Therapeutic oligonucleotides revisited: Focus on siRNA and antisense technologies

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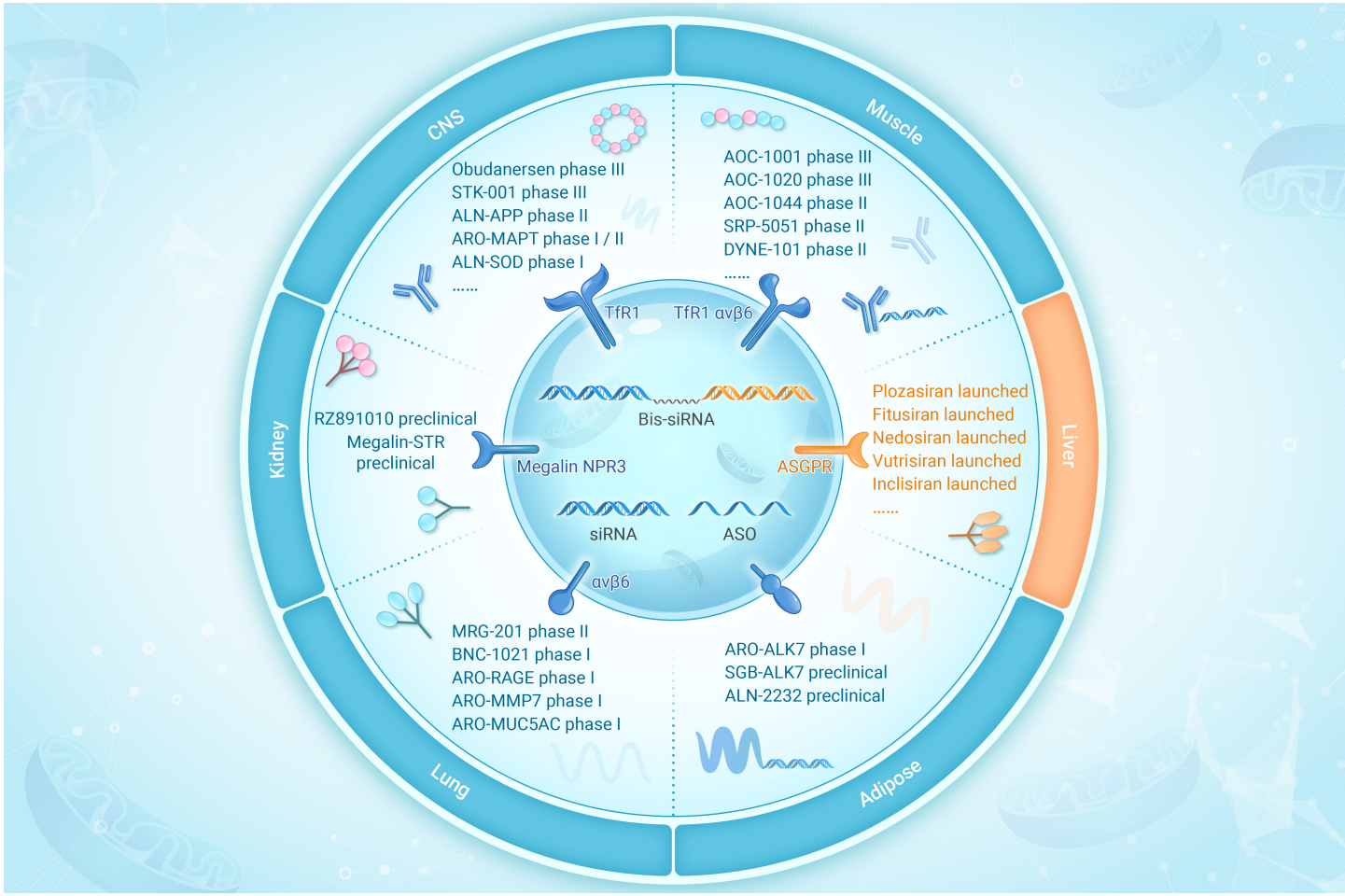
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- siRNA and antisense oligonucleotide can precisely “seal off” faulty-gene for disease treatment.
- They often offer long-lasting effects and can treat previously "undruggable" genetic disorders.
- Approved drugs now help patients with ATTR amyloidosis, hemophilia, and Duchenne muscular dystrophy.
- A key challenge is delivering these drugs beyond the liver to other tissues like brain.
- New delivery technologies and multi-targeting approach aim to enable broader application in future.

REVIEW Precision Medicine

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Oligonucleotide therapeutics have demonstrated unique advantages, including the capacity to target traditionally undruggable disease targets and achieve prolonged pharmacological effects that can last up to months. This fast-evolving drug modality has been gaining momentum over the past two decades, evidenced by regulatory approval on multiple entities which mostly target liver. Nevertheless, further extending its application to extrahepatic targets is largely confined by corresponding delivery systems. This review first provides a concise overview of the mechanistic principles underlying oligonucleotide function and commonly employed chemical modification techniques. It then highlights recent advancements in receptor-mediated delivery systems for extrahepatic targeting, and dual-targeting oligonucleotide engagement strategies encompassing design principles and outcomes in complex disease condition. Finally, we discuss key challenges and future directions in the field. Collectively, ongoing innovations in chemical modification and delivery technologies are expected to broaden the therapeutic scope of oligonucleotide agents, paving the way for improved clinical outcomes across a wider range of diseases.

INTRODUCTION

Oligonucleotide therapeutics represent an innovative class of pharmaceutical agents composed of short nucleotide sequences that modulate gene expression for therapeutic benefits.[1] Over the past two decades, the U.S. Food and Drug Administration (FDA) has approved 29 oligonucleotide-based therapies, including small interfering RNA (siRNA), antisense oligonucleotides (ASO), aptamers, and mRNA therapeutics. Notably, ASO and siRNA account for 14 and 8 approvals, respectively (Table 1). Currently, more than 300 clinical development programs are underway for ASOs and siRNAs, underscoring their status as the most rapidly advancing subclasses within the oligonucleotide therapeutic landscape (Figure 1). Thus, this article provides a comprehensive review of recent advancements in ASO and siRNA.

