases

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ABSTRACT

In December 2022, the Indonesian Ministry of Health projected that rare diseases might affect 8–10% of the population, equating to approximately 27 million people. These diseases, often congenital, are linked to genetic inheritance or mutations, leading to structural or functional defects. Despite advancements in diagnostic and treatment methods, many rare diseases remain challenging for healthcare practitioners. RNA interference (RNAi) presents a promising therapeutic approach by enabling the selective inhibition of genes responsible for genetic disorders. RNAi employs small RNA molecules, such as small interfering RNA (siRNA) and microRNA (miRNA), to bind specific mRNA molecules and prevent their translation into proteins. Current research showed that RNAi-based therapies have the potential to treat various genetic diseases, including acute hepatic porphyria (AHP) and primary hyperoxaluria type 1 (PH1). However, the mechanisms of RNAi in hereditary disorders like AHP and PH1 require further documentation. RNAi offers several advantages, including gene-specific targeting, versatility in treating diverse genetic disorders, and scalability for mass production. Nonetheless, challenges remain, such as side effects, difficulties in targeting specific cells, and high development cost. Despite these obstacles, RNAi-based therapy holds significant potential for revolutionize the treatment of genetic disorders.

Keywords: RNA interference, RNA therapy, genetic disease, acute hepatic porphyria, primary hyperoxaluria type 1

Introduction

Genetic diseases arise from mutations in particular genes, leading to alterations in the structure or function of the proteins synthesized by these genes [1]. These disorders could have various negative impacts on human health, including chronic disabilities, developmental disorders, and an increased risk of serious diseases. Although various treatment approaches, including enzyme replacement therapy and gene therapy, have been developed, challenges persist for some conditions and specific issues [2]. Currently, RNA interference (RNAi) has emerged as an intriguing approach to addressing these constraints.

RNA interference (RNAi) is a complex biological process involving various intricate molecular pathways. Key components of this process include Dicer, the RNA-induced silencing complex (RISC), small interfering RNA (siRNA), and microRNA (miRNA). RNAi functions by using small RNA molecules to inhibit gene expression. The mechanism was first discovered in *Caenorhabditis elegans* by Andrew Fire and Craig Mello [3], who were awarded the Nobel Prize in Physiology or Medicine in 2006 for this work. The RNAi mechanism involves Dicer cleaving double-stranded RNA (dsRNA) into siRNA or miRNA, which are then incorporated into RISC to guide the silencing of target genes [4].

A crucial aspect of RNAi therapy is balancing specificity with minimizing off-target effects [4] which occur when RNAi inadvertently affects the expression of unintended genes. Designing siRNA and other molecules with high specificity while developing strategies to reduce off-target effects remains a significant challenge in the field.

The discovery of the RNAi mechanism has revolutionized genetic therapy. RNAi is a natural process where short RNA molecules enable the regulation of gene expression by selectively suppressing protein synthesis. This technique has become the basis for the development of novel therapies targeting genetic diseases, taking significant steps in overcoming challenges that are difficult to address by conventional therapeutic methods. This review will discuss RNAi-based therapy for genetic diseases, focusing on how this approach has provided new insights into the treatment of some genetic disorders, such as acute hepatic porphyria (APH) and primary hyperoxaluria type 1 (PH1).

Basic concept of RNA interference

RNA interference (RNAi) was first discovered in *C. elegans* in 1998 by Fire and colleagues [3]. RNAi is an evolutionarily conserved cellular process triggered by single-stranded RNA (ssRNA), which leads to the inhibition of target gene expression by degrading complementary mRNA, thereby inhibiting the production of related proteins [5,6]. In 2001, Elbashir et al demonstrated for the first time that 21-nucleotide RNA molecules could inhibit gene expression in mammalian cell lines via the RNAi pathway, opening the chance to apply this method for studying gen function or therapeutic in mammalian cells [7].

RNAi has since emerged as a powerful tool, enabling researchers to investigate gene function by introducing short double-stranded RNA (dsRNA) into cells to silence specific gene targets. The two main types of small RNA molecules involved in RNAi, small interfering RNA (siRNA) and microRNA (miRNA), are the most efficient effectors in this process.

