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REVIEW

Clinical pharmacology of siRNA therapeutics: current status and future prospects

Ahmed Khaled Abosalha[a,b], Jacqueline Boyajian[a], Waqar Ahmad[a], Paromita Islam[a], Merry Ghebretatios[a], Sabrina Schaly[a], Rahul Thareja[a], Karan Arora[a] and Satya Prakash[a]

[a]Biomedical Technology and Cell Therapy Research Laboratory, Department of Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, Quebec, Canada; [b]Pharmaceutical Technology Department, Faculty of Pharmacy, Tanta University, Tanta, Egypt

ABSTRACT

Introduction: Small interfering RNA (siRNA) has emerged as a powerful tool for post-transcriptional downregulation of multiple genes for various therapies. Naked siRNA molecules are surrounded by several barriers that tackle their optimum delivery to target tissues such as limited cellular uptake, short circulation time, degradation by endonucleases, glomerular filtration, and capturing by the reticuloendothelial system (RES).

Areas covered: This review provides insights into studies that investigate various siRNA-based therapies, focusing on the mechanism, delivery strategies, bioavailability, pharmacokinetic, and pharmacodynamics of naked and modified siRNA molecules. The clinical pharmacology of currently approved siRNA products is also discussed.

Expert opinion: Few siRNA-based products have been approved recently by the Food and Drug Administration (FDA) and other regulatory agencies after approximately 20 years following its discovery due to the associated limitations. The absorption, distribution, metabolism, and excretion of siRNA therapeutics are highly restricted by several obstacles, resulting in rapid clearance of siRNA-based therapeutic products from systemic circulation before reaching the cytosol of targeted cells. The siRNA therapeutics however are very promising in many diseases, including gene therapy and SARS-COV-2 viral infection. The design of suitable delivery vehicles and developing strategies toward better pharmacokinetic parameters may solve the challenges of siRNA therapies.

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1. Introduction

Since its discovery in the late twentieth century by American pioneers, Andrew Fire and Craig Mello, RNA interference (RNAi) has revolutionized the medical and pharmaceutical fields drastically [1]. Its use became widespread as researchers around the world from numerous institutions began employing RNAi as a silencer for a wide variety of genes for the management of several related diseases. RNAi involves the utilization of 21–25-nucleotide, double-stranded RNA molecule known as 'siRNA' to regulate the post-transcriptional expression of the mRNA of the targeted gene. The siRNA is composed of two strands: a passenger strand (i.e. sense) and a guide strand (i.e. antisense) that is complementary to the mRNA of the gene of interest [2,3]. The siRNA with the aid of a multiprotein complex known as RNA-Induced Silencing Complex (RISC) recognizes the targeted mRNA and destroys it into nonfunctional moieties [4–6]. Despite the prominent mechanism of gene silencing via siRNA and its consistency for each target gene, only four siRNA products have been approved by FDA for human use. Patisiran was the first FDA-approved siRNA in 2018 [7], followed by the approval of Givosiran in 2019 [8] and Lumasiran as the third (2020) accredited siRNA [9]. Recently, Inclisiran has been approved for the treatment of hypercholesterolemia. The limited number of currently authorized formulations may be attributed to the difficulty of synthesizing a controlled drug delivery system that can carry siRNA safely and efficiently to the desired tissue while using a suitable concentration for a reproducible therapeutic effect.

The size of siRNA acts as a limitation in that it is large enough (7–8 nm in length) to penetrate cell membranes (about 5 nm in thickness) yet its relatively small size makes it vulnerable to glomerular filtration [10,11]. It is also highly susceptible to degradation by endonucleases, and it has a poor capacity for binding to circulating serum proteins and lipids. Moreover, siRNA can be easily retained by RES, endosomes, and lysosomes. The above-mentioned factors sharply decrease the half-life of siRNA to less than 10 minutes with limited distribution to the site of action [12–16]. Moreover, the physicochemical characteristics of siRNA represent an additional challenge for proper delivery. The siRNA possesses a negative charge due to the presence of phosphate groups on its backbone. Consequently, the cellular uptake of siRNA becomes diminished as a result of the electrostatic repulsion between siRNA and cell membranes [16]. Considering this, the efforts of many researchers have been oriented toward the development of suitable strategies to overcome such obstacles for the effective transport of siRNA to desired organs with an optimized pharmacokinetic pattern.

CONTACT Satya Prakash satya.prakash@mcgill.ca Biomedical Technology and Cell Therapy Research Laboratory, Department of Biomedical Engineering, Faculty of Medicine, McGill University, Montreal, Quebec H3A 2B4, Canada

Article highlights

- siRNA is a promising tool that targets the mRNA of the gene of interest and degrades it, providing effective management of gene-associated diseases at an early step.
- The delivery of siRNA has many challenges which restrict its therapeutic application.
- The chemical modification of original siRNA molecules may optimize the bioavailability, pharmacokinetic, and therapeutic efficacy of siRNA.
- Bioconjugation of ligands such as aptamers, peptides, lipids, and carbohydrates shows a targeted transport of siRNA to desired cells with a developed clinical pharmacology profile.
- The siRNA therapeutics approved by the United States FDA represent a new approach to controlling challenging genetic and other diseases.

Many techniques have been adopted to camouflage the characteristics of naked siRNA molecules to ensure appropriate transfer to targeted cells and to minimize reported barriers. Previous literature has focused on the chemical modification of the original siRNA molecule by adding functional groups to the ribose sugar, inter-nucleotide linkage, or nucleobases. Improved cellular uptake, high serum stability, low toxicity, and prolonged half-life have been recorded after chemical modification, demonstrating the probable outcome of this strategy for siRNA delivery [17,18]. Other research has shown motivation toward the conjugation of siRNA with specific ligands for pronounced targeting and higher cellular accumulation ratios. Several moieties have been implemented for bioconjugation with siRNA, including antibodies [19], peptides [20], aptamers [21], carbohydrates [22], lipids [23], and cell-penetrating peptides [24]. These ligands compromised an excellent affinity to several receptors in vivo, permitting an accurate and specific targeting of siRNA [16]. Recent advances in nanotechnology and nanoparticulate drug delivery systems have attracted the attention of numerous scientists to employ the basics of nanoscience in siRNA encapsulation. Diverse nanocarriers have been utilized to entrap siRNA and transfer it efficiently. Some of these carriers are fatty such as liposomes [25] and solid lipid nanoparticles [26], which exhibit good biocompatibility with biological fluids. Others are polymeric in nature, like poly(lactide-co-glycolide) (PLGA) nanoparticles [27] and chitosan-based nanocarriers [28], with a well-controlled and sustained release of siRNA, thereby prolonging its short half-life. Herein, we discuss the clinical pharmacology of various siRNA therapeutics either in literature or marketed products.

