## siRNA: Its Translation from Research to Therapeutic Applications

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#### 5 Introduction

Small interfering RNA (siRNA) is a short, double-stranded RNA molecule that has emerged 6 as a pivotal tool for gene silencing through the RNA interference (RNAi) pathway. It is a 7 powerful post-transcriptional gene-silencing molecule central to RNA interference (RNAi) 8 mechanisms<sup>1</sup>. Typically 21–25 nucleotides in length, it guides the RNA-induced silencing 9 complex (RISC) to a target messenger RNA (mRNA), enabling sequence-specific 10 degradation and effectively silencing gene expression<sup>2</sup>. This process is highly specific and 11 forms the foundation for siRNA's role in research and therapeutic interventions<sup>3</sup>. By guiding 12 the RNA-induced silencing complex (RISC) to target mRNA, siRNA enables post-13 transcriptional gene regulation with unparalleled precision. This targeted action holds 14 immense promise in treating genetic, infectious, and degenerative diseases<sup>4</sup>. Therapeutically, 15 siRNA offers unique advantages over traditional treatments, including the ability to target 16 undruggable proteins, rapid development timelines, and reduced systemic toxicity. These 17 18 advantages have catalyzed interest in siRNA for precision medicine and rare genetic disorders<sup>5</sup>. 19

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## 21 History and Discovery

The siRNA journey began in 1990 when gene silencing was first reported in plants (petunias). This phenomenon, termed "co-suppression," hinted at a broader biological principle<sup>6</sup>. The landmark discovery came in 1998 when Andrew Fire and Craig Mello demonstrated that injecting double-stranded RNA into *C. elegans* could silence specific genes — a mechanism later known as RNA interference<sup>7</sup>. This breakthrough revolutionized molecular genetics, and earned them the Nobel Prize in 2006. By 2001, Tuschl and colleagues synthesized siRNA and used it to silence genes in mammalian cells, marking a significant milestone in therapeutic development. This paved the way for siRNA's translation
from experimental research to clinical innovation<sup>8</sup>.

The discovery of small interfering RNA (siRNA) marked a paradigm shift in the 31 32 understanding of gene regulation. The roots of this breakthrough lie in observations from the early 1990s, where researchers noted unexpected suppression of transgenes in plants-termed 33 post-transcriptional gene silencing (PTGS)<sup>9</sup>. Similar mechanisms were later identified in 34 fungi and animals, suggesting a conserved biological process<sup>10</sup>. The key turning point came 35 in 1998, when Andrew Fire and Craig Mello demonstrated that introducing double-stranded 36 37 RNA (dsRNA) into *Caenorhabditis elegans* could silence specific genes far more effectively than single-stranded RNA. Their work, which earned the 2006 Nobel Prize in Physiology or 38 Medicine, confirmed that dsRNA triggered a potent and specific gene-silencing mechanism 39 now known as RNA interference (RNAi)<sup>11</sup>. 40

Subsequent research revealed that long dsRNA is processed by the enzyme Dicer into 21–23 nucleotide fragments, termed siRNAs. These siRNAs are incorporated into the RNA-induced silencing complex (RISC), which uses one strand as a guide to recognize and cleave complementary messenger RNA (mRNA), thereby suppressing gene expression. This mechanism, initially thought to be exclusive to lower organisms, was soon confirmed to operate in mammalian cells under certain conditions<sup>12</sup>.

siRNAs belong to a broader family of small RNAs that includes microRNAs (miRNAs) and
piwi-interacting RNAs (piRNAs). While siRNAs typically originate from exogenous or
synthetic sources and show perfect complementarity to their mRNA targets, miRNAs are
endogenously encoded and often act through partial pairing to suppress translation<sup>13</sup>. In
contrast, piRNAs function in germline cells to silence transposable elements and operate via
Dicer-independent mechanisms<sup>14</sup>.

The discovery of siRNA had profound implications for biomedical science. It provided a precise and reversible method to silence genes, which transformed functional genomics research<sup>15</sup>. Almost immediately, siRNAs were explored as potential therapeutics for a variety of genetic and infectious diseases. However, the translation from bench to bedside was not without hurdles—issues such as delivery efficiency, stability in vivo, off-target effects, and immune responses initially hampered clinical development<sup>16</sup>. Nonetheless, the foundational discovery of siRNA remains a landmark achievement in
molecular biology, laying the groundwork for the current and future era of RNA-based
therapeutics<sup>17</sup>.

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#### 64 Research Applications of siRNA

In biomedical research, siRNA serves as a critical tool for functional genomics by enabling
targeted gene knockdown. Scientists have employed siRNA to:

• Study cancer biology by silencing oncogenes or tumor suppressors.

• Model neurodegenerative diseases such as Alzheimer's and Parkinson's.

• Validate drug targets and understand disease mechanisms<sup>18</sup>.

In infectious disease research, siRNA has been used to dissect host-pathogen interactions by
selectively silencing host genes involved in viral entry, replication, or immune evasion—
contributing significantly to antiviral drug development. For instance, siRNAs targeting host
factors essential for HIV or influenza virus replication have identified novel therapeutic
targets<sup>19</sup>.