In 1978, Paul Zamecnik demonstrated that the introduction of specific synthetic single-stranded nucleic acids into chicken embryo fibroblasts infected with Rous sarcoma virus effectively suppressed viral replication, laying the foundation for antisense technology.[2] Subsequent research elucidated that ASO exerts effects by sequence-specific hybridization to target messenger RNA (mRNA) molecule, leading to modulation of gene expression through mechanisms operating in both the cytoplasm and the nucleus.[3] In 1998, Ionis Pharmaceuticals (formerly Isis Pharmaceutical) launched fomivirsen, the first ASO-based therapeutic approved worldwide, for the treatment of cytomegalovirus (CMV) retinitis in immunocompromised patients, heralding the dawn of a new era in gene-targeted drug discovery.[4]

In 1998, Fire et al. introduced exogenous double-stranded RNA (dsRNA) into Caenorhabditis elegans, demonstrating for the first time that dsRNA could efficiently and specifically silence the expression of homologous genes (Figure 2).[5] Later that year, further studies confirmed that this effect occurs post-transcriptionally through targeted mRNA degradation.[6] For their discovery of RNA interference (RNAi), Andrew Fire and Craig Mello were awarded the Nobel Prize in Physiology or Medicine in 2006. [7] Despite the therapeutic potential, the development of siRNA as a drug, faces several challenges. As a hydrophilic, negatively charged macromolecule, siRNA exhibits poor membrane permeability and is rapidly degraded by serum nucleases, resulting in a short plasma half-life due to renal clearance.[8,9] Moreover, upon cellular uptake, most siRNA remains trapped in endosomal compartments (e.g., endosomes and lysosomes), with limited release into the cytoplasm where it mediates target mRNA cleavage via RNA-induced silencing complex (RISC).[10,11] These challenges historically hindered its clinical application. Nevertheless, sustained progress in oligonucleotide chemical modifications, formulation strategies, and targeted delivery systems has progressively addressed these challenges. Patisiran (marketed as Onpattro®), the world's first siRNA therapeutic, received approval from FDA for the treatment of hereditary transthyretin-mediated amyloidosis, marking a landmark achievement of clinical era of RNAi-based therapies.[12]

Currently approved oligonucleotide therapeutics are mainly used to treat hereditary neurological disorders and liver-associated metabolic diseases.[13] Most of siRNAs employ N-acetylgalactosamine (GalNAc)-conjugation technology, limiting their application to hepatic related indications or targets of interest. In contrast, most ASOs typically require either local (e.g., intraocular, intrathecal) or systemic administration, relying on endogenous distribution pathways that often necessitates higher doses and are associated with hepatotoxicity and nephrotoxicity.[14,15] Consequently, achieving safe and efficient delivery of oligonucleotide drugs to extrahepatic tissues remains a major challenge. Current efforts are largely directed towards developing novel targeting ligands for extrahepatic delivery, with several candidates now advancing into clinical trials.[16]

Chronic diseases, including diabetes and cardiovascular disease, often involve dysregulation in multiple pathological pathways and signaling mechanisms, which makes single-target oligonucleotide therapies insufficient for addressing the complex pathophysiology.[17] In contrast, multi-target oligonucleotide drugs can simultaneously target multiple critical molecular targets, offering a more comprehensive therapeutic strategy, particularly for conditions with multifactorial etiologies.[18] Among these, dual-targeting oligonucleotide therapeutics are progressing rapidly, with several candidates already in clinical development. This review summarizes the mechanisms of action and chemical modification strategies for oligonucleotides, highlights advances in receptor-mediated extrahepatic delivery systems, discusses recent progress in dual-targeting oligonucleotide therapeutics, along with current challenges and future perspectives.

MECHANISM OF OLIGONUCLEOTIDES

Oligonucleotides can regulate target genes either up or down through various mechanisms (Figure 3).[19] ASOs primarily function via three mechanisms, including RNase H1-dependent mRNA degradation, splicing modulation, and translation inhibition, with first two being the most prominent.[20] In the RNase H1-dependent process, ASOs hybridize with target mRNA to form DNA-RNA double strands.[21] This hybrid is specifically recognized and activated by intracellular RNase H1, which cleaves the RNA segment of the hybrid, leading to mRNA degradation and subsequent gene silencing.[22] Alternatively, through steric hindrance mechanism, ASOs bind to specific mRNA regions, such as translation initiation or splicing junctions, thereby blocking ribosomal assembly or progression, or disrupting spliceosome recognition of canonical splicing sites. This results in altered pre-mRNA slicing patterns or reduced protein translation, enabling precise modulation of gene expression.[23,24] ASOs, beyond their role in degrading mRNA to down regulate target protein expression, can also enhance mRNA translation efficiency and elevate protein expression levels by targeting upstream open reading frames (uORFs) or translation-inhibitory elements within the 5' untranslated regions (5'UTRs).[25,26]

siRNA achieves sustained gene silencing primarily by facilitating efficient cytoplasmic mRNA cleavage via RISC. The core mechanism relies on exogenously introduced short double-stranded RNA to achieve sequence-specific suppression of target gene expression within cells.[27] Upon cellular entry, the