2. Mechanism of action

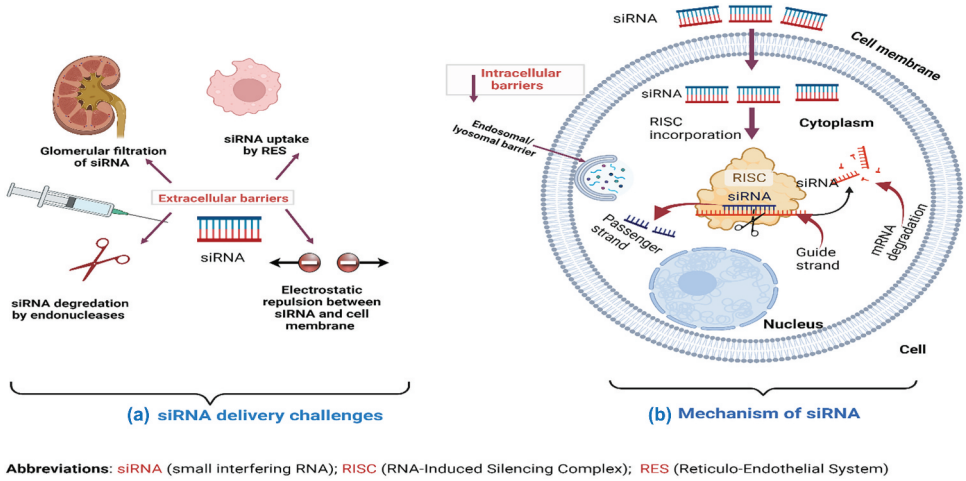
Simply, siRNAs are designed to block the expression of targeted genes at the post-transcriptional stage by degrading the mRNA that governs the regulation of these genes. Therefore, siRNAs are designated as small double-stranded RNA duplexes with a 21–23 nucleotide length. One strand of this duplex is complementary to the mRNA of the gene of interest and known as the 'guiding' or 'antisense' strand. This guiding strand can easily and specifically recognize the mRNA of the targeted gene and degrade it, a process known as 'gene silencing.' Gene silencing begins when a long double-stranded RNA (dsRNA) is cleaved by a specific dicer enzyme, a member of the RNAase family, into short siRNAs. Then, these siRNAs are incorporated into a multi-protein complex termed, ' (RISC).' Subsequently, siRNA binds to argonaute-2 receptors, a crucial component of RISC, with a consequent cleavage of siRNA duplex into two strands: the passenger (sense) strand and the guiding strand. The sense strand is degraded while the guiding strand recognizes the complementary mRNA of the gene of interest and destroys it into numerous nonfunctional units. This process downregulates the expression of targeted genes and proteins as demonstrated in Figure 1 [29–32].

3. Adverse effects of siRNA

It was previously and erroneously thought that RNAi functions at a high level of specificity. It has now been shown that this is not the case, in fact, a high portion of siRNA molecules have off-target sites. Adverse effects of siRNA can present themselves at numerous levels throughout signaling pathways; knocking down a gene can lead to unforeseen downstream changes, such as off-target gene silencing, innate immune system activation, and delivery system toxicity. Most of the off-target gene silencing occurs due to partial sequence homology [33]. Off-target effects can also be a consequence of immune system activation; toll-like receptors can recognize the RNA and trigger the release of cytokines which can alter gene expression. A potential method to correct for off-target effects is through chemical modifications. Certain chemical modifications can reduce the off-target effects and immunostimulatory activity whilst preserving the target sequence specificity [34,35]. This shows that it is crucial to design siRNA therapeutics with a high degree of specificity to avoid adverse effects that can occur downstream of the signaling pathway.

4. Barriers of siRNA delivery limiting siRNA therapies

The process of utilization of a specific drug or active pharmacological ingredient for the treatment of a certain disease does not end at its discovery or synthesis. It should be followed by the appropriate incorporation of the active component into a suitable drug delivery system that can deliver the drug with the required concentration to the targeted tissue while causing minimum side effects. The pathway of siRNA to the site of action represents a challenge toward its therapeutic efficacy. Numerous barriers interfere with siRNA before it arrives in the cytoplasm of targeted cells [36]. The RES is composed of noncellular, and cellular components originating from monocytes that play an integral role in the phagocytosis of foreign bodies and particles. Most of these cells are concentrated in the liver, spleen, and lymph nodes [37]. siRNAs molecules undergo extensive uptake by RES, limiting their biodistribution to targeted cells and minimizing their circulation time [38,39]. Several approaches were adopted to evade the opsonization of particles by RES especially through the surface modification using polyethylene glycol (PEG) polymer [40]. Nanoparticles (NPs) have been widely utilized as drug carriers for siRNA



Abbreviations: siRNA (small interfering RNA); RISC (RNA-Induced Silencing Complex); RES (Reticulo-Endothelial System)

Figure 1. Mechanism of action of siRNA and its delivery-associated challenges. Created with BioRender.com.

delivery to overcome its delivery barriers [41]. The surface charge of NPs should be controlled properly to be able to escape RES engulfment efficiently [42].

Degradation of DNA or RNA therapies following systemic administration is considered one of the most significant challenges in gene delivery or gene silencing. Unfortunately, siRNA is highly susceptible to catalytic degradation by nucleases. Therefore, the proper delivery of siRNA candidates requires a certain degree of resistance against serum and tissue endonucleases. Otherwise, it will be rapidly cleared from systemic circulation before its cellular internalization [12]. Additionally, the small size of siRNA makes it an ideal substrate for renal clearance by glomerular filtration [43]. The accumulation of siRNA in both kidneys after intravenous administration was reported to be approximately 40 times higher than in other tissues [44]. Moreover, the disposition and elimination of siRNA by the liver have also been reported [45]. The above-mentioned factors result in rapid clearance of siRNA candidates from the blood with a sharp decrease in its plasma concentration and subsequent failure of gene silencing activity.

Furthermore, siRNAs possess negative surface charges as a result of the phosphate backbone. Thus, the cellular uptake of these molecules is highly limited due to the repulsion between the negatively charged lipid bilayers of cell membranes and siRNAs. Although the size of siRNA is relatively small, it remains larger than cell membrane thickness (approximately 5 nm), making it unable to penetrate cell membranes [10]. Finally, upon cellular entry, siRNA is challenged by the acidity of the endo/lysosomal compartment, which may result in further degradation [46]. Such factors may help explain the current limited number of approved siRNA therapies, despite the prominent gene silencing activity of these candidates.

5. Strategies to overcome the barriers of siRNA delivery

As we discussed above, various obstacles interfere with the efficient delivery of siRNA to the intended sites of action. The administration of naked siRNA shows a diminished pharmacokinetic profile, which is characterized by poor absorption, incomplete distribution, limited cellular uptake, and rapid systemic clearance. Therefore, the proper delivery of siRNAs should be mediated by one or more of the following strategies. There are three major strategies to deliver siRNA therapies: chemical modification, bioconjugation, and nanocarrier-mediated delivery. Recent advances in nanotechnology have broadened the incorporation of a wide range of nanocarriers in siRNA delivery. Liposomes, niosomes, dendrimers, solid lipid nanoparticles, and polymeric nanoparticles are commonly utilized nanocarriers for siRNA delivery. Recently, new nanovesicles (exosomes) released from cells after the fusion of a multivesicular body with the plasma membrane are also introduced. Exosomes are well known for their ability to penetrate the blood–brain barrier and deliver the encapsulated drug to the brain. Additionally, it can be easily functionalized by different ligands to direct their delivery to the targeted tissue. Interestingly, it has been established that exosomes comprise elements of the RNA-induced silencing complex, such as argonaute-2 and its interrelating partner GW182, suggesting that they are intrinsically involved in miRNA-induced gene silencing [47]. Figure 2 represents common strategies for siRNA delivery.

