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*Minireview*

# Clinical Pharmacology of RNA Interference–Based Therapeutics: A Summary Based on Food and Drug Administration–Approved Small Interfering RNAs

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## ABSTRACT

**RNA-based oligonucleotide therapeutics are revolutionizing drug development for disease treatment. This class of therapeutics differs from small molecules and protein therapeutics in various ways, including both its mechanism of action and clinical pharmacology characteristics. These unique characteristics, along with evolving oligonucleotide-associated conjugates allowing specific tissue targeting, have fueled interest in the evaluation of RNA-based oligonucleotide therapeutics in a rapidly increasing number of therapeutic areas. With these unique attributes as well as growing therapeutic potential, oligonucleotide therapeutics have generated significant interest from a clinical pharmacology perspective. The Food and Drug Administration (FDA) previously published results of a survey that summarized clinical pharmacology studies supporting oligonucleotide therapies approved and in development between 2012 and 2018. Since the first approval of a small interfering RNA (siRNA) therapeutic in 2018, this class of modalities has gained momentum in various therapeutic areas. Hence, a comprehensive examination of the clinical pharmacology of FDA-approved siRNA therapeutics would benefit the path forward for many stakeholders. Thus, in this current review, we thoroughly examine and summarize clinical pharmacology data of the FDA-approved siRNA therapeutics approved from 2018 (year of first approval) to 2022, aimed at facilitating future drug development and regulatory decision making.**

## SIGNIFICANCE STATEMENT

**This review systematically summarizes the clinical pharmacology information of Food and Drug Administration (FDA)-approved small interfering RNAs (siRNA) therapeutics. SiRNAs are revolutionizing the drug development field. Unique clinical pharmacology characteristics represent a differentiating factor for this class of therapeutics. The FDArecently published a draft guidance for clinical pharmacology considerations for developing oligonucleotide therapeutics. As clinical development of this class of therapeutics is fast growing, this review will inform discovery and clinical-stage evaluation of upcoming siRNA-associated drug candidates.**

## Introduction

For many decades, the focus of drug development has been to target disease-causing proteins, for instance, proteins from invading pathogens or proteins with abnormal functions or expression levels. However, neither small molecule drugs nor protein therapeutics directly target the underlying genes that are the root cause of many human diseases. In addition to this inherent limitation, small molecules and protein therapeutics cannot access a significant number of "undruggable" targets (Li et al., 2022). Knowledge of how detrimental upstream genetic sources translate into downstream biologic malfunction fueled the emergence of the gene therapy concept (Friedmann, 1992). Fundamentally different from the strategy of modulating the protein target, gene therapies modify the DNA sequence that is the source of disease pathology. Since the first regulatory approval in 1990, the application of gene therapy in medical intervention has increasingly gained momentum, as highlighted by the invention of the clustered regularly interspaced short palindromic repeat tool in the recent decade (Friedmann, 1992; Wirth et al., 2013; Knott and Doudna, 2018). Although DNA-based gene therapies hold the potential to cure diseases through genome manipulation, the potential for off-target editing could result in irreversible destructive consequences (Knott and Doudna, 2018).

RNA-targeting therapy, often aimed at amending the mRNA expression to prevent synthesis of the cognate problematic protein, by contrast, not only directly modulates biologic function at posttranscriptional level without irreversibly revising the human genome but also bypasses the challenge of developing a pharmacologic agent with sufficient specificity and affinity to the protein target of interest. This RNA-targeting concept can be traced to more than 2 decades ago, when there were

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**ABBREVIATIONS:** ASGPR, asialoglycoprotein receptor; DDI, drug-drug interaction; FDA, Food and Drug Administration; GalNAc, N-acetylgalactosamine; P450, cytochrome P450; PD, pharmacodynamic; PK, pharmacokinetic; PK/PD, pharmacokinetic/pharmacodynamic; RISC, RNA-induced silencing complex; RNAi, RNA interference; siRNA, small interfering RNA.