Table 1. Approved nucleic acid drugs

Drug name	Indication	Target	Organization	Year of approval	Delivery system	Modality
Donidalorsen	HAE	KLKB1	Ionis	2025	GalNAc	ASO
Olezarsen	FCS	ApoC-III	Ionis	2024	GalNAc	
Imetelstat	MDS	hTR	Geron	2024	Lipid	
Eplontersen	ATTRv-PN	TTR	Ionis	2024	GalNAc	
Tofersen	ALS	SOD1	Biogen	2023	Naked	
Casimersen	DMD	Exon 45	Sarepta	2021	Naked	
Viltolarsen	DMD	Exon 53	Nippon Shinyaku	2020	Naked	
Golodirsen	DMD	Exon 53	Sarepta	2019	Naked	
Volanesorsen	FCS	ApoC-III	Ionis	2019	Naked	
Inotersen	hATTR	TTR	Ionis	2018	Naked	
Nusinersen	SMA	Exon 7	Biogen & Ionis	2017	Naked	
Eteplirsen	DMD	Exon 51	Sarepta	2016	Naked	
Mipomersen	HoFH	ApoB-100	Ionis	2013	Naked	
Fomivirsen	Infection	CMV UL123	Ionis	1998	Naked	
Plozasiran	FCS	ApoC-III	Arrowhead	2025	GalNAc	siRNA
Fitusiran	HEMO	SERPINC1	Alnylam	2025	GalNAc	
Nedosiran	PH1	LDHA	Dicerna & Novo Nordisk	2024	GalNAc	
Vutrisiran	hATTR-PN	TTR	Alnylam	2022	GalNAc	
Inclisiran	FH	PCSK9	Alnylam & Novartis	2021	GalNAc	
Lumasiran	PH1	HAO1	Alnylam	2020	GalNAc	
Givosiran	AHP	ALAS1	Alnylam	2019	GalNAc	
Patisiran	hATTR	TTR	Alnylam	2018	LNP	
Lzervay	GA	C5	IVERIC	2023	N.A.	Aptamer
Pegaptanib	AMD	VEGF-165	EyeTech & Pfizer	2005	N.A.	
Kostaive	COVID-19	SARS-CoV-2	Arcturus	2024	LNP	mRNA
mRESVIA	RSV	RSV	Moderna	2024	LNP	
Elasomeran	COVID-19	SARS-CoV-2	Moderna	2020	LNP	
Comirnaty	COVID-19	SARS-CoV-2	BioNTech & Pfizer	2020	LNP	
Exagamglogene Autotemcel	BCL11A	SCD	Vertex & CRISPR	2023	N.A.	CRISPR/Cas

HAE: hereditary angioedema; KLKB1: kallikrein B1; FCS: familial chylomicronemia syndrome; ApoC-III: apolipoprotein c-iii; MDS: myelodysplastic syndromes; hTR: human telomerase RNA; ATTRv-PN: transthyretin amyloid polyneuropathy; TTR: transthyretin; ALS: amyotrophic lateral sclerosis; SOD1: superoxide dismutase 1; DMD: duchenne muscular dystrophy; SMA: spinal muscular atrophy; HoFH: homozygous familial hypercholesterolemia; ApoB-100: apolipoprotein b-100; CMV: cytomegalovirus; HEMO: hemophilia; SERPINC1: serpin family c member 1; PH1: primary hyperoxaluria type 1; LDHA: lactate dehydrogenase a; FH: familial hypercholesterolemia; PCSK9: proprotein convertase subtilisin/kexin type 9; HAO1: hydroxyacid oxidase 1; AHP: acute hepatic porphyria; ALAS1: aminolevulinate synthase 1; GA: geographic atrophy; C5: complement component 5; AMD: age-related macular degeneration; VEGF: vascular endothelial growth factor; COVID: coronavirus disease; RSV: respiratory syncytial virus; BCL11A: b-cell lymphoma/leukemia 11a; SCD: sickle cell disease; LNP: lipid nanoparticle.

double-stranded siRNA is recognized by the RISC loading complex, where it is unwound. The antisense strand, also called guide strand, is selectively retained, while the complementary strand is degraded.[28] The guide strand then associates with the core effector protein Argonaute-2 (Ago2), forming the active RISC complex. This complex identifies target mRNA through complementary base pairing, allowing Ago2, an endo-ribonuclease, to cleave the mRNA at specific sites. The resulting mRNA fragments are rapidly degraded by intracellular nucleases, leading to effective and specific gene silencing.[29,30]

CHEMICAL MODIFICATION OF OLIGONUCLEOTIDES

Chemical modification represents a pivotal strategy for addressing key challenges in the development of oligonucleotide therapeutics. Systematic modification to the phosphate backbone, ribose, or nucleobase can substantially enhance nuclease resistance, target binding affinity, mitigate immunogenicity, and improve pharmacokinetic profiles.[31,32] This section reviews the major chemical modification approaches and corresponding impacts on the physicochemical and biological properties of oligonucleotide therapeutics, offering a theoretical foundation for rational design (Figure 4).

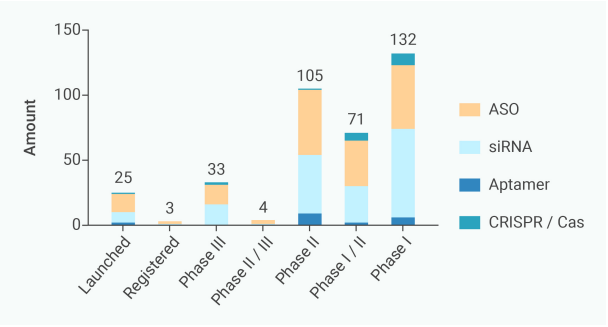


Figure 1. Statistics on marketed and clinical-stage oligonucleotide drugs

Backbone modifications, typically entail substitution of the non-bridging oxygen in the phosphodiester linker with a sulfur atom, yielding a phosphorothioate (PS) linkage.[33] PS modifications significantly enhance the resistance of oligonucleotides to exonucleases, improving oligonucleotide stability,[34] and promote the binding to serum proteins, thereby prolonging the in vivo half-life. Consequently, PS modifications are extensively employed in ASOs and siRNAs,[35] Additionally, introduction of a 5'-(E)-vinylphosphonate (5'-(E)-VP) at the 5' end of the antisense strand can further enhance siRNA stability and gene silencing potency, which is frequently employed in combination with extrahepatic delivery system.[36] Regarding base modifications, 5-methylcytosine is commonly incorporated into ASOs, where it strengthen the hybridization affinity for target mRNA and increases duplex thermal stability, thus augmenting gene silencing efficacy.[37]