5.1. Chemical modifications of siRNA for targeted siRNA delivery and therapies

Chemical modification acts as a significant strategy to optimize the delivery of naked siRNAs to overcome some delivery obstacles. The negatively charged phosphodiester skeleton of siRNA represents a powerful barrier to its cellular uptake through the anionic lipid bilayers of the cell membrane. Furthermore, the original structure of siRNA candidates makes them highly susceptible to degradation by endonucleases with a poor pharmacokinetic profile. Also, hazardous off-target side effects such as the unintended block of expression of other genes have been reported besides triggering the host immune response [48]. Consequently, chemically modified siRNA therapeutics can offer a high degree of cellular uptake and resistance against endonucleases in addition to minimizing the harmful off-target

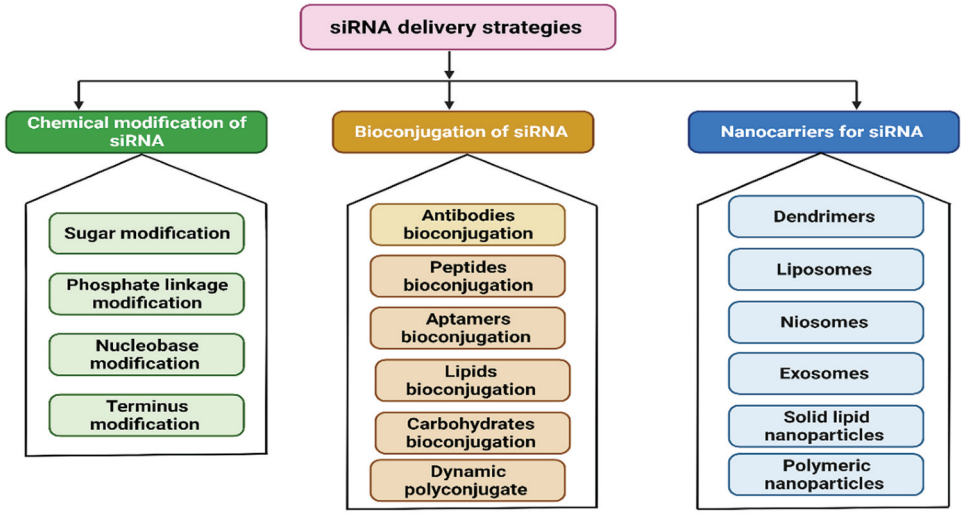


Figure 2. Delivery strategies for siRNA. Created with BioRender.com.

effects and antigenicity. Generally, both DNA and RNA are composed of nucleotides as building blocks. Nucleotides compromise a ribose or 2′-deoxyribose sugar moiety with 1′-nucleobase and 3′-phosphate groups. Four sites of chemical modifications to siRNA molecules were previously proposed, including the ribose sugar, nucleobase, phosphate link, and strand terminus [17].

The 2′-OH modification of the sugar was extensively studied due to its pronounced outcome on nuclease resistance and cellular uptake without affecting the gene silencing potential of modified siRNAs [49]. Some researchers substituted the 2′-OH group with 2′-o-methyl, 2′-o-methoxyethyl, or 2′-deoxy-2′-fluoro, whereas other studies experimented substitution with bicyclic compounds such as locked nucleic acids (LNA) or acyclic compounds like unlocked nucleic acids (UNA) [50]. The common goal of nucleobase modifications is to optimize the duplex stability while preserving the base pair recognition which is essential for the RNAi mechanism. Substitution of Ura with 5-bromo-Ura and 5-iodo-Ura substitutes are common examples of this strategy [17,51].

The phosphodiester inter-nucleotide linkage of siRNA makes it highly vulnerable to degradation by serum and cellular nucleases. Inter-nucleotide modifications were widely discussed to prolong the circulation time of siRNA and enhance its cellular internalization. Substitution of one phosphate oxygen with sulfur showed a significant improvement in the nuclease stability of the modified siRNA [52]. Concerning chemical modifications to the strand terminus, it is well established that the 5′-terminal of the guiding strand is not recommended for modifications due to the integral role of this region (i.e. the seed region) for the biological activity of siRNA. On the other hand, 3′ and 5′ terminus of the sense strand or 3′-terminal of antisense strand can be suitable alternative sites for strand chemical modifications [18,43]. Common examples for siRNA chemical modifications are illustrated in Figure 3.

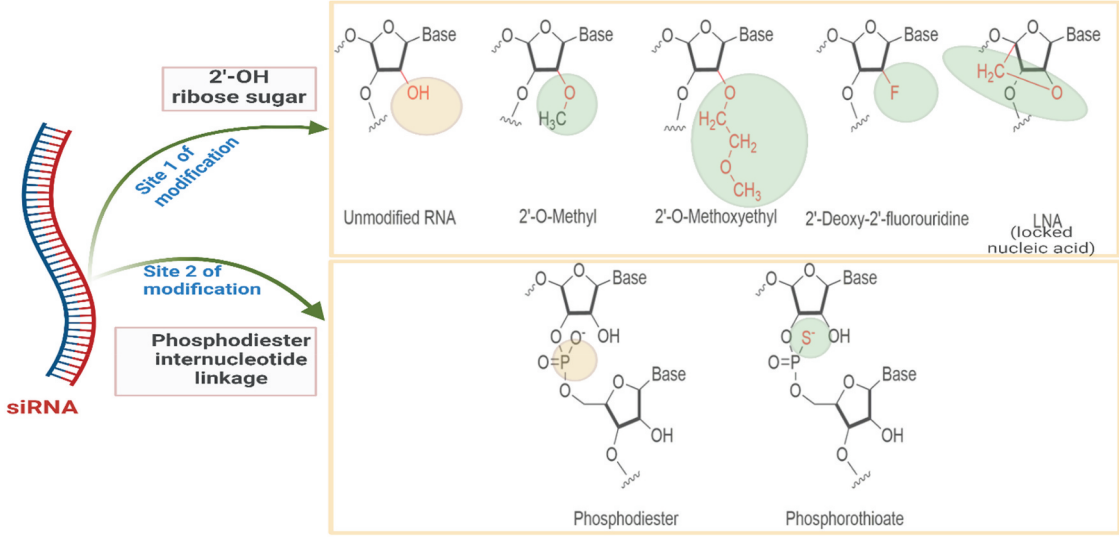


Figure 3. Examples of siRNA chemical modifications for targeted delivery. Created with BioRender.com.

5.1.1. Clinical pharmacology of chemically modified siRNA therapies

Notably, chemically modified siRNAs exhibit developed pharmacological and pharmacokinetic behaviors in terms of increased potency, improved serum stability, lower off-target adverse effects, and minimized immunostimulatory effects when compared to their unmodified counterparts. In a particular study, a fully modified siRNA composed of a passenger strand made of 2′-F-RNA pyrimidine and DNA purines while the guide strand was built from 2′-F-RNA pyrimidine, 2′-O-methyl purines, and one phosphorothioate linkage at the 3′-terminus. This modified siRNA showed improved potency in a hepatitis B virus (HBV) mouse model as compared to the unmodified siRNA. Both siRNAs experienced a dose-dependent reduction of serum viral DNA. The fully modified siRNA showed a 3.7 $\log_{10}$ decrease in serum HBV DNA with respect to a 2.2 $\log_{10}$ reduction with the unmodified siRNA at a dose of 1 µg. Surprisingly, the serum half-life of the modified molecule was 2 to 3 days, as compared to 3 to 5 minutes of the original siRNA [53]. Wang et al. synthesized a chemically modified siRNA by introducing 2′-fluoro and phosphorothioate to siRNA duplex and examined the influence of such modifications on the knockdown of human Cu-Zn superoxide dismutase enzyme (SOD). The authors concluded that modified siRNA retained the same gene silencing potential as the unmodified siRNA. On contrary, the modified molecule showed a prolonged retainability within cells for up to 6 days, while the naked siRNA was rapidly diffused out of cells after a few minutes of transfection. The down regulation of the mutant SOD gene may represent a new approach toward the control of neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) [54]. Based on the sustained circulation time of the chemically modified siRNA, these therapies are expected to show better distribution profiles throughout the central nervous system (CNS), especially to distant cells to slow the progression of ALS. Also, these neurodegenerative disorders are chronic and require frequent administration of high doses of the drug, making the use of chemically modified siRNA therapies with prolonged durations of action highly desirable.