RNA interference is a complex process involving multiple intricate molecular pathways. The RNAi mechanism begins with the enzyme Dicer, which cleaves long double-stranded RNA into small RNA molecules. These small RNA molecules are then incorporated into the RNA-induced silencing complex (RISC), which guides them to complementary mRNA targets, leading to their degradation and preventing gene expression [8]. In RNA therapy, achieving high specificity while minimizing off-target effects is critical, as these factors significantly influence the efficacy and safety of the treatment.

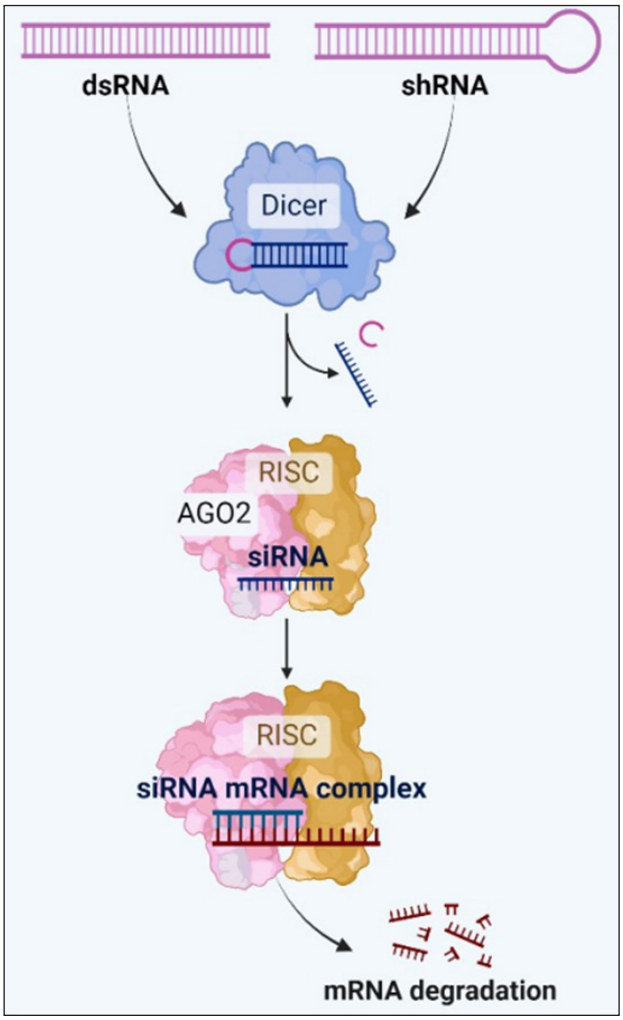

Figure 1. Mechanism of siRNA action in inhibiting gene expression through mRNA degradation. siRNA (small interfering RNA), dsRNA (double stranded RNA), shRNA (short hairpin RNA), RISC (RNA-induced silencing complex), AGO2 (argonaute 2)

Small interfering RNA (siRNA)

Small interfering RNA (siRNA), also known as short interfering RNA, is a double-stranded RNA molecule typically 20–27 base pairs in length. siRNA is a type of non-coding RNA (ncRNA) that is not translated into protein. Long double-stranded RNA (long dsRNA), encoded in the genome, serves as the precursor to siRNA. This long dsRNA is cleaved into siRNA fragments by Dicer, a cytoplasmic RNase