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Additionally, in cardiovascular research, siRNA has been employed to study genes involved in lipid metabolism, atherosclerosis, and hypertension. Its ability to downregulate diseaseassociated genes in animal models has enabled the preclinical evaluation of gene-targeted therapies<sup>20</sup>.

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Collectively, siRNA continues to be a cornerstone of translational research, bridging basic molecular biology and therapeutic innovation. Its versatility and effectiveness in modeling diseases and validating targets underscore its enduring value in the biomedical research landscape<sup>21</sup>.

85 It enables the systematic silencing of genes, aiding in the elucidation of gene function and the 86 identification of disease-related pathways. siRNA has facilitated target validation, pathway dissection, and the modeling of genetic disorders in vitro and in vivo. It has been instrumental
in studying cancer biology, virology, neurodegenerative diseases, and cardiovascular
conditions. The simplicity of designing siRNAs against virtually any gene, coupled with their
robust knockdown capabilities, underscores their utility in preclinical research<sup>22</sup>.

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### 93 From Research to Therapeutic Applications

94 siRNA's transition from research to therapy materialized with the advent of FDA-approved
95 drugs such as Patisiran (ONPATTRO®) (2018) for hereditary transthyretin-mediated
96 amyloidosis and Givosiran (GIVLAARI<sup>TM</sup>) (2019) for acute hepatic porphyria. These drugs
97 demonstrated the clinical potential of siRNA in silencing harmful genes systemically with
98 minimal off-target effects<sup>23</sup>.

99 The advent of Inclisiran, an siRNA targeting PCSK9 to lower cholesterol, further exemplifies 100 siRNA's therapeutic utility<sup>24</sup>. Therapeutic siRNA must navigate biological barriers, remain 101 stable in circulation, and target specific tissues. Advances in nanocarrier systems, including 102 lipid nanoparticles (LNPs), have greatly enhanced siRNA delivery, enabling their therapeutic 103 success in clinical trials and real-world use. Lipid nanoparticles (LNPs) and conjugate 104 chemistries have enabled safe and effective delivery, making siRNA a cornerstone of modern 105 therapeutics<sup>25</sup>.

#### 106 **Table.1: siRNA delivery system and its therapeutic outcome**<sup>26</sup>

Sl.no	Disease	<b>Target Genes</b>	Delivery Systems/	Therapeutic Outcomes
			Strategies	
1	Cancer	KRAS, c-Myc,	- Lipid nanoparticles	- Tumor suppression
		PD-L1	- 2'-O-methylation	- Immune activation
			- Advanced targeting	- Reversal of drug
			modifications	resistance
2	Rheumatoid	NF-κB p65,	- PEI-based	- Reduced joint
	Arthritis	STAT1	nanocomplexes (Chen	inflammation

			et al.)	- Targeted
			- RGD-modified	macrophage/dendritic cell
			PLGA NPs	suppression
			(Scheinman et al.)	
3	Ulcerative	NF-κB p65 and	- MPEG-PCL-	- Suppressed
	Colitis	related	CH2R4H2C NPs	inflammatory mediators
		cytokines	(Ibaraki)	- Improved clinical &
			- Silica-coated CaP	histopathological scores
			NPs (Muller et al.)	in colitis models
4	Brain	Inflammatory	- Poly(β-amino ester)	- Inflammation control
	Inflammation	genes (e.g.,	NPs	- Effective BBB
		post-ICH)	- Receptor-mediated	penetration
			transcytosis	- Neuroprotection and
				tumor inhibition
			Intranasal/intracerebral	
			delivery	
			- LNPs, viral vectors	

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## 108 Limitations of siRNA

109 Despite the significant therapeutic potential of siRNA, several limitations must be addressed110 for its successful clinical application:

- Instability in Biological Fluids: Naked siRNA is highly susceptible to degradation
   by nucleases present in blood and tissues, limiting its half-life and therapeutic
   efficacy<sup>27</sup>.
- Rapid Renal Clearance: Due to its small molecular size and hydrophilic nature,
   unmodified siRNA is quickly eliminated via the kidneys, reducing its systemic
   bioavailability<sup>28</sup>.
- Off-Target Effects: siRNAs can inadvertently silence genes with partial sequence
   similarity, leading to unintended gene knockdown and potential toxicity<sup>29</sup>.

- Immune Stimulation: Certain siRNA sequences may activate innate immune
   responses through Toll-like receptors (e.g., TLR3, TLR7/8), triggering inflammation
   or cytokine release<sup>30</sup>.
- Poor Cellular Uptake: siRNA cannot easily cross cell membranes due to its negative
   charge and hydrophilicity, requiring specialized delivery systems to ensure
   intracellular access<sup>31</sup>.
- Limited Tissue Specificity: Achieving targeted delivery to specific tissues or cell
   types remains challenging, particularly in non-hepatic tissues<sup>32</sup>.
- Risk of Saturating the RNAi Machinery: High doses of exogenous siRNA may
   overload components of the endogenous RNAi pathway, potentially disrupting normal
   microRNA function<sup>33</sup>.