5.2. Bioconjugation of siRNA delivery and therapies

The bioconjugation of siRNA to various functional moieties has emerged as an ideal strategy to counteract different barriers to siRNA delivery. The theory of siRNA bioconjugation imparts a high degree of flexibility in selecting the appropriate ligand or multiligand depending on desired characteristics. Wide ranges of conjugating agents are available for linkage to naked siRNA therapies. Bioconjugation may be employed using a single ligand or a combination of more than one ligand for efficient siRNA delivery. The siRNAs may be conjugated with targeting agents such as antibodies, peptides, and aptamers to transfer them specifically to desired tissues through a definite ligand-receptor uptake [55,56]. This type of bioconjugation ensures the active targeting of siRNA to intended cells with minimum off-target effects. Other researchers conjugated siRNA to lipids, such as cholesterol, docosanoic acid, lithocholic acid, and α-tocopherol (vitamin E). Lipid bioconjugates display a better pharmacokinetic profile due to the effective distribution of siRNA in various tissues based on the high binding affinity with serum low-density lipoprotein (LDL), high-density lipoprotein (HDL), and albumin which prolong the circulation time of siRNA. Moreover, it shows better cellular uptake as compared to unconjugated siRNA as a result of the high partitioning between conjugated lipids and lipid bilayers of cell membranes [57,58]. Another strategy of bioconjugation links siRNA to specific types of peptides known as 'cell-penetrating peptides.' These peptides are cationic and can penetrate cell membranes readily and deliver the conjugated siRNA to the cytosol of targeted cells due to the ionic attraction between these lipids and anionic cell membranes. Transportan, penetratin, and TAT are excellent examples of cell-penetrating peptides [59,60]. Another school of research experimented the bioconjugation of siRNA to small molecules of low molecular weight as folic acid or galactose derivatives. This method resulted in a targeted uptake of siRNA therapeutics by specific cells due to the overexpression of surface receptors for these ligands like folic acid receptor and asialoglycoprotein receptor [61,62]. Other studies focused on polymer-siRNA bioconjugates using certain polymers such as PEG which sustains the half-life of siRNA and increases its homogeneity with serum and body fluids [63]. An interesting recent strategy of siRNA bioconjugation comprises the incorporation of two or more ligands into polymeric skeleton known as 'dynamic polyconjugates.' This theory has changed the overview of bioconjugation toward facing more than one barrier by the same delivery system. The first dynamic polyconjugate was synthesized from a poly(butyl amino vinyl ethers) as a polymeric backbone with attached PEG and N-acetylgalactosamine ligands [64].

5.2.1. Clinical pharmacology of bioconjugated siRNA therapies

Biscans et al. designated a novel siRNA-docosanoic acid bioconjugate to deliver siRNA efficiently to skeletal and cardiac muscles to regulate the expression of the myostatin (muscle growth regulation) gene after systemic injection. They investigated the distribution, gene silencing potential, and off-target inflammatory effects of the administered siRNA. Interestingly, they found that this siRNA lipid conjugate was able to block the regulation of the targeted gene by about 55% and 88% in muscle tissue and heart muscles, respectively. They also reported the prolonged gene silencing behavior of this bioconjugate over 1 month, including a 50% increase in muscle volume after 1 week of injection. In addition to the minimized off-target effects of this delivery system demonstrated by no cytokine stimulation up to a 100 mg/kg dose [23]. Wolfrum et al. examined the potential role of lipid bioconjugates in optimizing siRNA pharmacokinetic and gene silencing efficacy. They illustrated that the developed and prolonged distribution profiles of siRNA-lipid bioconjugates are mainly attributed to their higher affinity for binding to circulating lipoproteins, especially HDL and LDL. Wolfrum et al. synthesized two bioconjugates: cholesterol-siRNA and HDL-cholesterol-siRNA. Subsequently, they studied the effect of both bioconjugates on the regulation of the apoB gene in a mice animal model.

Their findings showed that the plasma level of apoB was significantly reduced by 8 to 15-fold with HDL-cholesterol-siRNA complex as compared to cholesterol-siRNA, demonstrating the potential outcome of binding to lipoproteins in the optimization of distribution and gene silencing of siRNA therapeutics [58]. Another study conducted by Nair et al. targeted the delivery of siRNA to hepatocytes by conjugating siRNA to N-acetylgalactosamine to deliver it specifically to the liver through asialoglycoprotein receptors. This complex achieved a 5-fold increase in gene silencing as compared to the unconjugated siRNA after subcutaneous administration. As a result, the administration of siRNA was facilitated at relevant doses and a suitable volume (≤1 ml). Weekly administration of this conjugate exhibited a sustained gene silencing potential over 9 months. Such types of bioconjugation open the door toward the efficient treatment of hepatic diseases that involve the misregulation of liver genes [62].

5.3. Nanotechnology-based siRNA delivery and therapies

Richard Feynman introduced the term 'nanotechnology' in 1959 to describe the application of materials at a nanoscale size range. It is well-established that nanosized materials exhibit outstanding features as compared to their coarse size range. Scientists started to incorporate the basics of nanotechnology into their diverse research interests to get the maximum benefit of this new theory. Medical and pharmaceutical researchers experimented the proper utilization of nanosized materials for the treatment, diagnosis, and prophylaxis of numerous clinical complications. Regarding the delivery of siRNA, which is tackled by several intracellular and extracellular barriers, may nano-based drug delivery systems aid to overcome some or even all of these challenges? Below we discuss some of the employed nanocarriers to deliver siRNAs efficiently to target organs.

5.3.1. Polymers-based nanocarriers for siRNA delivery

The small size of nanoparticles is the key determinant of their characteristics. Nanocarriers show small particle size, high surface area, better solubility [65,66], higher tissue penetration ability [67], prolonged systemic circulation [68,69], and a well-developed pharmacokinetic profile [70,71]. Polymers are natural or synthetic macromolecules composed of several building blocks called 'monomers.' Polymers were reported extensively for the delivery of various active pharmaceutical ingredients to optimize their therapeutic efficacy. Polymers are diverse and have different properties which enable researchers to select the proper polymer efficiently based on the desired goal. Some polymers are hydrophilic while others are lipophilic or amphiphilic. Certain polymers show immediate release of the encapsulated drug whereas others display a sustained release behavior. PLGA, poly(lactic acid) (PLA), poly(glycolide) (PLG), Eudragit RS100, Eudragit S100, and chitosan are some examples of polymers that have been investigated for drug delivery. PLGA is the most popular polymer to encapsulate siRNA and deliver it properly to target cells. PLGA was approved by FDA for clinical applications due to its prominent biodegradability, biocompatibility, safety, and prolonged systemic circulation [72]. Both siRNA and PLGA are anionic, meaning that the encapsulation of siRNA may act as a challenge toward the effective entrapment within PLGA nanoparticles. One possible solution is to add a small amount of positively charged polymer, such as polyethyleneimine to increase the entrapment efficiency [73]. Chitra et al. formulated siRNA-loaded PLGA nanoparticles using poly-L-lysine as a complexing agent in order to overcome multidrug-resistant ovarian cancer by suppressing genes responsible for the efflux of chemotherapeutics [74]. Chitosan is a well-known natural polysaccharide approved by FDA for clinical use and drug delivery. It carries a positive charge which may enable it to bind efficiently to the negatively charged siRNA with an improved encapsulation efficiency [74]. Additionally, chitosan is water-soluble which can limit the use of toxic inorganic solvents during the formulation of nanoparticles [75]. Also, it shows mucoadhesive properties which prolong its contact time with the mucosa at the site of action, thereby enhancing its absorption. Moreover, chitosan improves the paracellular and transcellular transport of loaded drugs by opening the tight junctions of the epithelium, acting as a permeability enhancer [76]. 'Dendrimers,' another unique polymer-based nanocarriers, were introduced by Tomalia in 1985. Dendrimers show a specific three-dimensional geometry involving peripheral functional moieties radiating from a central core in a shape that resembles a tree. Poly(amidoamine) dendrimers were the first synthesized type of dendrimers [77]. Meanwhile, cationic dendrimers may represent an effective delivery system that is able to carry anionic siRNA molecules successfully to target cells. Peripheral functionalities of dendrimers may undergo further decorations to counteract several mentioned barriers [78]. Chen et al. developed an amphiphilic dendrimer to distribute siRNA to the challenging primary immune cells, such as macrophages, natural killer cells, T cells, and B cells. The siRNA-loaded dendrimer was simply synthesized by mixing siRNA and dendrimer in a buffer solution at room temperature [79].