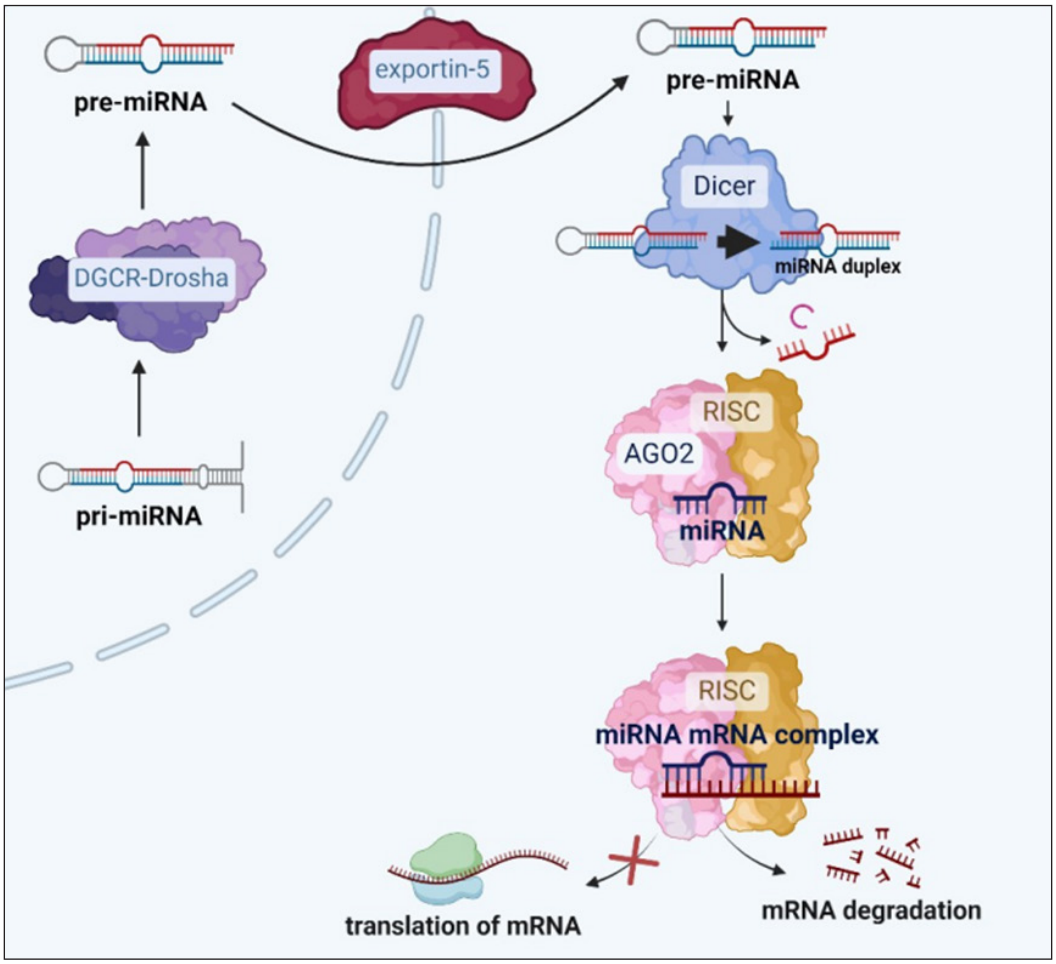

Figure 2. Mechanism of miRNA action in inhibiting gene expression through mRNA degradation and prevention of mRNA translation in the ribosome. miRNA (microRNA), DROSHA (class 2 ribonuclease III enzyme), DGCR8 (DiGeorge Syndrome Critical Region 8), RISC (RNA-induced silencing complex), AGO2 (argonaute 2)

specific to dsRNA. Each resulting siRNA fragment is 20–25 nucleotides in length, with characteristic two-nucleotides overhangs at the 3' end and a 5'UTR phosphate group [9,10].

Once processed, the siRNA is incorporated into a complex structure consisting of the RISC and Argonaute 2 (AGO2) protein, the core catalytic component of this complex. Within this complex, the antisense strand of the siRNA binds to the complementary sequence on the specific target mRNA, while the sense strand is released from the complex and degraded. If the binding between the antisense siRNA strand and the target mRNA is highly complementary, AGO2's endoribonuclease activity is triggered, cleaving the target mRNA into small fragments (Figure 1). This cleavage effectively prevents the translation of mRNA into functional proteins [11,12].

Currently, synthetic siRNAs are designed to target specific genes for various biomedical applications, including the development of gene-based therapies. These synthetic siRNAs, when introduced exogenously into cells or tissues, directly integrate into the RISC-AGO2 complex, mimicking the natural products of Dicer cleavage. This process results in the efficient degradation and inhibition of the target mRNA, thus silencing gene expression [13–15].