Addressing these limitations necessitates the development of advanced delivery platforms
 (e.g., lipid nanoparticles, polymeric carriers) and chemical modifications (e.g., 2'-O-methyl,
 phosphorothioate linkages) to improve stability, specificity, and safety<sup>34</sup>.

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## 134 Approaches to overcome limitations of siRNA as a therapeutic

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The clinical translation of siRNA has been significantly advanced through the development
of innovative strategies aimed at overcoming its biological and pharmacokinetic limitations.
These approaches span chemical engineering, delivery system design, and molecular
optimization<sup>35</sup>:

#### 140 **Table.2: Strategies to Enhance siRNA Therapeutics**<sup>36</sup>

Sl.no	Strategy	Description
1	Chemical Modifications	-2'-O-Methyl (2'-OMe) & 2'-Fluoro (2'-F): Improve
	to Enhance Stability and	nuclease resistance and reduce immunostimulation.
	Reduce Immunogenicity	-LNA: Locked conformation enhances stability and
		binding.
		-Phosphorothioate Linkages: Improve plasma

		stability and degradation resistance.
		Note:-These modifications improve
		pharmacokinetics, reduce dosing frequency, and
		enhance safety profiles.
2	Advanced Delivery	- Lipid Nanoparticles (LNPs): Prevent degradation
	Systems	and support cellular uptake.
		- Polymeric Carriers (e.g., PLGA, PEI): Controlled
		release and targeted delivery.
		- Peptide-Based Systems: Tissue-specific targeting
		via CPPs and ligands.
3	Hybrid &	- Combinatorial Systems: Integrate lipids, polymers,
	Multifunctional	and targeting ligands for synergistic benefits.
	Platforms	- Can include imaging agents for theranostics and
		monitoring therapeutic efficacy.
4	Sequence Optimization	- Bioinformatics Tools: Aid in designing siRNAs
	and Design	with minimal off-target effects.
		- Guide Strand Modifications: Prevent unintended
		mRNA interactions and improve specificity.
5	siRNA Pooling	- Smart Pooling: Combines multiple siRNAs
	Strategies	targeting different regions of the same gene.
		- Reduces off-target effects while maintaining
		silencing potency—useful in screening and early
		therapy stages.

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# 142 Challenges in Development and Deployment of siRNA 143 Therapeutics

Several challenges continue to hinder siRNA's widespread therapeutic use. Efficient and
targeted delivery remains a bottleneck, especially for extrahepatic tissues. Formulating stable,
biocompatible delivery vehicles that can navigate the immune system and reach target cells is
critical<sup>37</sup>. Additionally, large-scale manufacturing, cost, and regulatory hurdles affect clinical

translation. Dosing regimens, long-term effects, and patient variability must be addressed

through rigorous clinical studies. Despite these challenges, the expanding siRNA therapeutic

150 pipeline reflects strong industry and academic interest in overcoming these barriers<sup>38</sup>.

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## 154 **Table.3: Key Challenges at a Glance – siRNA Therapeutics**<sup>39</sup>

Sl.no	Challenges	Description
1	Targeting Beyond the Liver	siRNA therapies work well in the liver, but
		efficient delivery to organs like the brain, lungs,
		or joints remains a major hurdle.
2	Building the Perfect Carrier	Requires stable, biocompatible vehicles that
		evade the immune system, resist degradation,
	0	and accurately reach target cells.
3	Scale-Up Struggles	Large-scale production faces challenges in
		maintaining consistency, stability, and cost-
		efficiency for clinical deployment.
4	Cost and Complexity	High production and formulation costs limit
		affordability and accessibility, especially in low-
		resource healthcare settings.
5	Regulatory Roadblocks	Complex and evolving regulatory requirements
		demand robust safety and efficacy data, slowing
		clinical progress.
6	Dosing and Duration	Determining effective dosing and assessing
	Dilemmas	long-term impacts is essential, with concerns
		around toxicity and therapeutic duration.
7	Patient-Specific Variability	Genetic and physiological differences require
		tailored strategies to ensure consistent

	therapeutic outcomes across diverse patient
	populations.

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#### **156 Future Prospects of siRNA Therapeutics**

The future of siRNA lies in expanding beyond liver targets through innovative delivery
 methods and personalized approaches, positioning it as a key player in next-generation
 precision medicine<sup>40</sup>.

#### 160 **Conclusion**

siRNA has revolutionized gene silencing, transitioning from a powerful research tool to a clinical therapeutic platform. Despite inherent limitations, continuous innovations in molecular design and delivery systems have substantially enhanced its viability. The success of drugs like Inclisiran and Patisiran underscores the therapeutic promise of siRNA. Future breakthroughs in targeting, safety, and scalability will determine its role in mainstream medicine. As the field evolves, siRNA is poised to reshape the landscape of disease management and personalized therapy.

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