5.3.1.1. Clinical pharmacology of siRNA-loaded polymeric nanocarriers.

Polymeric nanocarriers have emerged as competent drug delivery systems to transfer siRNA with a pronounced gene silencing activity. Kwak et al. investigated the effect of siRNA loaded PLGA nanoparticles to block the expression of programmed cell death protein 1 (PD1) and programmed cell death protein ligand 1 (PDL1). Simply, PD1 is overexpressed on the surface of tumor-infiltrating T lymphocytes, while PDL1 is its ligand. The interaction between PD1 and PDL1 results in depletion of effector T cells with a subsequent impairment of the host immune system, facilitating the escape of tumor cells. Silencing of PD1 and PDL1 may offer a significant strategy to stimulate tumor cell death. PLGA nanoparticles encapsulating siRNA were prepared via the reported double emulsion solvent evaporation technique. The findings revealed a significant uptake of siRNA-PLGA nanoparticles in CD8 + T cells and tumor cells, demonstrating the role of nanoparticles to optimize the cellular internalization of siRNA. The transfection with siRNA-

PLGA nanoparticles reduced the expression of PDL1 in MC38 cells by 75.4% 72 h after transfection. Additionally, the expression of PD1 decreased 24 h after transfection in the anti-CD3 /CD28-stimulated CD8 + T cells in a dose-dependent manner, compared to the control CD8 + T cells [80]. In an interesting study by Woensel et al., the delivery of siRNA to CNS was targeted through the intranasal route of administration for the management of glioblastoma multiforme. The prepared siRNA-chitosan suspension could successively deliver siRNA, resulting in a significant reduction in Galectin-1 (Gal-1) protein that is highly expressed in glioblastoma multiforme by more than 50% within a few hours following administration. These findings knock the door toward targeting siRNA to the brain and lungs through the nasal cavity and may aid to face the aforementioned delivery barriers [81]. Jain et al. formulated siRNA loaded Eudragit E 100 (dimethyl aminoethyl methacrylate copolymer) nanoparticles to deliver siRNA to the cytosol of macrophages for targeting bacterial species (e.g. *Mycobacterium tuberculosis* (MT)) within macrophages. The endogenous host gene Bfl1/A1 promotes the survival of MT inside the macrophage of the host, allowing its knockdown to stimulate its death and provide an efficient strategy to manage this lethal infection. The siRNA-Eudragit E100 nanoparticles showed a 5-fold increase in Bfl1/A1 silencing as compared to free siRNA administration [82]. Notably, Dong et al. established a unique siRNA dendrimer to transport siRNA to cancer cells and decorated its surface with RGDK peptide that can bind with a high affinity to integrin and neuropilin-1 receptors overexpressed on cancer cells. This technique of active siRNA targeting showed a noticeable cellular uptake, higher stability, and endosomal escape of the encapsulated siRNA with a consequent stronger gene silencing potential and minimum off-target harmful effects [83]. Thus, we may conclude that polymeric nanocarriers appear to be a safe, stable, targeted, and sustained delivery system for siRNA encapsulation. Additionally, the availability of a wide range of polymers gives a higher degree of flexibility to researchers looking forward to optimizing the delivery of naked siRNA therapies.

5.3.2. Lipid-based nanocarriers for siRNA delivery

As we discussed before, the potential role of lipid-siRNA bioconjugates in optimizing the delivery of siRNA, and here we will demonstrate the probable outcome of utilizing lipid-based nanocarriers in the enhancement of the bioavailability and gene silencing activity of siRNA therapies. Liposomes and solid lipid nanoparticles are common examples of fatty nanocarriers that have been incorporated earlier in drug delivery. Liposomes have been extensively reported as vehicles for increasing the bioavailability and stability of drugs due to their excellent biocompatibility. Liposomes are lipid nanovesicles characterized by a bilayer structure with a hydrophilic core and hydrophobic bilayers. This unique morphology enables it to encapsulate a wide range of aqueous, lipophilic, and amphiphilic biologically active components [84]. Natural or synthetic phospholipids are the major component of liposomal vesicles, offering a high degree of biocompatibility with phospholipid components of cell membranes [25]. Liposomes increase the stability of loaded siRNA, prevent its degradation, prolong its systemic circulation, and deliver it passively to target cells, especially in cases of cancer diseases that show an enhanced permeability and retention effect [85]. Unfortunately, the stability of liposomes acts as a challenge due to the inclusion of high amounts of fatty phospholipids in their structure that are readily susceptible to oxidative degradation. Niosomes are similar vesicles composed of nonionic surfactants as a replacement for phospholipids and show an excellent degree of physical stability, making them a suitable alternative to liposomes [86]. Niosomal vesicles have also been reported for optimized siRNA delivery [87]. Solid lipid nanoparticles (SLNs) represent an advanced class of lipid nanocarriers compromising specialized lipids that remain in the solid form at body temperature. SLNs show superior stability, entrapment efficiency, and sustained release of the encapsulated siRNA therapeutics [26,88]. Table 1 summarizes the major differences between nano-based drug delivery systems [89,90]

5.3.2.1. Clinical pharmacology of siRNA-loaded lipid nanocarriers.

In a particular trial to target breast cancer cells, Bedi et al. prepared siRNA-entrapped liposomal vesicles and investigated their effects on gene silencing of proline-rich domain proteins-14 (PRDM14) gene and its translated protein in MCF-7 cells. The formulated liposomes were coated with PEG polymer to increase their stability and experienced further linkage to the 'DMPGTVLP' phage coat protein to allow the targeted delivery of siRNA to MCF-7 cells through the interaction between the phage protein and its surface receptor on breast cancer cells. The phage protein affinity to MCF-7 showed more than 2000 times higher than the binding of a control group, indicating the advanced specificity of liposomal targeting by a phage protein. This siRNA formula was able to silence PRDM by 44% after 72 hours of administration [91]. In another study conducted by Song et al., they evaluated the potential outcome of silencing of integrin β6 protein, which is highly expressed in malignant colon cancer cells on the growth and metastasis of colon carcinoma. They prepared immunoliposomes encapsulating β6-siRNA and measured its cellular uptake, gene silencing, and its effect on tumor growth. The findings showed a pronounced gene silencing ability of about 75% as compared to the control group. Additionally, they reported high cellular internalization with a subsequent significant tumor growth inhibition [92]. Recently, Hanafy et al. synthesized siRNA loaded SLNs to downregulate the expression of PD1 protein in macrophages and cancer tissues in mice and examined its effect on tumor growth. The siRNA was successfully entrapped within SLNs, recording 98.9% encapsulation efficiency. The release of siRNA was in a sustained manner over 16 days. This prolonged-release profile is highly advantageous because it decreases the frequency of administration of siRNA therapies, especially in cases of chronic diseases. The in vitro results revealed that siRNA-SLNs could significantly inhibit tumor growth. Additionally, immunohistostaining showed that the expression of PD1 in the tumor tissues from mice treated with the siRNA-SLNs was decreased, as compared with the control group that received PBS. This study demonstrates the potential role of synthesized SLNs to inhibit tumor growth and cancer metastasis [93].

Table 1. Major differences between nano-based drug delivery systems.