Micro RNA (miRNA)

MicroRNAs (miRNAs) are endogenous non-coding RNA molecules that are evolutionarily conserved and play a key role in mediating RNA interference. The biogenesis of miRNA begins in the cell nucleus, where miRNA genes are transcribed by RNA polymerase II to produce a primary miRNA (pri-miRNA) with

a stem-loop or hairpin structure of approximately 33 nucleotides. This pri-miRNA is then processed by a complex composed of DROSHA, a class 2 class 2 ribonuclease III enzyme, and a cofactor DGCR8, into a precursor miRNA (pre-miRNA) that is about 70 nucleotides long.

The pre-miRNA is subsequently transported from the nucleus to the cytoplasm by exportin 5, aprotein transporter. Exportin 5 forms a complex with pre-miRNA and guanosine triphosphate (GTP), enabling its passage through nuclear pores. Once in the cytoplasm, Dicer, an endoribonuclease, cleaves the pre-miRNA into shorter, functional miRNA molecules, each approximately 22 nucleotides in length [16].

miRNAs regulate gene expression through two main mechanisms after binding to their target mRNA. First, they can inhibit translation by preventing ribosomes from interacting with the target mRNA, thereby hindering protein synthesis. Second, miRNAs can facilitate the selective degradation of the target mRNA, effectively destroying it and preventing the production of proteins (Figure 2). These mechanisms are crucial role for regulating gene expression and are fundamental to various biological processes.

Delivery systems in RNA interference-based therapy

The development of RNA interference (RNAi) therapies requires the design and synthesis of RNA strands that can effectively bind to specific sequences on target mRNA strands. This process commonly involves a combination of computational modeling and experimental validation to identify siRNA sequences with optimal strength and specificity. Once validated in vitro, siRNA strands are further stabilized by chemically modifying nucleotides to enhance their efficacy. Following this, various delivery formulation strategies are developed to ensure efficient and targeted delivery of siRNA.

Delivery systems for RNAi-based therapies can be broadly categorized into virus-based and non-virus-based methods, each with varying levels of success and clinical utility. Virus-based siRNA delivery systems employ different viral vectors, such as retroviruses, lentiviruses, and adenoviruses, which offer intrinsic advantages in efficiently transfecting target cells [17]. Non-virus-based delivery methods currently utilized in siRNA therapy include two main approaches: the conjugation of siRNA with ligands to enhance the targeting of specific tissues and cells, and the encapsulation of siRNA into vesicles for improved delivery [10].

Genetic disease

Acute hepatic porphyria

Genetic diseases are medical conditions caused by changes or mutations in DNA, directly related to the central dogma of molecular biology, which encompasses DNA replication, transcription, and translation. During DNA replication, mutations in genes that regulate this process or in the enzymes involved can lead to genetic changes that may trigger the development of genetic diseases [18]. In the transcription stage, mutations in specific genes can lead to the production of incorrect or incomplete mRNA, leading to defective or non-functional proteins. Messenger RNA (mRNA) molecules serve as crucial intermediaries between DNA and proteins, and mutations in the genes encoding mRNA can disrupt the production of essential proteins, as observed in genetic diseases like thalassemia.

Acute hepatic porphyria (AHP) is a group of rare genetic diseases caused by deficiencies in enzymes crucial for hepatic heme biosynthesis [19]. AHP comprises four types: acute intermittent porphyria (AIP), variegate porphyria (VP), hereditary coproporphyria (HCP), and aminolevulinic acid dehydratase (ALAD) deficiency porphyria (ADP). Among these, AIP is the most prevalent, accounting for approximately 80% of AHP cases [20]. AIP is characterized by a mutation in the *hydroxymethylbilane synthase (HMBS)* gene, which leads to a deficiency in this enzyme (Figure 3). This deficiency results in reduced heme production and increased demand for hepatic heme, causing elevated levels of delta-aminolevulinic acid synthase 1 (ALAS1) enzyme and the overproduction of toxic