attempts to exploit this strategy to fight hepatitis B virus infection(Korba and Gerin, 1995). Adding to the attraction of RNA-targeting strategy is the existence of a natural biologic pathway that governs sequence-specific suppression of gene expression, a mechanism called RNA interference (RNAi) (Wilson and Doudna, 2013). The approval of the first small interfering RNA (siRNA) drug in 2018 has demonstrated that siRNAs are a valid and emerging class of therapeutic moieties (Wilson and Doudna, 2013; Rossi and Rossi, 2021). To date, the Food and Drug Administration (FDA) has approved five siRNA therapeutics. SiRNA therapeutics, often 20–30 nucleotides long, harness the human argonaute 2 (Ago 2) protein to form the RNA-induced silencing complex (RISC), which, in turn, mediates cleavage of targeted mRNA (Wilson and Doudna, 2013). In addition to the distinct mechanism of action, unique physiochemical and pharmacological properties distinguish siRNA therapeutics from small molecules and protein therapeutics. For example, unlike small molecules and protein therapeutics that are metabolized and catabolized, respectively, siRNA molecules are degraded by endogenous human RNases (endonucleases and exonucleases). Therefore, not considered either biologics or traditional small molecules, siRNAs may have unique pharmacologic considerations (Kanasty et al., 2012).

Further differentiating siRNA therapeutics are their unique pharmacokinetic/pharmacodynamic (PK/PD) relationships. The pharmacodynamic (PD) effects of siRNAs are both spatially and temporally separate from their systemic exposure [systemic pharmacokinetics (PK)]. Within systemic circulation, siRNAs undergo both rapid cellular uptake, facilitated by either chemical conjugates or delivery technologies, and phagocytosis by the phagocytic cells of the immune system, resulting in a short half-life in plasma (Huang et al., 2016; Bajan and Hutvagner, 2020; Dammes and Peer, 2020). After tissue uptake into cytoplasm, the site of action of siRNAs, it is the concentration of intracellular siRNA-loaded RISC complex, not that of the systemic siRNA, that directly correlates with the level of targeted mRNA degradation, contributing to the notably long PD half-life and thus low dosing frequency of siRNA drugs (McDougall et al., 2022). Additionally, siRNA off-target effects, which could lead to toxicity, mediated by base paring with unintended sequences of endogenous RNAs, can be concentration dependent (Caffrey et al., 2011). These unique clinical pharmacology characteristics need to be considered when developing and evaluating siRNA therapeutics at different stages of drug development.

Although orphan neurodiseases dominate the indications of the currently FDA-approved siRNAs, siRNAs are being evaluated in a wider range of therapeutic areas such as infectious diseases, oncology, ocular disorders, and metabolic diseases (Kulkarni et al., 2021). For instance, several siRNA candidates have entered clinical trials targeting hepatitis B virus, for which a functional cure has been a challenge using current antiviral strategies (Soriano, 2018; van den Berg et al., 2020). Cancer treatment may also benefit from the RNAi mechanism, and a few siRNA candidates are being evaluated in clinical trials (Wang et al., 2022). In addition, development of new conjugates to siRNAs may enable the accessibility of this class of therapeutics to broader disease indications (Mullard, 2022).

Previously, the FDA surveyed clinical pharmacology studies that were conducted to support oligonucleotide therapy development between 2012 and 2018, a period proximate to the first siRNA approval (Rogers et al., 2021). In addition, the FDA recently published a draft guidance to provide recommendations for clinical pharmacology evaluations of oligonucleotide therapeutics (https://www.fda.gov/media/159414/download). All aforementioned advancements in this field attest to the importance of timely and in-depth understanding of clinical pharmacology–related features of siRNAs. To this end, through this review, we summarized the clinical pharmacology–relevant information of FDA-approved siRNA therapeutics from 2018 (year of first approval) to 2022.