	Polymeric nanoparticles	Solid lipid nanoparticles	Liposome	Dendrimer
Characteristics	Solid, nanosized (10–1,000 nm) colloidal particles made up of biodegradable polymers	Synthesized from lipids that are solid at room temperature	Spherical vesicles having an aqueous core enclosed by lipid bilayers	Distinctive molecular weight, increased number of branching, multivalency, spherical shapes and monodispersed macromolecules (1.5–14.5 nm)
Advantages	• Better stability on storage and in vivo • Higher drug payload • More homogeneous particle size distribution • Better and controllable physicochemical properties • Higher drug circulation times • More controlled drug release	• Controlled drug delivery • No biotoxicity • High drug payload • Improved bioavailability of poorly water-soluble drugs • High stability • less expensive • large-scale production	• Enhanced drug delivery • Protection of active drug from environmental factors • Protecting the encapsulated drug from early degradation • Cost-effective formulations of expensive drugs • Improved pharmacokinetic properties compared to free drugs	• Easy drug loading via hydrogen bonds, chemical linkages, or hydrophobic interactions. • Contact precision at cell walls and biologically active sites
Disadvantages	• High cost • Require advanced techniques of fabrication	• Fast elimination from the blood flow • Poor encapsulation of hydrophilic and ionic drugs	• Physical instabilities • Poor encapsulation of Aqueous and ionic drugs	• Low aqueous solubility • Nonspecific toxicity

5.4. Cation-free siRNA based spherical nucleic acids

Cation-free delivery of siRNA is a promising approach that enables efficient siRNA delivery while avoiding cation-associated cytotoxicity and plasma protein interaction which can result in rapid siRNA clearance [94,95]. For example, Zheng et al. [96] created a siRNA vehicle using hydrophilic and thermal-and-intracellular reduction sensitive hydrophobic layers for controlled loading and release of the siRNA. The vehicle could also be loaded with both doxorubicin hydrochloride and anti-P-glycoprotein siRNA as an additive treatment for resistant cancer cells. Comparatively, Jensen et al. [97] created spherical nucleic acid nanoparticles consisting of gold and siRNA duplexes capable of crossing the blood–brain barrier and targeting oncogenes overexpressed in Glioblastoma multiforme. The silencing of antiapoptotic signaling led to a reduction in tumor burden and no adverse side effects in the treated mice. Similarly, self-assembly of siRNA-disulfide-poly(N-isopropyl acrylamide) (siRNA-SS-PNIPAM) diblock copolymers, creating a siRNA micelle, also demonstrated blood-brain-barrier (BBB) penetration, inhibited tumor growth, increased survival time, and did not cause any adverse effects in mice [98]. Recently, a phase 0 human clinical study investigating SNAs consisting of gold-nanoparticles incorporating siRNA, directed against Bcl2L12, revealed promising results for glioblastoma. Specifically, the SNAs penetrated the BBB and reached the glioblastoma, reduced the expression of Bcl2L12 protein, and did not cause adverse effects [99]. Examples of Targeted siRNA delivery are summarized in Table 2.

6. The siRNA therapeutics in clinical trials

Nowadays, there are several promising siRNA therapeutics in clinical trials. These siRNAs are designed to target many genetic disorders, providing a future perspective on the predictable outcome of this strategy of RNA interference in gene therapy [9]. Table 3 summarizes some of the siRNA therapeutics in clinical trials.

7. Clinical pharmacology of currently used siRNA therapeutics

7.1. Patisiran

Patisiran was the first authorized siRNA therapy for the treatment of hereditary variant transthyretin amyloidosis (ATTRv) in August 2018. Simply, ATTRv is a rare genetic disease that affects approximately 5000–1000 people worldwide [109]. Patients with ATTRv are characterized by amyloidosis due to

Table 2. Examples of targeted siRNA delivery.

siRNA	Delivery strategy	Targeted organ/cell	References
siRNA-antiCD71	Bioconjugation to antibodies	Skeletal and cardiac muscle cells	[55]
siRNA- atherosclerotic plaque-specific peptide-1 (AP1)	Bioconjugation to peptides	Solid tumors	[100]
siRNA- N-acetylgalactosamine (GalNAc)	Bioconjugation to carbohydrates	Hepatocyte	[62]
siRNA- anti-spike protein aptamer	Bioconjugation to aptamers	SARS-COV-2 infected cells	[101]
Hsp27 siRNA	Dendrimers	Prostate cancer cells	[102]
cRGD- anti STAT3-siRNA	Liposomes and peptide bioconjugation	Melanoma cells	[103]
siRNA- EphA2	Solid lipid nanoparticles	Prostate cancer cells	[104]
siRNA-chitosan	Polymeric nanoparticles	CNS	[81]
siRNA- Eudragit E 100	Polymeric nanoparticles	Macrophages	[82]

Table 3. siRNA therapeutics in clinical trials.

S. No	siRNA	Delivery strategy	Diseases	Phase trials	References
1	Vutrisiron	Bioconjugation with *N*-acetylgalactosamine (GalNAc)	Hereditary transthyretin mediated amyloidosis	Phase 3 trials Elios-A (NCT03759379)	[105]
2	Nedosiran	Bioconjugation with *N*-acetylgalactosamine (GalNAc)	Primary hyperoxaluria	Phase 3 trials PHYOX 3 (NCT04042402)	[9]
3	Fitusiran	Chemical modifications and bioconjugation with *N*-acetylgalactosamine (GalNAc)	Hemophilia A & B	Phase 3 trials ALTAS-A/B (NCT03417245)	[106]
4	Teprasiran	Chemical modifications	Acute kidney injury Delayed graft functions	Phase 3 trials REGIFT (NCT02610296)	[9]
5	Cosdosiron	Chemical modifications	Non-arterictic anterior ischemic optic nueropathy	Phase 2/3 trials (NCT02341560)	[107]
6	Tivanisiran	Naked siRNA molecule	Dry eyes Ocular pain	Phase 3 trials Helix (NCT03108664)	[108]

the deposition of insoluble mutant transthyretin protein fibrils [110]. Transthyretin protein is produced by the liver and is known to be a transport protein for the thyroid hormone and retinol-binding protein [111]. Gene mutation of the transthyretin protein is the leading cause of precipitation of this mutant misfolded protein, leading to cardiomyopathy and polyneuropathy [112].

7.1.1. Pharmaceutical dosage form

Naked siRNA delivery is tackled by several obstacles that can restrict its therapeutic efficiency and lead to failure of treatment. Patisiran is delivered via a liposomal nano-based drug delivery system. Patisiran is loaded within liposomes fabricated from ionizable positively charged lipids (DLin-MC3-DMA), phospholipid, and cholesterol, with a surface decoration by PEG. Mixing of these ingredients under acidic conditions results in a spontaneous assembly of pH-dependent liposomal vesicles. Under neutral pH, liposomes solidify into solid lipid nanoparticles which experience better stability and entrapment efficiency characteristics [113–115]. Additionally, patisiran structure exhibited two chemical modifications, 2-methoxy-modified ribose residues, and 2-deoxythymidine residues, in order to optimize its stability and minimize its harmful adverse effects [113,116]. The adopted drug delivery system ensures higher stability of the encapsulated siRNA against endonucleases and targeted delivery to hepatocytes.

7.1.2. Pharmacodynamic

Patisiran is designated to block the expression of the transthyretin protein gene to decrease the deposition of insoluble protein fibrils. It follows the general mechanism of RNAi therapies by degrading the mRNA of this gene through its complementary guide strand as discussed previously. Following its systemic administration, the circulating PEG-coated patisiran is recognized by serum lipoproteins, specifically apolipoprotein E (apo E) due to the interaction between apo E and the cholesterol component of lipid nanoparticles. This complex targets patisiran directly to the liver where transthyretin protein is translated. This active drug targeting hepatocytes increases the concentration of the drug in the liver while minimizing its off-target side effects. Hepatocytes efficiently uptake apo E-decorated lipid nanoparticles and deliver them to the endosomes [116,117]. The acidic environment of endosomes converts anionic DLin-MC3-DMA lipids into cationic molecules that stimulate osmosis inside endosomal vesicles with subsequent rupture [117,118]. Finally, patisiran nanoparticles are released within the cytosol and begin the pathway toward the downregulation of transthyretin gene. The systemic administration of patisiran demonstrated a decrease in the serum level of transthyretin protein by almost 80% within 10 to 14 days after a single dose of 0.3 mg/kg through intravenous infusion once every 3 weeks in patients with ATTRv amyloidosis. After repeating doses within 9 and 18 months, transthyretin serum level was reduced by 83% and 84%, respectively, and reached the maximum reduction by 88% after 18 months [119].