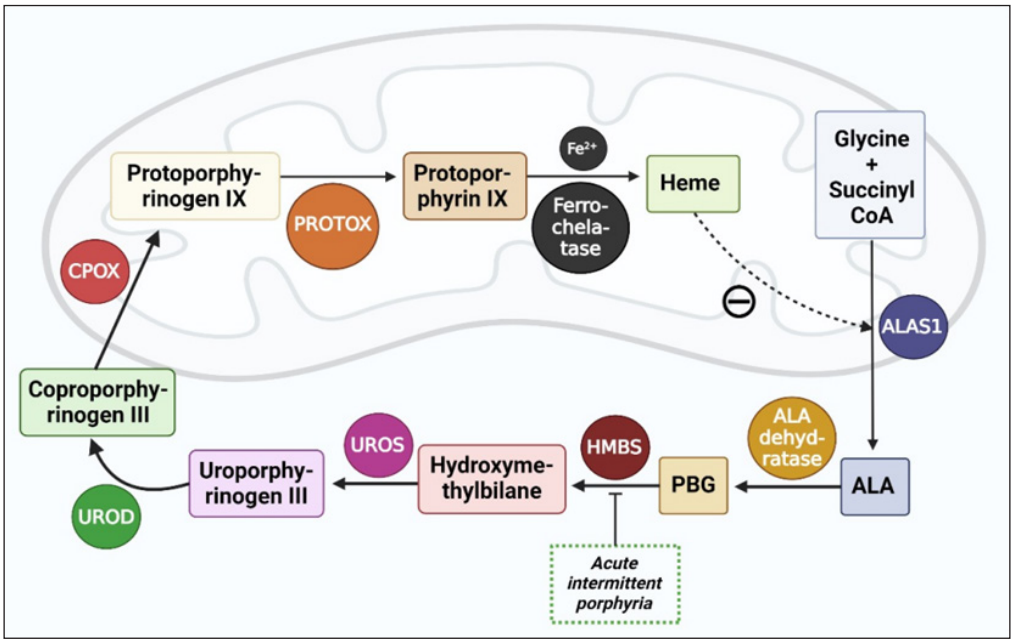

Figure 3. Pathogenesis of acute hepatic porphyria (AHP), with a focus on acute intermittent porphyria (AIP), the most common type of AHP. AIP is characterized by a mutation or deficiency in the HMBS, leading to increased levels of ALA and PBG. ALAS1 (aminolevulinic acid synthase 1), ALA (aminolevulinic acid), PBG (porphobilinogen), HMBS (hydroxymethylbilane synthase), UROS (uroporphyrinogen III synthase), UROD (uroporphyrinogen decarboxylase), CPOX (coproporphyrinogen oxidase), PROTOX (protoporphyrinogen oxidase)

heme precursors, such as delta-aminolevulinic acid (ALA) and porphobilinogen (PBG) [21]. The accumulation of ALA and PBG is thought to primarily damage the nervous system, as well as the liver and kidneys [22].

AHP typically manifests in women aged 20 to 40 years [23], with an estimated symptomatic prevalence of 1 in 100,000 individuals. While most symptomatic patients experience sporadic attacks throughout their lives, up to 8% endure recurrent attacks, defined as four or more attacks per year [24].

The heme biosynthesis pathway begins in the mitochondria with the condensation of glycine and succinyl CoA into ALA by ALA synthase (ALAS). This is followed by four enzymatic steps in the cytoplasm, before returning to the mitochondria for the final three steps that produce heme molecules [25]. ALAS is the rate-limiting enzyme in this pathway when the catalytic capacity of other enzymes is normal. There are two isoforms of ALAS: ALAS1 and ALAS2. ALAS2 is exclusively expressed in erythroid cells, whereas ALAS1, the housekeeping isoform, is expressed in all tissues and is controlled by negative feedback from heme. In the liver, certain drugs and chemicals that induce microsomal cytochrome P450 oxidases (CYPs) can increase ALAS1 activity [26]. In individuals with a deficiency in the porphobilinogen deaminase/HMBS enzyme, the liver produces excessive ALA and PBG, which then leak into the bloodstream, enter the nervous system and damage neurons [20].