## Information Sources

This review focuses on information pertinent to the clinical pharmacology of five siRNAs FDA approved from 2018 (year of first approval) to 2022, as listed in Table 1. The latest prescribing information (drug labels) and FDA clinical pharmacology reviews from Drugs@FDA (https://www.accessdata.fda.gov/scripts/cder/daf/) for these siRNAs were retrieved. In addition, selected publicly available literature (PubMed) pertinent to clinical pharmacology of siRNAs are also included as references for the discussions in the review.

## Clinical Pharmacology Results

All five approved siRNAs are double-stranded RNA sequences composed of a 21-nucleotide sense strand and a 21- to 23-nucleotide antisense strand. In cytoplasm, the two strands unwind, and the antisense strand integrates into the RISC (Wilson and Doudna, 2013). As described above, siRNAs are metabolized by nucleases to oligonucleotides of shorter lengths, which could include some pharmacologically active species. Except for patisiran, the approved siRNAs are conjugated with N-acetylgalactosamine (GalNAc) for efficient delivery to the liver because the asialoglycoprotein receptor (ASGPR) that recognizes the GalNAc moiety is mainly expressed on hepatocytes (Springer and Dowdy, 2018). These siRNAs use the RNAi mechanism and directs catalytic breakdown of the mRNA of interest. In addition, the four GalNAc-conjugated siRNAs are given through subcutaneous administration, whereas patisiran is given through intravenous administration (Table 1).

**PK.** After subcutaneous administration, the GalNAc-conjugated siRNAs were absorbed in systemic circulation with time to maximum concentration at approximately 3 to 4 hours after dosing (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210922Orig1s000MultiR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/214103Orig1s000IntegratedR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/214012Orig1s000ClinPharmR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/215515Orig1s000ClinPharmR.pdf). Except for patisiran, which distributes primarily to liver, plasma protein binding of these GalNAc-conjugated siRNAs was generally concentration dependent, ranging from approximately 80% to 90% at therapeutic doses. Systemic exposure to these siRNAs was generally dose proportional within the range of tested dose levels, without apparent accumulation after repeat dosing. Elimination half-lives of these four GalNAc-conjugated siRNAs was less than 10 hours, which was much shorter than the dosing intervals. Unchanged parent siRNA accounted for less than 30% of administered doses. These data indicated efficient delivery of GalNAc-conjugated siRNAs to hepatocytes and significantly longer half-lives in the liver, as reflected by the subsequent prolonged target mRNA suppression. Because there is significant temporal dissociation between systemic exposure and PD of siRNA therapeutics, conventional exposure (in plasma)-response assessments may not be applicable to inform optimal dose selection. For instance, the dose regimen for givosiran was supported by dose-response analysis instead of plasma exposure-response (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf). Furthermore, except body weight, no intrinsic factors that may impact PK were identified by population PK analyses for these five FDA-approved siRNAs. Regarding quantification of drug concentrations in serum and urine, different bioanalytical approaches (Table 1) were used for these five siRNAs. Both sense and antisense strands were monitored, with final siRNA concentrations reported based on the antisense concentrations for lumasiran and vutrisiran, double-stranded concentration for givosiran, antisense/sense ratio for inclisiran, and full-length double strand for patisiran (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210922Orig1s000MultiR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/

TABLE 1

Summary of clinical pharmacology information of FDA-approved siRNA therapeutics

*The information in the table for each individual siRNA is retrieved from the latest drug label and clinical pharmacology reviews, which can be accessed through Drugs@FDA (https://www.accessdata.fda.gov/scripts/cder/daf/). In addition, the detailed chemical structure of each siRNA is also provided in the drug label.*