Chemical modification strategies for ribose in nucleic acids encompass a range of strategies. For siRNA, the most prevalent modification is substitution of 2'-hydroxyl group of ribose with a 2'-methoxy group (2'-OMe), which enhances nuclease resistance and reduces immunogenicity.[38,39] Another widely employed modification is the 2'-fluoro (2'-F) substitution, which confers high binding affinity to target mRNA and excellent nuclease resistance, making it particularly suitable for complete sequence modification of siRNA.[40-42] To mitigate off-target effects mediated by the seed region, Alnylam introduced a glycol nucleic acid (GNA) modification at position 7 of the antisense strand.[43] This adjustment maintains the target gene silencing activity while reducing antisense strand binding to off-target mRNAs, thereby effectively lowering off-target risks.[44] In the field of ASOs, one of the most successful ribose modifications is the 2'-O-methoxyethyl (2'-MOE) substitution, which significantly boosts target affinity and nuclease resistance.[45] Additionally, locked nucleic acid (LNA) and constrained ethyl (cEt) increases binding affinity to complementary sequences by introducing a methylene bridge between the 2'-oxygen and 4'-carbon of the ribose, which restricts the ribose's conformational flexibility. LNA/cEt is commonly utilized to shorten ASO length, enhance efficacy, and reduce immunogenicity.[46,47]

DELIVERY OF OLIGONUCLEOTIDES

Oligonucleotide drugs have achieved significant success in liver delivery, enabled by advanced platforms such as lipid nanoparticles (LNP) and GalNAc-conjugation. These technologies enable efficient and selective hepatic uptake, contributing to the approval and commercialization of several oligonucleotide-based therapeutics.[48] However, effective delivery to most extrahepatic tissues, such as kidneys, muscles, and central nervous system, remains a major challenge, severely restricting the broader application of oligonucleotide therapies across diverse disease indications.[49]

To address this challenge, various strategies for extrahepatic delivery of oligonucleotides have been explored, among which receptor-mediated oligonucleotide delivery has garnered increasing attention and achieved notable progress.[50] This method involves conjugating oligonucleotides with ligands that bind specifically to cell surface receptors. Through receptor-ligand recognition and endocytosis, this approach enables efficient and selective delivery of therapeutic nucleic acids to target cells or tissues.[51] It successfully addresses major challenges such as poor cell membrane penetration, nonspecific distribution, and potential off-target toxicity of oligonucleotide drugs, thereby enhancing the precision and efficacy of oligonucleotide therapeutics. Thus, receptor-mediated delivery holds particular promise for targeting tissues beyond the liver, including the central nervous system, tumors, muscle, and lungs.[52] This section offers a systematic review of recent advances in receptor-mediated delivery strategies across diverse extrahepatic tissues.

Liver

The liver is currently the most effective target organ for oligonucleotide delivery, with the majority of marketed and clinically developed oligonucleotide drugs utilizing GalNAc-mediated hepatic targeting (Figure 5).[53-57] Given the abundance of existing systematic reviews on this topic, our discussion will focus primarily on extrahepatic delivery strategies rather than hepatic applications.

It is noteworthy that although the liver is a key target organ for many chronic diseases, these conditions often involve multiple pathogenic genes.[58] Consequently, interventions targeting a single pathway are frequently inadequate for effective disease management.[59] Therefore, the development of dual-targeting oligonucleotide therapeutics, capable of simultaneously modulating multiple targets, has emerged as a crucial strategy in the treating complex chronic diseases.[60] Advances in this field will be discussed in detail in the following sections.

Muscle

Skeletal muscle plays a vital role in crucial mechanical functions such as posture, movement, and respiration.[61] Dysfunction in this tissue is often linked to several rare hereditary myopathies, including Duchenne muscular dystrophy (DMD), Myotonic dystrophy type 1 (DM1), and Facioscapulohumeral muscular dystrophy (FSHD).[62] While oligonucleotide therapeutics