Primary hyperoxaluria type 1

Primary hyperoxaluria type 1 (PH1) is a rare genetic disorder caused by mutations in the *AGXT* gene, which encodes the enzyme alanine-glyoxalate aminotransferase (AGT) [27]. These mutations impair oxalate metabolism in the body, leading to the accumulation of oxalate because glyoxylate cannot be converted to glycine. Instead, glyoxylate is converted to oxalate via oxidation catalyzed by lactate dehydrogenase (LDH) (Figure 4). The oxalate is then transported from the liver to the kidneys for excretion, resulting in hyperoxaluria. Elevated levels of oxalate in the kidneys and urine lead to the formation of calcium oxalate (CaOx) crystals in the kidneys and urine lead to urolithiasis and/or

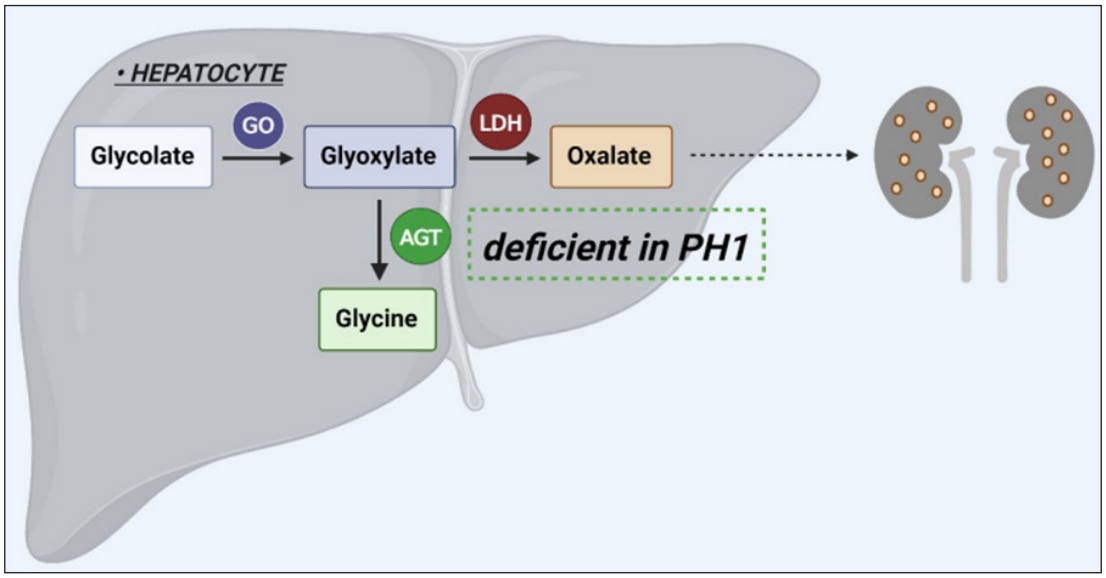

Figure 4. Pathogenesis of primary hyperoxaluria type 1 (PH1). A mutation or deficiency in the AGT leads to the accumulation of oxalate, because glyoxylate cannot be converted to glycine. Instead, glyoxylate is converted to oxalate via oxidation, catalyzed by LDH. AGT (alanine-glyoxalate aminotransferase), GO (glycolate oxidase), LDH (lactate dehydrogenase)

nephrocalcinosis. As kidney function progressively declines, the kidneys become less able to effectively remove oxalate from the bloodstream, potentially leading to systemic oxalosis—a condition in which CaOx crystals form outside the kidneys and damage other organs, including bones, heart, skin, and eyes [28].

Primary hyperoxaluria (PH) encompasses three types, with PH type 1 (PH1) being the most prevalent and extensively studied, accounting for approximately 80% of PH cases [29]. These conditions are autosomal recessive and characterized by an excessive oxalate production within the body. The estimated prevalence of PH is fewer than three cases per million individuals, with PH1 being a notable contributor. Symptoms typically manifest in childhood and can progress to end-stage renal disease (ESRD) at any age, from infancy to the sixth decade of life. PH is responsible for 1–10% of pediatric ESRD cases, with higher incidences observed in North African and Middle Eastern populations [30].