| | | Drug (Year of Approval) | | | | |
|---|---|---|---|---|---|---|
| Clinical Pharmacology Information of Interest | | Patisiran (2018) | Givosiran (2019) | Lumasiran (2020) | Inclisiran (2021) | Vutrisiran (2022) |
| Route of administration | | Intravenous | Subcutaneous | Subcutaneous | Subcutaneous | Subcutaneous |
| Chemical conjugate | | None | GalNAc | GalNAc | GalNAc | GalNAc |
| Sense/antisense length (nt) | | 21/21 | 21/23 | 21/23 | 21/23 | 21/23 |
| Chemical modification | | 2'-O-methyl | 2'-O-methyl or 2'-fluro | 2'-O-methyl or 2'-fluro | 2'-O-methyl or 2'-fluro | 2'-O-methyl or 2'-fluro |
| Site of action | | Hepatocyte | Hepatocyte | Hepatocyte | Hepatocyte | Hepatocyte |
| Body weight–based dosing | | Yes (if <100 kg); No (if ≥100 kg) | Yes | Yes | No | No |
| Plasma PK | $T_{max}$ | 70 min | 3 h (parent), 7 h (active metabolite) | 4.1 h | 4 h | 4 h |
| | Serum protein binding | < 2.1% | 90% concentration dependent | 77 to 85% concentration dependent | 87% | 80% concentration dependent |
| | $T_{1/2}$ | 3 d | 6 h | 5.2 h | 7 h | 5.2 h |
| | $V_d$ | 0.26 L 18.2 | 10.4 L | 4.9 L | 500 L | 10.1 L |
| What intrinsic factors impact PK | | Body weight | Body weight | Body weight, renal function | Renal function | No identified |
| Dose adjustment | | Body weight | Body weight | Body weight | No | No |
| PK/PD relationship assessment | | Indirect $I_{max}$ response PK/PD model | 1. Nonclinical PK/PD $E_{max}$ model best described relationship between RISC-loaded active siRNA and target mRNA degradation, enabling $IC_{50}$ estimation; 2. Nonclinical model to predict liver active siRNA in human; 3. Predicted RISC-loaded siRNA concentrations were modeled to have PD effect | RISC-loaded PK concentrations in human liver derived from nonclinical PK/PD model; RISC-loaded PK/PD model for PD markers | Abbreviated PK in PK/PD modeling (population PD model) | PK/PD based on phase 1 data in healthy subjects |
| DDI | In vitro P450 or transporter study performed | Yes | Yes | Yes | Yes | Yes |
| | P450 enzyme(s) or transporter(s) affected by the study drug in clinical studies | None | CYP1A2, CYP2D6, CYP2C19, CYP3A4, CYP2C9[a] | No studies conducted | None | No studies conducted |
| | Dedicated clinical DDI study conducted | No | Yes | No | No | No |
| | Population PK approach used | Yes | No | No | Yes | No |
| | Actionable recommendation in labeling | No | Avoid concomitant use with sensitive CYP1A2 and CYP2D6 substrates | No | No | No |
| HI | Dedicated HI study performed/ population PK approach used | Population PK | Population PK | Population PK | Dedicated HI study | Population PK |
| | clinically meaningful impact on PK | No | No | No | No | No |
| RI | Dedicated RI study performed/ population PK approach used | Population PK | Population PK | Population PK | Dedicated RI study | Population PK |
| | clinically meaningful impact on PK | No | No | No | No | No |