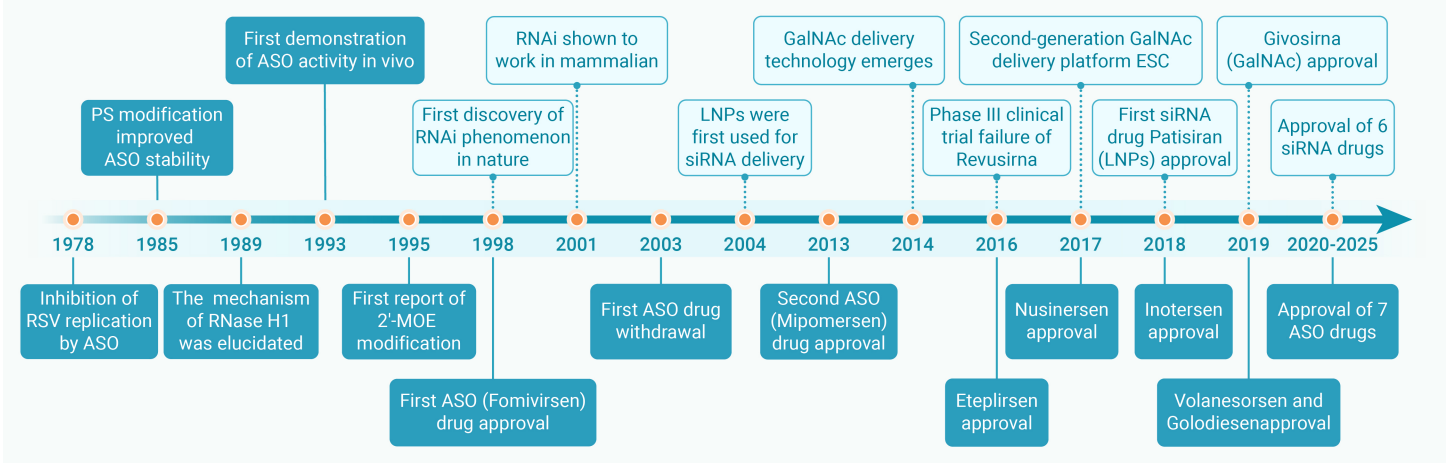


Figure 2. Milestones in the development of ASO and siRNA

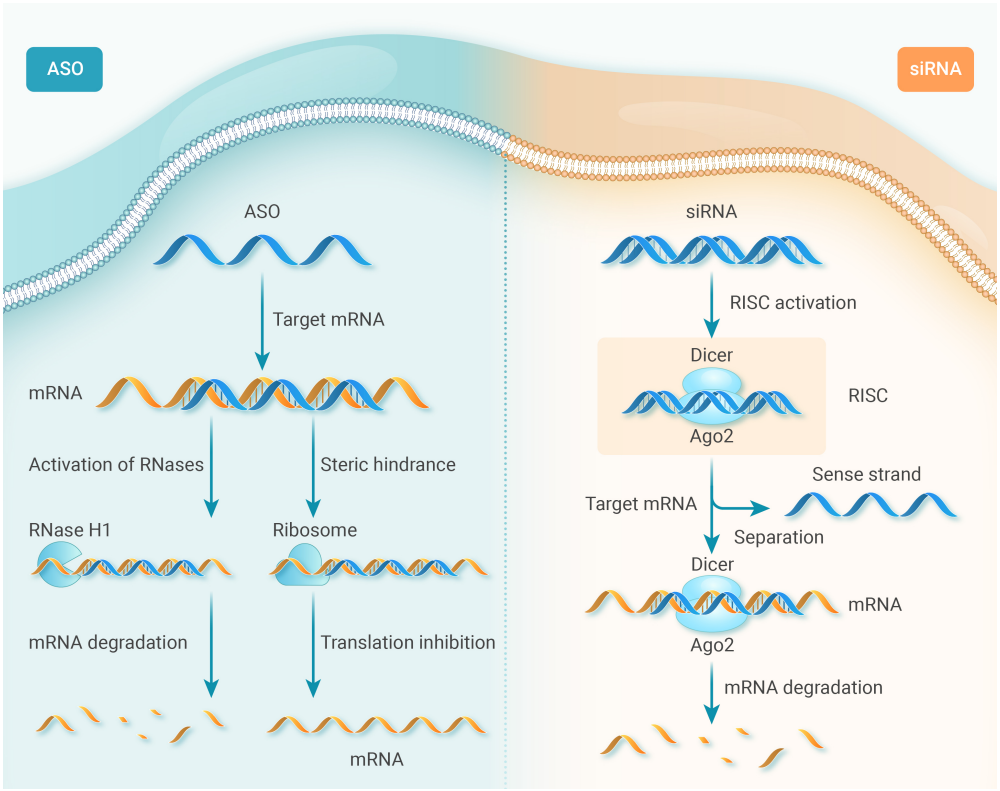


Figure 3. Mechanism of ASO and siRNA ASO: antisense oligonucleotide; siRNA: small interfering RNA; RISC: RNA-induced silencing complex; Ago-2: argonaute 2; Dicer: endonuclease; RNase H1: ribonuclease H1, possesses both endonuclease and exonuclease activity.

hold great promise for treating these muscular disorders, efficient delivery to skeletal muscle remains a significant challenge.[63] Recently, receptor-mediated strategies for delivering oligonucleotides to muscle tissues have made notable progresses.

TfR1 is one of the well-established and promising targets for receptor-mediated delivery, with a notably high expression level observed in skeletal muscle.[64] Avidity Biosciences' antibody-oligonucleotide conjugate (AOC) platform features a monoclonal antibody (mAb, Figure 4) directed at TfR1, a non-cleavable linker, and one or more oligonucleotides.[65] Utilizing this platform, Avidity has developed several oligonucleotide therapeutics for muscle disorders that have entered clinical development.[66] The most advanced candidate, AOC 1001 (Table 2), is anticipated to be submitted for regulatory approval in the United States, European Union, and Japan in the second half of 2026.[67]

DM1: myotonic dystrophy type 1; DMPK: dystrophia myotonica protein kinase gene; FSHD1: facioscapulohumeral muscular dystrophy 1; DUX4: double homeobox 4; CPP: cell-penetrating peptide; TfR1: transferrin receptor 1; αvβ6: integrin alphav beta6; AS: angelman syndrome; UBE3A: ubiquitin protein ligase E3A; DS: down syndrome; SCN1A: sodium voltage-gated channel alpha subunit 1; SCA2: spinocerebellar ataxia type 2; ATXN2: taxin 2; AD: alzheimer's disease; MAPT: microtubule-associated protein tau; CAA: cerebral amyloid angiopathy; APP: amyloid beta precursor protein; ACO: auxiliary oligonucleotide; HD: huntington's disease; HTT: huntingtin protein; IPF: idiopathic pulmonary fibrosis; MiR-29: microRNA 29; TGF-β1: transforming growth factor beta 1; COPD: chronic obstructive pulmonary disease; MUC5AC: mucin 5AC; IPD: interstitial lung disease; RAGE: receptor for advanced glycation end products; matrix metalloproteinase 7; OB: obesity; ALK7: activin receptor-like kinase 7; IV: intravenous administration; IT: intrathecal administration; SC: subcutaneous administration; INH: inhalation administration.

Dyne Therapeutics' FORCE™ platform employs a conjugate consisting of an antigen-binding fragment (Fab, Figure 5) specifically targeting TfR1, a cleavable linker, and a therapeutic oligonucleotide, enabling precise delivery to tissues such as skeletal and cardiac muscle.[68] Using this platform, Dyne has advanced its candidate, DYNE-101, into clinical development for DM1.[69] Preliminary clinical data demonstrate sustained improvement across multiple functional endpoints, and the company plans to submit an accelerated approval application in the United States in the first half of 2026.[70]

Beyond antibodies, substantial progress has been made in the receptor-mediated delivery to muscles using cyclic peptides targeting the TfR1. In a recent study, Ionis and Bicycle employed phage display technology to identify and refine a high-affinity, human TfR1-specific bicyclic peptide known as BCY17901 (Figure 5). This peptide was covalently linked to a MALAT1 ASO and HPRT siRNA. In vivo experiments showed a 71% and 86% reduction in mRNA levels in mouse quadriceps after a single subcutaneous dose.[71]