Standard supportive treatments, such as increased fluid intake and agents that inhibit crystallization, help enhance the solubility of oxalate and prevent the formation of calcium oxalate crystals. However, these treatments do not address the underlying pathophysiology of PH1. Pyridoxine (vitamin B6) can stabilize specific defective AGT variants, potentially increasing enzyme activity and showing efficacy in about 30% of patients [31]. In advanced stages, treatment may involve intensive dialysis as a temporary action before transplantation or when other treatments are not feasible. Liver transplantation offers a way to correct the metabolic defect in PH1, normalizing oxalate levels and preventing the progression of kidney failure. However, this procedure carries significant risks of morbidity and mortality and often requires simultaneous kidney transplantation to restore renal function in patients with existing kidney failure. Given the limitations of current treatment options, several innovative therapies for PH1 are being developed, including RNAi drugs that reduce the expression of oxalate-producing proteins and a bacterial preparation that degrades oxalate in the intestines [28,32].

RNA interference therapy with FDA approval: givosiran and lumasiran

Currently, the FDA has approved three siRNA-based therapies for treating various diseases. Significant progress in lipid-based siRNA therapy was marked by the approval of patisiran in 2018,

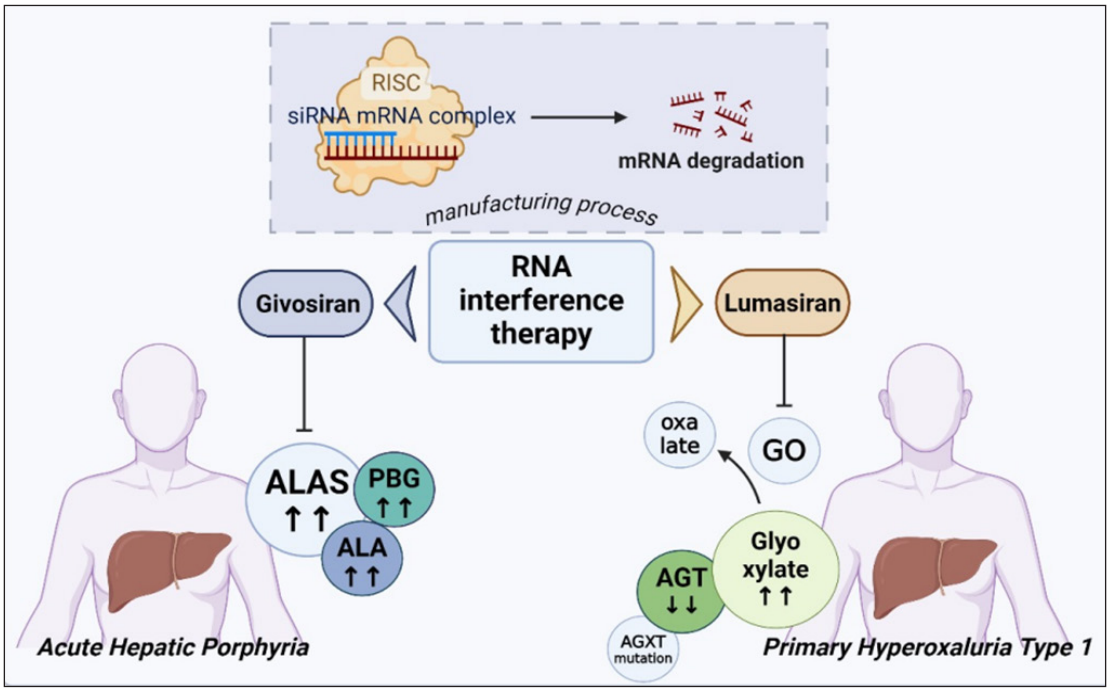

Figure 5. RNA interference therapy with FDA approvals: givosiran and lumasiran. Givosiran targets ALAS1 mRNA for AHP treatment, while lumasiran is approved for treating PH1

which became the first RNAi therapy receive approval from both the FDA and the European Commission for treating polyneuropathy in patients with hereditary transthyretin amyloidosis [33]. Following this milestone, the second siRNA drug, givosiran, targeting ALAS1 mRNA, was approved by the FDA in 2019 for treatment of AHP [34]. In 2020, the FDA approved lumasiran as the first treatment specifically for PH1 (Figure 5) [35].