TABLE 1 *continued*

| Clinical Pharmacology Information of Interest | | Drug (Year of Approval) | | | | |
|---|---|---|---|---|---|---|
| | | Patisiran (2018) | Givosiran (2019) | Lumasiran (2020) | Inclisiran (2021) | Vutrisiran (2022) |
| ADA | ADA incidence rate | 3.6% | 0.9% | 6% | 1.7% | 2.5% |
| | Labeling language | Impact of ADA on efficacy/safety is not identified but not conclusive due to limited data | No clinically meaningful impact on PK, PD, efficacy, or safety | No clinically meaningful impact on PK, PD, or safety | No clinically meaningful impact on PD, efficacy, or safety, but long-term consequences are unknown | Impact of ADA on PK, efficacy/safety is not identified, but not conclusive due to limited data |
| QT | In vitro hERG assay performed | Yes | Not mentioned in review or label | No | Not mentioned in review or label | No |
| | Dedicated QT study performed | No | No | No | Yes | No |
| | Clinical ECG-monitoring performed | Yes | Yes | No | Yes | Yes |
| | Labeling language | None | No large increase in QTc detected at recommended dose | No clinically relevant QT interval prolongation at recommended dose | No clinically relevant QT interval prolongation at super-therapeutic dose | Does not prolong the QT interval |
| PK bioanalytic | Drug concentration reported as | Not mentioned in review or label | Full-length double-stranded siRNA | Antisense concentration | Antisense/ sense ratio | Antisense concentration |
| | Method for drug concentration measurement | LC with fluorescent detection | LC-MS/HRAM and LC-MS/MS | LC-TOF-MS | LC-TOF-MS | LC/MS/ HRAM |

ADA, antidrug antibody; $E_{max}$, maximum response; hERG, the human Ether-à-go-go–Related Gene; HI, hepatic impairment; LC-MS/HRAM, liquid chromatography–mass spectrometry/high-resolution accurate mass; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LC-TOF-MS, liquid chromatographic time-of-flight mass spectrometry; nt, nucleotide; QTc, QT corrected for heart rate; RI, renal impairment; $T_{1/2}$, elimination half-life; $T_{max}$, time to maximum concentration; $V_d$, volume of distribution.

[a]Effects of givosiran on substrates of CYP1A2 (caffeine), CYP2D6 (dextromethorphan), CYP2C9 (losartan), CYP2C19 (omeprazole), and CYP3A4 (midazolam) were evaluated in a dedicated clinical drug interaction study. All of these studied drugs had exposure changes in concomitant use with givisiran. However, except for substrates of CYP1A2 and 2D6, these changes in exposure were not considered clinically relevant.

[b]Patisiran: the mean steady state $V_d$ was 0.26 L/kg (18.2 L for a 70-kg body weight); the other four siRNAs: population estimate of apparent $V_d$.

2019/212194Orig1s000MultidisciplineR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/214103Orig1s000IntegratedR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/214012Orig1s000ClinPharmR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/215515Orig1s000ClinPharmR.pdf).

**Exposure-Response Relationship Assessments.** The PK/PD characterization programs for these drugs are distinguished from conventional strategies for small molecules or protein therapeutics because, as elaborated earlier, serum PK of siRNAs is not linked with PD effects (at the site of action) or clinical efficacy data for PK/PD characterization or dose selection justification (McDougall et al., 2022). For instance, because the duration of the PD effects of inclisiran was significantly longer than its serum PK half-life, a population PD model that accounted for the temporal dissociation between PK and PD was used (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/214012Orig1s000ClinPharmR.pdf). In case of givosiran and lumasiran, nonclinical data have been leveraged to predict clinical RISC-loaded PK of the siRNA at the site of action (hepatocyte) for subsequent PK/PD assessments (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/214103Orig1s000IntegratedR.pdf). Briefly, for givosiran, a nonclinical PK/PD model was developed to describe the relationship between observed liver concentrations of active siRNA species (givosiran and its pharmacologically active metabolite), RISC-loaded active siRNA levels, and PD effects in rats. Subsequently, the obtained PK parameters were used to predict liver active siRNA levels in humans, which, along with observed PD, were used to estimate concentrations of human RISC-loaded active siRNAs (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf). It has been noted in literature that physiologically based pharmacokinetic modeling could also be a powerful tool to characterize local (tissue) exposure of siRNA and thus facilitate more relevant PK/PD assessment (Fairman et al., 2021). In addition, the application of other modeling applications including, but not limited to, compartmental and mechanistic PK/PD and population PK/PD modeling methods have also been reviewed and discussed in literature (Jeon et al., 2022).