7.1.3. Pharmacokinetic

According to patisiran full prescribing information by FDA, patisiran is administered via intravenous infusion at a dose of 0.3 mg/kg once every 3 weeks. The plasma concentration–time curve of patisiran exhibits a dose-dependent increase in the mean steady-state concentration and area under the curve (AUC), following its infusion. It takes about 6 months to reach the steady-state plasma concentration with a 3.2-fold AUC value as compared to the first dose. The estimated mean steady-state concentration and AUC are equal to 7.15 ± 2.14 μg/mL and 184 ± 159 μg.hr/mL, respectively. Patisiran is distributed principally to hepatocytes where the gene expression knockdown occurs with a volume of distribution equal to 0.26 ± 0.20 L/kg and a limited plasma protein binding (≤2.1%). It is metabolized mainly by the liver by endogenous endonucleases and shows a total body clearance of about 3.0 ± 2.5 mL/hr/kg. Less than 1% of the drug is excreted unchanged in the urine and the elimination half-life is 3.2 ± 1.8 days [119].

7.2. Givosiran

Givosiran was the 2nd approved siRNA therapy by FDA for the treatment of acute hepatic porphyria (AHP) in 2019 [8]. AHP is a rare, genetic, and progressive disease that is caused by the overexpression of certain enzymes involved in heme biosynthesis [120]. Heme biosynthesis in the liver is primarily catalyzed by δ-aminolevulinic acid synthase 1 (ALAS1) enzyme. The upregulation of hepatic ALAS1 results in buildup of toxic heme intermediates, δ-aminolevulinic acid (δ-ALA), and porphobilinogen (PBG) [121,122]. These intermediates are highly

neurotoxic, leading to lethal and acute porphyria attacks in addition to chronic manifestations.

7.2.1. Pharmaceutical dosage form

Givosiran is a synthetic siRNA conjugated to N-acetylgalactosamine that actively targets it to hepatocytes for the downregulation of ALAS1, thereby decreasing plasma levels of δ-ALA and PBG [123]. Givosiran is marketed as a sterile water-soluble solution for subcutaneous injection that contains 189 mg of givosiran in a single dose.

7.2.2. Pharmacodynamics

After subcutaneous administration, the circulating givosiran is selectively targeted to hepatocytes through a ligand–receptor interaction between N-acetylgalactosamine and asialoglycoprotein receptors on hepatocytes, stimulating endocytosis [124]. After that, givosiran is incorporated into RISC with a consequent unwinding into two strands. The guiding strand binds to the complementary mRNA of ALAS1, leading to its degradation and the block of ALAS1 protein translation [123]. A significant reduction in the level of δ-ALA and PBG in urine by 83.7% and 75.1%, respectively, was observed after 2 weeks following the first dose of givosiran 2.5 mg/kg once monthly in patients with AHP. Peak reductions in δ-ALA and PBG levels were reached around the third month, with a median reduction of 93.8% for δ-ALA and 94.5% for PBG, and it was sustained subsequently with recurrent once-monthly dosing [125]. Chan et al. demonstrated the gene silencing of hepatic ALAS1 by siRNA-loaded solid lipid nanoparticles in animals using a mouse model of acute intermittent porphyria. The findings showed that givosiran significantly ($p < 0.05$) reduced the hepatic and serum levels of ALAS1 mRNA at 24 hr and 48 hr after treatment [126].

7.2.3. Pharmacokinetic

Based on its full prescribing information by FDA, pharmacokinetic parameters of givosiran and its active metabolite AS (N-1)3′-givosiran were measured in patients with AHP. Givosiran is administered via subcutaneous injection at a dose of 2.5 mg/kg and 5 mg/kg. The plasma concentration–time curve of givosiran and its metabolite shows a dose-dependent increase in the mean steady-state concentration and AUC over a dose range of 0.35 mg/kg to 2.5 mg/kg by 0.14 to 1-fold the approved prescribed dosage when administered once monthly. Moreover, C_{max} and AUC for both have a slightly greater increase at doses over 2.5 mg/kg once monthly. The estimated mean steady-state concentrations for givosiran and AS(N-1)3′-givosiran were 321 ng/mL and 123 ng/mL, respectively. Whereas, the AUC for givosiran and its metabolite are estimated to be 4130 ng. hr/mL and 1930 ng. hr/mL, respectively. T_{max} was estimated by approximately 3 hr for givosiran and by around 7 hr for the metabolite. Givosiran is distributed mainly to hepatocytes with a volume of distribution of about 10.4 L/kg and shows an extensive plasma protein binding (90%). It is principally metabolized by endogenous endonucleases to shorter oligonucleotides and shows a total body clearance of about 35.1 L/hr. Nearly, 5–14% of the drug and 4%–13% of the metabolite are excreted in urine [125].

7.3. Lumasirnan

Lumasiran is the most recently approved siRNA therapy by the FDA for the management of primary hyperoxaluria type 1 (PH1) in 2020. PH1 is a rare, genetic, and progressive disorder caused by a metabolic defect in the hepatic alanine–glyoxylate aminotransferase (AGT) production. AGT converts glyoxylate to glycine, the downregulation of this enzyme results in the oxidation of glyoxylate directly to oxalate by the glyoxylate oxidase enzyme [127,128]. This metabolic defect causes an increased plasma level of oxalate with subsequent kidney failure as a result of the excretion of high amounts of this toxic mediator. Additionally, the resulted systemic oxalosis leads to a further precipitation of oxalates in other tissues, such as skin, retina, bone, and heart [129,130]. The adopted strategies for the management of this lethal disease involve hyperhydration, calcium oxalate precipitation inhibitors, hemodialysis and may extend to liver transplantation [131–133]. Lumasiran was designated to block the expression of hepatic glyoxylate oxidase enzyme by degrading its mRNA, preventing the conversion of glyoxylate to oxalate with subsequent accumulation of the readily excreted glyoxylate intermediate [134,135].

7.3.1. Pharmaceutical dosage form

Lumasiran is supplied in the form of 94.5 mg/0.5 mL clear solution for subcutaneous injection. The siRNA is covalently linked to N-acetylgalactosamine to deliver it directly to the liver.

7.3.2. Pharmacodynamics

Lumasiran targets the mRNA of hydroxyacid oxidase 1 enzyme and degrades it to reduce the level of glycolate oxidase via RNAi. The pharmacodynamic effects of lumasiran were investigated through the administration of different doses with variable frequency to adult and pediatric patients with PH1. The urinary oxalate level showed a significant reduction in a dose-dependent manner. The onset of reduction was observed 2 weeks after the administration of the first dose with the maximal decline in urinary oxalate level after 2 months [136]. In a specific study by Garrelfs et al., they conducted a random study on 39 patients, 26 of them were treated with lumasiran and the other 13 received a placebo. They reported a significant decrease in the 24-h urinary oxalate excretion by 53.5% as compared to the placebo group during the 6 months of treatment. Most of the patients experienced normal or pre-normal levels of urinary oxalate at month 6. These findings were supported by the decline in plasma oxalate level, indicating the potential outcome of RNAi by lumasiran [137].