Givosiran is an RNAi therapy using synthetic siRNA to reduce ALAS1 mRNA levels, delivered specifically to the liver with the help of N-acetyl-galactosamine [36]. Givosiran was approved in November 2019 for the treatment of adults with AHP in the United States and received a positive opinion in January 2020 in the European Union for use in adults and adolescents aged 12 and older [34]. By inhibiting liver ALAS1 synthesis, givosiran reduces the concentrations of toxic heme precursors, such as porphobilinogen and δ-ALA [37].

Evidence from phase III randomized controlled trials (NCT03338816) showed a 74% reduction in the rate of acute attack rates with givosiran treatment compared to placebo. The treatment was also associated with sustained reductions in urinary δ-ALA and porphobilinogen levels, decreased pain scores, and improved quality of life over a six-month study period [38]. Furthermore, monthly administration of givosiran has been shown to significantly and sustainably reduce liver ALAS1 levels, thereby lowering neurotoxic intermediate heme levels, including δ-ALA and porphobilinogen, to near-normal levels [39].

Lumasiran is a synthetic double-stranded siRNA conugated with the carbohydrate N-acetylgalactosamine (GalNAc), specifically designed to target the *hydroxyacid oxidase 1 (HAO1)* gene in liver cells. This siRNA therapy inhibits the translation of HAO1 mRNA, thereby reducing the production of the glycolate oxidase enzyme. Glycolate oxidase, located in hepatocyte peroxisomes, facilitates the conversion of glycolate into glyoxylate, the precursor of oxalate. By reducing glycolate oxidase expression leads to decreased oxalate production and increased glycolate levels.

Lumasiran is designed for the treatment of PH1, a condition characterized by the overproduction of oxalate due to a deficiency in the peroxisomal enzyme alanine-glyoxylate aminotransferase (AGT),

which normally converts glyoxylate, the precursor of oxalate, into glycine. The reduction of HAO1/glycolate oxidase by lumasiran treatment results in decreased level of metabolites that are precursors to toxic oxalate [40].

Preclinical studies reported that lumasiran effectively inhibited *HAO1* gene expression by more than 95% at all tested doses and nearly eliminates urinary oxalate production in a rat model of PH1 [41]. Phase I/II clinical trials of lumasiran showed that the drug was well tolerated in both adult and pediatric patients with PH1, with no serious adverse events reported (NCT02706886). Currently, lumasiran is being further evaluated in one Phase II clinical trial (NCT03350451) and three Phase III clinical trials (NCT03681184, NCT03905694, and NCT04152200) ongoing to evaluate its efficacy, safety, pharmacokinetics, and pharmacodynamics in adults, children, and infants [42].

Conclusion

RNA interference (RNAi)-based therapy represents a promising new approach for treating genetic diseases by inhibiting the expression of disease-causing genes. RNAi is a natural process that regulates gene expression by utilizing small RNA molecules to target and reduce the expression of specific genes. This method allows for precise intervention at the genetic level, potentially halting the production of faulty or excessive proteins that underlie various genetic disorders. While challenges remain, particularly in the areas of efficient delivery and long-term effects, RNAi-based therapies offer the potential to revolutionize the management of genetic diseases by providing more targeted and effective treatments.

Although further research and development are needed to overcome certain technical hurdles, RNAi therapy has opened new avenues for understanding of genetic mechanisms and intervening at a more fundamental level. These advancements suggest a paradigm shift in the treatment of genetic diseases, where RNAi-based therapies could provide more effective and sustainable solutions for patients with conditions that have difficult to treat with conventional methods.

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Declaration of interest

The authors declare no conflict of interest.

Author contributions

MUA: conceptualization, writing – original draft; SD: supervision, writing – review and editing.

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