**Drug-Drug Interactions.** Some have suggested that GalNAc-conjugated siRNAs are unlikely to interact with drug-metabolizing enzymes and transporters because they efficiently distribute to target tissue and do not modulate cytokines (Ramsden et al., 2019). In addition, because GalNAc-conjugated siRNAs are administered by subcutaneous injection, the potential of interaction with P-glycoprotein transporter in the gastrointestinal tract is low. In in vitro studies, these five FDA-approved siRNAs did not function as inhibitors, inducers, or substrates of cytochrome P450 (P450) enzymes or inhibitors and substrates of transporters. Except for givosiran, no clinical drug-drug interaction (DDI) studies were conducted for these approved siRNAs (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210922Orig1s000MultiR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/214103Orig1s000IntegratedR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/214012Orig1s000ClinPharmR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/215515Orig1s000ClinPharmR.pdf).

However, there are other possible mechanisms for indirect modulation of P450 enzymes or transporter activity by siRNAs (and other oligonucleotide therapeutics) (https://www.fda.gov/media/159414/download). For instance, via off-target hybridization with mRNA transcripts of P450 enzymes or transporters, siRNAs (and other types of oligonucleotide therapeutics) can modulate P450 enzymes or transporters. In addition, the pharmacological

effects of liver-targeting siRNAs on hepatic biologic functions may need to be considered for assessing DDI liability. For instance, given the effects of givosiran on the heme biosynthesis pathway in hepatocytes, it has a potential to reduce the activity of P450 enzymes in the liver (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf). In a dedicated DDI study, givosiran increased the exposures of substrates of CYP1A2, 2C9, 2C19, and 3A4 (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf). As described in a literature report, givosiran likely exerted more profound inhibition on CYP2C9 than originally considered (Bins et al., 2022). It has been argued that givosiran's minimal effect on CYP2C9 was based on the exposure change of losartan, which is not considered a sensitive CYP2C9 substrate (Vassiliou et al., 2021; Bins et al., 2022). Furthermore, it has been described that two patients who took givosiran with vitamin K antagonists (warfarin and acenocoumarol) experienced severely potentiated anticoagulant effects and, therefore, more thorough assessment of the DDI potential of siRNAs may be warranted (Bins et al., 2022). Although not occurring for the five approved siRNAs, the PK of siRNAs may be influenced by alteration of non P450-mediated pathways by small molecules. For instance, it has been reported that some small molecules can bind to chemically modified siRNAs and, in turn, increase their cellular uptake (Juliano 2016).

**Immunogenicity.** It has been suggested that siRNAs can induce innate immune responses (Lam et al., 2015) and that both siRNAs and their delivery vehicles can be immunogenic (Kanasty et al., 2012). However, the currently approved siRNAs had low immunogenicity incidence rates (≤6%) without meaningful impact on PK, PD, efficacy, or safety. It should be noted that the immunogenicity rates are difficult to compare across programs given the sensitivity/specificity of the immunogenicity assays used. Among these five approved siRNAs, only patisiran was evaluated for immunogenicity potential by measuring antibodies specific to 1,2-dimyristoyl-rac-glycero-3-carbonylaminoethyl-$\omega$-methoxypolyethylene glycol-2000, a lipid component exposed on the surface of patisiran, whereas immunogenicity assessment for the other GalNAc-conjugated siRNAs was to detect antidrug antibodies (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210922Orig1s000MultiR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/214103Orig1s000IntegratedR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/214012Orig1s000ClinPharmR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/215515Orig1s000ClinPharmR.pdf). It has been demonstrated that unmodified siRNAs can trigger immune responses, which can be reduced by siRNA sequence optimization or RNA chemical modifications including, but not limited to, the 2′-O-methyl, 2′-fluoro, 2′-deoxy, or locked nucleic acid (Marques and Williams, 2005; Jackson and Linsley, 2010; Lam et al., 2015). Indeed, all five FDA-approved siRNAs possess one or multiple chemical modifications in the RNA strands.