Similarly, Eli Lilly developed a series of TfR1-targeting cyclic peptides using phage display technology. Their leading compound binds with high affinity to human TfR1 without interfering with the natural ligand transferrin (Tf). In mouse models, administration of this cyclic peptide-oligonucleotide conjugate achieved approximately 60% silencing of target mRNA in muscle tissue.[72] Compared to antibodies, cyclic peptides offer advantages such as smaller molecular size, ease of synthesis, and lower production costs, underscoring their potential for oligonucleotide delivery to muscle.[73]

The integrin receptor αvβ6 is highly expressed in skeletal muscle and presents a promising target for oligonucleotide delivery to muscle tissues.[74] Arrowhead Pharmaceuticals reported a muscle delivery system utilizing a peptide targeted at αvβ6 (αvβ6-pep1-LP238b, Figure 5), combined with a lipid module, to optimize pharmacokinetics and pharmacodynamics (PK/PD).[75] Their investigational drug, ARO-DM1, achieved over 80% silencing of DMPK mRNA in skeletal muscle following a single dose in non-human primates, with sustained effects lasting beyond 85 days.[76] ARO-DM1 is currently being evaluated in Phase 1 clinical trials for the treatment of Duchenne muscular dystrophy.[77]

Alnylam utilizes a small-molecule ligand targeting integrin αvβ6 for targeted delivery to muscle.[78] To improve PK/PD properties, a C22 lipid chain is incorporated at positions 6 of the siRNA sense strand.[79] In studies with non-human primates, a single subcutaneous dose of the small-molecule-siDMPK conjugate (3 mg/kg) achieved approximately 80% silencing of DMPK mRNA in skeletal muscle by day 32.[80]

Central nervous system

Central nervous system (CNS) disorders, including Parkinson's disease, Alzheimer's disease, Huntington's disease, and other hereditary neurodegenerative diseases, remain significant unmet clinical treatment needs.[81] A major challenge to treating these conditions is the blood-brain barrier (BBB), which impedes the delivery of oligonucleotide therapeutics to the CNS.[82] Recently, significant advancements have been made in both localized CNS administration and systemic delivery of oligonucleotides.[83]

Local administration is a crucial strategy for delivering oligonucleotides to the CNS.[84] ASOs exhibit intrinsic cellular uptake abilities, allowing them to function without reliance on complex delivery systems.[85] In 2016, Ionis Pharmaceuticals pioneered nusinersen, the first oligonucleotide drug for treating spinal muscular atrophy (SMA), which targets the CNS via intrathecal (IT) administration.[86] Subsequently, Alnylam developed a method of covalently attaching a C16 lipid chain (C16, Figure 5) to the sense strand ribose of siRNA targeting amyloid precursor protein (APP) mRNA. This modification, combined with intrathecal administration, resulted in prolonged suppression of APP mRNA within the CNS.[87] The candidate drug is now in Phase II clinical trials for the treatment of cerebral amyloid angiopathy (CAA).[88] Atalanta Ther-