7.3.3. Pharmacokinetics

The plasma concentration–time curve of lumasiran exhibits a dose-dependent increase in the mean steady-state concentration and AUC over doses ranging from 0.3 mg to 6 mg/kg and time-independent pharmacokinetics with multiple doses of 1 and 3 mg/kg, administered once monthly or 3 mg/kg

quarterly. The calculated mean steady-state concentration is 462 ng/mL and the AUC is estimated by about 6810 ng. hr/ml. T_{max} is valued by about 4 hr. Lumasiran is distributed primarily to the liver with a volume of distribution of about 4.9 L/kg and a plasma protein binding of 85%. It is mainly metabolized by endogenous and exogenous nucleases to shorter oligonucleotides and exhibits a total body clearance of about 26.5 L/hr. Less than 26% of lumasiran dose is excreted unchanged in the urine within 1 day [136].

7.4. Inclisiran

Inclisiran is an exciting new therapy that was recently approved by the FDA as the first siRNA pharmacotherapy. Unlike the majority of other siRNA therapies, inclisiran is unique in its indication to treat hypercholesterolemia. Inclisiran lowers circulating low-density lipoprotein cholesterol (LDL-C) by preventing the translation of protein convertase subtilisin/kexin type 9 (PCSK9) mRNA. Typically, PCSK9 binds and degrades LDL receptors. Inhibition of PCSK9 leads to increased LDL-C receptors and subsequent binding, thereby decreasing serum levels of LDL-C. Although statins are the standard of care for managing cholesterol levels, many patients fail to meet their therapeutic goals. Inclisiran is a promising new approach that acts as an adjunct to diet and maximally tolerated statin therapy for the treatment of heterozygous familial hypercholesterolemia (FH) or clinical atherosclerotic cardiovascular disease (ASCVD) in adults who require additional lowering of LDL-C. It is administered as a subcutaneous injection (284 mg/1.5 mL) into the abdomen, upper arm, or thigh initially, again at 3 months, and then every 6 months.

Late phase-three clinical trials by Novartis demonstrated great success with inclisiran in reducing LDL-C and PCSK9 levels in patients with heterozygous FH, ASCVD, or ASCVD risk factors. Inclisiran also showed favorable safety and tolerability with mild to moderate adverse events that were similarly frequent between treatment and placebo study groups. Inclisiran offers a novel approach to inhibiting PCSK9 that is more cost-effective with a convenient dosage for patients, compared to its anti-PCSK9 monoclonal antibody competitors (e.g. evolocumab). Overall, the success of inclisiran as a therapy for hypercholesterolemia has extended the clinical application of siRNA therapeutics far beyond orphan diseases and strengthened the role of RNAi in modern pharmacology. Inclisiran is conjugated on the passenger strand with triantennary N-Acetylgalactosamine to facilitate uptake by hepatocytes [9,138–141]. Table 4 demonstrates the pharmacokinetic parameters of inclisiran [142].

8. Conclusion and future prospects

Despite the demonstrated prominent efficacy of siRNA, its optimal utilization is limited by the challenge of delivering to the target site of action. Previous literature has focused on designing suitable techniques for siRNA protection and targeting such as the chemical modification or linkage with specific ligands. Other studies experimented various carrier-mediated systems like liposomes, niosomes, and polymer-based nanoparticles. The siRNA therapeutics may represent a new approach to the treatment of several progressive genetic diseases caused by the defective regulation of underlying genes. The knockdown of the expression of causative genes may help to control these complications at an early step. Many types of cancer diseases depend on growth-promoting proteins that enable them to grow and spread rapidly. The downregulation of such types of proteins may represent an effective strategy to slow the progression of tumors earlier. Glioblastoma multiforme, one of the most widespread and lethal type of primary brain tumor may be targeted by siRNAs [143]. Also, siRNA therapeutics may decrease the progress of neurodegenerative diseases by silencing signal mediators responsible for cell degeneration [144]. Various viral infections may be treated with siRNA therapeutics by designing an appropriate siRNA sequence that targets the mRNA of viral genes, thereby inhibiting its replication. Currently, COVID-19 is considered a milestone for media and news all over the world. Severe complications such as acute respiratory distress syndrome, multiple organ failure and death, especially for the elderly and those who experience chronic diseases, have been recorded as a result of this viral infection. This extremely transmissible virus rapidly becomes a pandemic with more than 400 million confirmed cases and more than 5 million deaths reported worldwide (WHO report, February 2022). The competition to design suitable vaccines for SARS-COV-2 started quickly and is enduring. However, the continual appearance of multiple viral variants has limited the efficacy of some vaccines against these variants [145,146]. The virion of SARS-COV-2 comprises four structural proteins: spike (S), membrane (M), nucleocapsid (N), and envelope (E) proteins. The spike glycoprotein mediates the fusion and entry of SARS-COV-2 into the host cells through its S1 and S2 subunits. Also, the spike protein stimulates the host immune system to produce neutralizing antibodies, making it an important target for vaccination strategies. The silencing of SARS-COV-2 expression and replication using RNAi theory was proposed and some studies previously discussed the possibility of utilizing siRNA candidates for the management of the COVID-19 outbreak

Table 4. Pharmacokinetic parameters of inclisiran.

Pharmacokinetic Parameters	Dosage regimen	284 mg subcutaneous injection
	C_{max}	509 ng/mL
	AUC	7980 ng*h/mL
	T_{max}	4 hours
	Volume of distribution	500 L
	The main organ of distribution	liver
	Plasma protein binding	87%
	Elimination half-life	9 hours
	Urinary excretion	16%

[147]. While designing the appropriate siRNA sequence that may block SARS-COV-2 expression, researchers must consider current barriers regarding its delivery to the target site as well as potential strategies to overcome them. Unfortunately, there is a current lack of optimum carriers to transfer these candidates to lung tissue and the passive administration of these molecules may result in several side effects. These clear observations suggest that the door is still open for designing new and effective vaccines for SARS-COV-2 using siRNA phenomena in the future. Therefore, there is a tremendous need to develop novel, effective siRNA drug delivery systems.

9. Expert opinion

Since the evolutionary identification of siRNA as a gene-silencing agent, it has been investigated extensively for the treatment of various diseases including liver, cancer, skeletal muscles, and cardiac complications. The siRNA has gained attention from several researchers as a potential therapy for many undruggable diseases by regulating the post-transcriptional destruction of mRNAs of various genes. However, currently, only four siRNA products have been approved by the FDA for clinical applications. For the siRNA to be effective, an efficient delivery system must deliver it to its intended site. Unfortunately, intracellular and extracellular barriers hurdle the pathway of siRNA delivery starting from its administration until it reaches the cytosol of targeted cells. Great efforts have been oriented toward finding a suitable technique to protect siRNA along its path to the target site, thereby optimizing its therapeutic efficacy. Indeed, studies have reported significant enhancement of siRNA delivery, following chemical modifications. Other researchers investigated the effectiveness of conjugation of siRNA to different types of ligands that have a definite binding ability to special receptors on surfaces of selected cells. Additionally, nanocarriers such as liposomes, niosomes, PLGA nanoparticles, and SLNs may encapsulate siRNA and deliver it properly. Most of the adopted techniques showed a significant optimization of siRNA therapeutics represented by higher serum stability, improved cellular uptake, prolonged systemic circulation, better gene silencing behavior, and fewer off-target effects of the modified therapies. However, further optimization and improvement in siRNA delivery strategies to achieve advanced gene silencing activity with minimum adverse effects are still required. The combination of reported strategies should be increased in the future to overcome obstacles to siRNA delivery efficiently. Recent advances in nanotechnology-based delivery systems such as the utilization of different polymers with different characteristics within the same formula should be also considered. Finally, the cost of applied ingredients and ease of synthesis should be considered during the design of suitable formulations to ensure the productive scale-up of these molecules in the future. Additionally, the cost of the final product should be reasonable to ensure equal distribution of these therapies around the world. This would be especially beneficial as most of these medicines target chronic diseases which need frequent administration of drugs over a long period.

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