**Impact of Hepatic and Renal Impairment.** Except for inclisiran, no dedicated renal or hepatic impairment studies have been conducted for the other approved siRNAs. Instead, the impact of mild and/or moderate renal and hepatic impairment on PK was evaluated as part of population PK analyses for these four siRNAs, and the results showed no clinically meaningful differences on the PK of these siRNAs observed in enrolled trial participants with various degrees of hepatic or renal impairment. For inclisiran, PK analysis of data from a dedicated hepatic impairment study reported increases in $C_{max}$ and area under the curve in patients with mild and moderate hepatic impairment, relative to patients with normal hepatic function; however, PD effects were similar between the groups of patients with normal and mild hepatic function (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/214012Orig1s000ClinPharmR.pdf). In patients with moderate hepatic impairment, baseline biomarker levels were lower, and PD effects were less than those observed in patients with normal hepatic function. No dose adjustment is necessary in patients with mild and moderate hepatic impairment. PK analysis of data from a dedicated renal impairment study reported increases in $C_{max}$ and area under the curve in patients with mild, moderate or severe renal impairment, relative to patients with normal renal function; however, PD effects were similar across all groups based on renal function (https://www.accessdata.fda.gov/drugsatfda_docs/label/2021/214012lbl.pdf).

Given that siRNAs are primarily degraded by endonucleases and exonucleases, are not substrates of P450 enzymes, and have short half-lives in plasma, in general, there have been no significant concerns with the clinically meaningful impact of hepatic impairment (regardless of the extent) on metabolism of the approved siRNAs. On the other hand, it has been indicated that ASGPR expression levels may be significantly impacted by liver diseases such as cirrhosis and hepatocellular carcinoma (Witzigmann et al., 2016). However, it has also been suggested that a therapeutic dose of GalNAc-conjugated siRNA may not be able to saturate the capacity of even 50%-reduced ASGPR level (Willoughby et al., 2018).

**QTc Liability.** Among these five siRNAs, only inclisiran had a dedicated QT study, which showed no QT interval prolongation at a supertherapeutic dose (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/214012Orig1s000ClinPharmR.pdf). A safety pharmacology study of givosiran indicated that the QTc interval was decreased by 12.4 ms (5%) in one of the five tested males cynomolgus monkeys. In safety pharmacology studies of patisiran and lumasiran in monkeys, there were no observed effects on the ECG parameters (including QT intervals) (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210922Orig1s000MultiR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2020/214103Orig1s000IntegratedR.pdf). In addition, as mentioned in the review for patisiran, a thorough QT study was waived because patisiran has a low likelihood of direct ion channel interactions (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2018/210922Orig1s000MultiR.pdf). Moreover, in clinical trials with incorporated ECG monitoring, the other approved siRNAs did not cause clinically relevant QT interval prolongation (https://www.accessdata.fda.gov/drugsatfda_docs/nda/2019/212194Orig1s000MultidisciplineR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/214012Orig1s000ClinPharmR.pdf; https://www.accessdata.fda.gov/drugsatfda_docs/nda/2022/215515Orig1s000ClinPharmR.pdf). Overall, the concern with the clinical QT interval elongation effect of siRNA therapies have been minimal.

## Conclusion

SiRNAs as therapeutic agents represent a paradigm shift in the field of drug development. A thorough evaluation of unique efficacy, safety, and clinical pharmacology attributes of this emerging class of therapeutics is being actively pursued. As elaborated in this review, the clinical pharmacology characteristics of FDA-approved siRNA therapeutics are unique compared with those of small molecules and protein-based therapeutics. As the global pipeline of RNA-based oligonucleotide therapeutics continues to rapidly expand, we envision that the topics covered in this brief summary will contribute to further understanding and evaluation of this class of therapeutic modality.

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### Authorship Contributions

*Participated in research design:* Jing, Arya, Reynolds, Rogers.

*Performed data analysis:* Jing.

*Wrote or contributed to the writing of the manuscript:* Jing, Arya, Reynolds, Rogers.

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