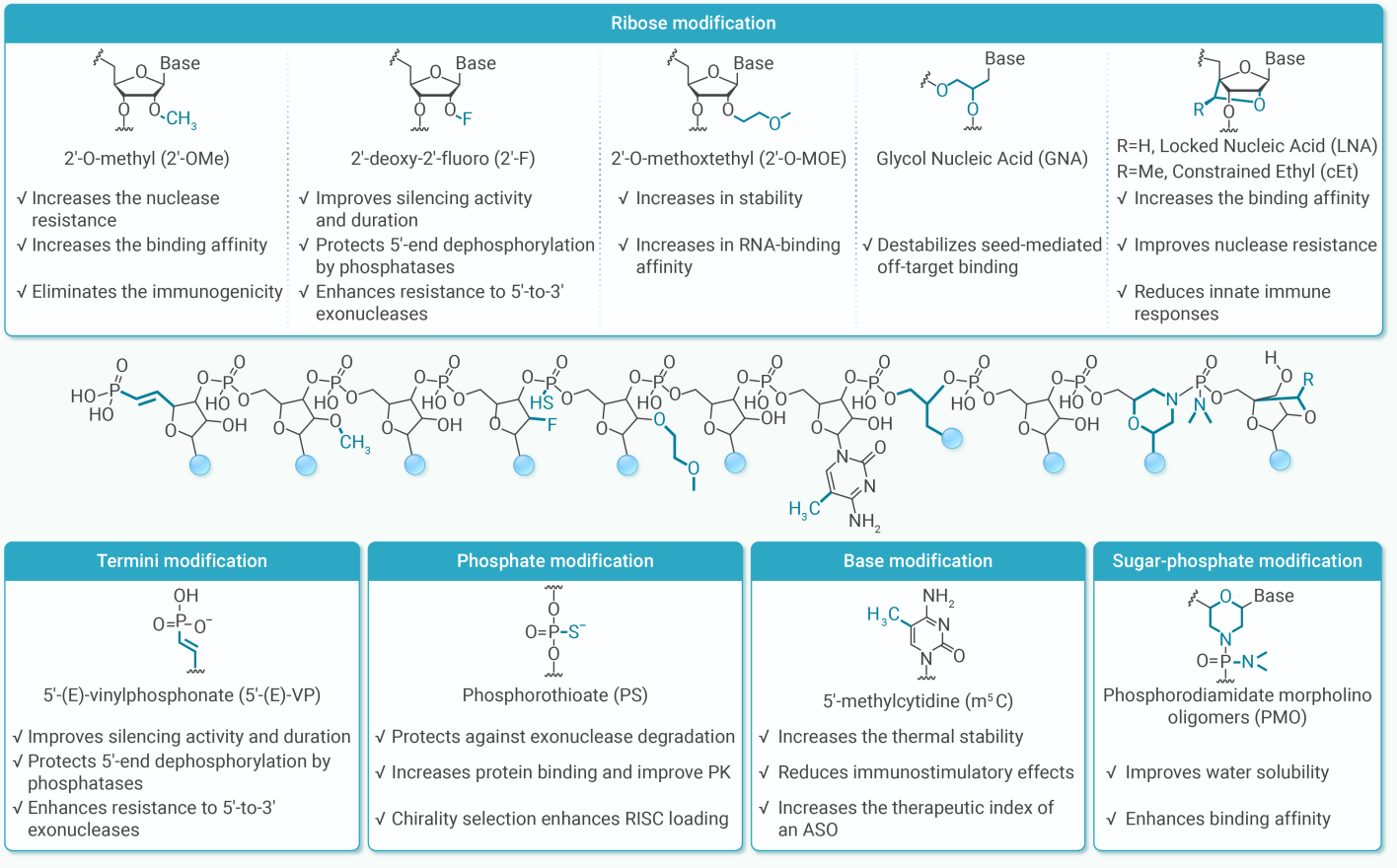


Figure 4. Chemical modification strategies of nucleic acid monomers

apeutics advanced the development by creating a dual siRNA (Di-siRNA) structure. This involves linking two fully chemically modified siRNAs with a linker and increasing the proportion of PS modifications, thereby achieving extended RNA silencing effects in the CNS.[89] Recently, Fu et al. and colleagues utilized click chemistry to attach a high-affinity TfR1 cyclopeptide to an oligonucleotide (hTfR894-N3-1, Figure 5). This local administration approach effectively silenced a target gene mRNA in the CNS, further demonstrating the potential of receptor-mediated strategies for targeted CNS delivery.[90]

Compared to local administration, systemic delivery not only effectively silences target gene mRNA in deep brain tissues but also significantly enhances patient medication adherence.[91] Conjugate approach reported by Arrowhead Pharmaceuticals' TRiM™ BBB platform consists of a TfR1-specific Fab fragment, a non-cleavable polyethylene glycol (PEG) linker, and an oligonucleotide. Administered via subcutaneous injection, siRNA is delivered to deep brain regions with a favorable safety profile, supporting monthly or quarterly dosing regimens.[92] In a study involving non-human primates (NHP), researchers conjugated the platform's ligand with siRNA targeting MAPT and administered it subcutaneously at a dose of 3 mg/kg weekly for three consecutive weeks. Measurements taken on day 29 demonstrated up to 85% maximum silencing of MAPT mRNA in the cortex, with the effects lasting for over three months.[93] Recently, Arrowhead entered into a collaboration with Novartis, granting Novartis exclusive global rights to develop, manufacture, and commercialize Arrowhead's preclinical candidate ARO-SNCA.[94]

Vect-Horus has made significant advances in CNS delivery developing a TfR1-targeting single-domain antibody (VHH, Figure 5, ~14 kDa). Owing to its small molecular size and enhanced tissue penetration, this VHH effectively crosses the blood-brain barrier.[95] Fusion with the IgG1 Fc domain and conjugation to oligonucleotides enables targeted CNS delivery. In NHP studies, weekly intravenous administering (1.5 mg/kg for three consecutive weeks) resulted in up to 70% maximal silencing of HPRT mRNA in deep brain tissues by day 28. [96]

Denali Therapeutics' Oligonucleotide Transport Vehicle (OTV) platform utilizes an engineered TfR1 antibody to efficiently traverse the BBB (OTV, Figure 5).[97] In both mouse and macaque models, this platform achieved greater than a 10-fold increase in brain ASO concentrations, resulting in over 50% reduction in target gene expression.[98] Two programs, DNL628 (targeting MAPT/Tau for Alzheimer's disease) is undergoing Phase I clinical trials and DNL422 (targeting SNCA/α-synuclein for Parkinson's disease), is close to entering clinical stage.[99]

Lilly has demonstrated enhanced CNS delivery of double-stranded RNA oligonucleotides using a full-length TfR1 antibody in cynomolgus macaques (mAb, Figure 5). Following a single intravenous administration of TBP5-dsRNA-DAR1 (10 mg/kg), greater than 80% silencing of APP mRNA was observed in the hippocampus and cortex by day 28, with sustained silencing exceeding 50% still present at three months post-dose.[100]

Lung

The lungs serve as the primary organs responsible for gas exchange in the human body, comprising approximately 300 million alveoli lined by a single layer of epithelial cells.[101] Effective, safe, and precise delivery of nucleic acid therapeutics to the lungs is essential for harnessing their therapeutic potential in major respiratory diseases such as lung cancer, cystic fibrosis, and chronic obstructive pulmonary disease (COPD).[102]

Arrowhead Pharmaceuticals is at the forefront of oligonucleotide pulmonary delivery technology with its tripartite platform consisting of a small-molecule ligand targeting integrin αvβ6 receptor which highly expressed on pulmonary epithelial cells, a linker, and an oligonucleotide payload (Tri-SM6.1-αvβ6, Figure 5).[103] In rat models, a single inhalation of a 0.5 mg/kg rat-specific RAGE-siRNA conjugate resulted in up to 90% RAGE mRNA knockdown in lung tissue, with the silencing effects lasting for over two months.[104] The candidate drug derived from this platform, ARO-RAGE, is currently in Phase I clinical trials.[105] Another drug using the same delivery system, ARO-MMP7, targets matrix metalloproteinase 7 (MMP7) for the

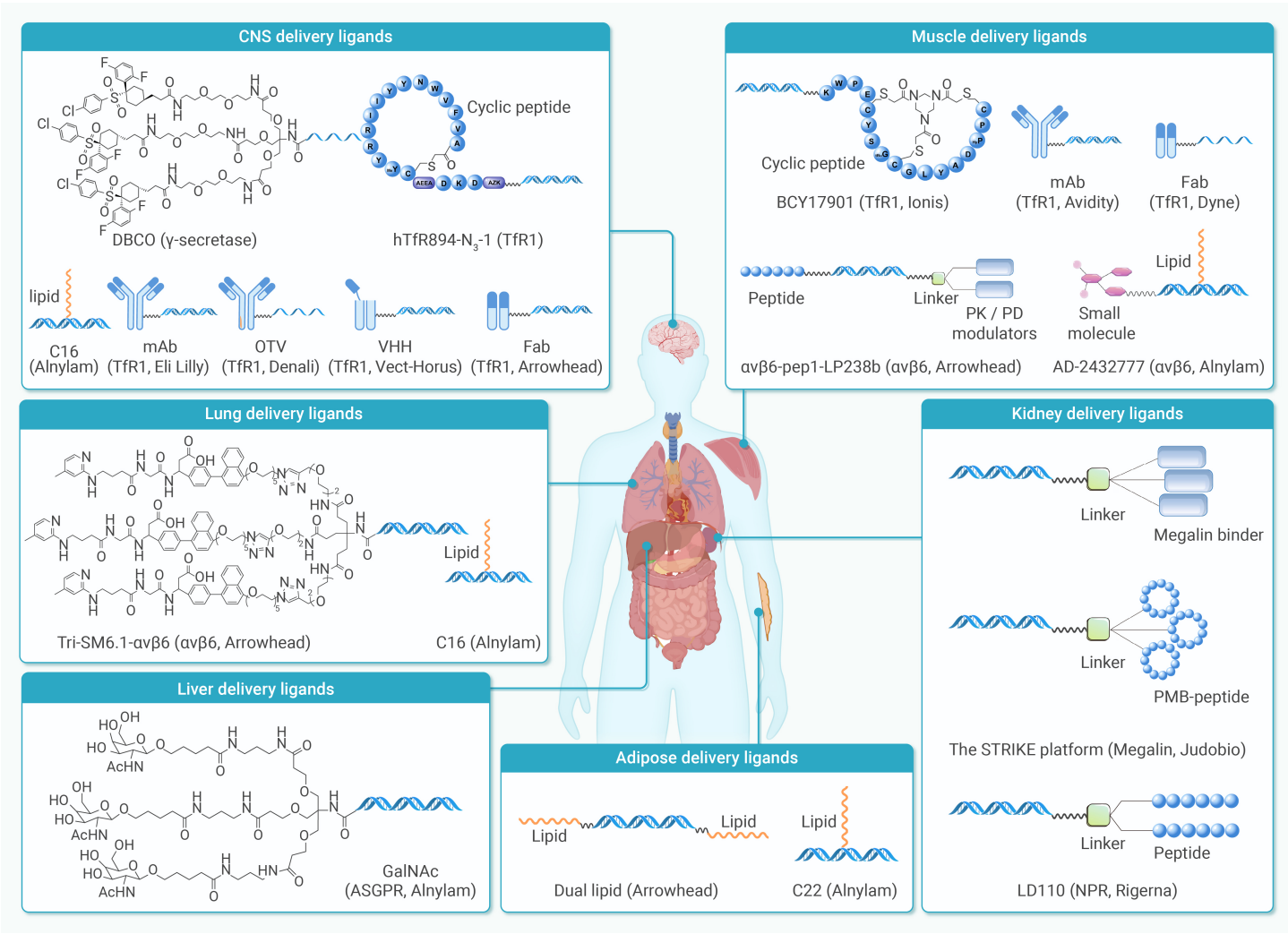


Figure 5. Receptor-mediated oligonucleotide delivery system TfR1: transferrin receptor 1, mAb: monoclonal antibody, OTV: oligonucleotide transport vehicle, VHH: variable domain of heavy-chain antibody, also known as nanobody, Fab: fragment of antigen binding, αvβ6: integrin alphaV/beta6, megalin: also known as low-density lipoprotein receptor-related protein 2, NPR: natriuretic peptide receptor, ASGPR: asialoglycoprotein receptor, GalNAc: N-acetylgalactosamine.

treatment and prevention of Idiopathic pulmonary fibrosis (IPF). The global development and commercialization rights for this compound have been licensed to Sarepta Therapeutics.[106] Furthermore, patents disclosed in 2024 and 2025 by Alnylam and Lilly, respectively, reveal small-molecule ligands targeting αvβ6 for pulmonary-targeted delivery of oligonucleotides.[107,108] Beyond receptor-mediated strategies, Alnylam's C16-modified siRNA (C16, Figure 5) also achieves efficient uptake by alveolar and bronchiolar epithelial cells, enabling potent gene silencing in lung tissue.[109]

Adipose

Adipose tissue is the largest endocrine organ and a central hub for energy metabolism in the human body, playing a critical role in obesity and associated cardiometabolic diseases.[110] Oligonucleotide therapeutics offer promising strategies for precise intervention in these conditions.[111]

Arrowhead Pharmaceutical is a pioneer in adipose-targeted delivery, through a proprietary technology involving dual covalent modification of siRNA sense strands with lipid moieties at both the 3′ and 5′ ends (Dual lipid, Figure 5). This dual-lipid modification enables efficient and sustained silencing of target mRNA within adipocytes.[112] Their candidate drug, ARO-ALK7, is a bilipid-modified siRNA conjugate designed to specifically target and silence activin receptor-like kinase 7 (ALK7) mRNA. Preclinical studies show that a single subcutaneous injection of ARO-ALK7 (3 mg/kg) in non-human primates results in up to 95% silencing of ALK7 mRNA, with efficacy lasting up to 6 months.[113] In obese mouse models, ARO-ALK7, combined with low-dose semaglutide (0.07 mg/kg), led to weight loss comparable to high-dose semaglutide (0.21 mg/kg), while minimizing muscle loss.[114] ARO-ALK7 is currently undergoing Phase I clinical trials.[115] Alnylam has also achieved precise delivery of oligonucleotides to adipose tissue by covalently attaching C22 lipid chains (C22, Figure4) to siRNA sense strands. In non-human primate studies, a single subcutaneous injection of this lipid-siRNA conjugate (3 mg/kg) resulted in over 90% silencing of ALK7 mRNA and protein levels in both white and brown adipose tissues, with effects lasting at least 85 days.[116]

Kidney

The kidneys are central in maintaining body homeostasis.[117] However, their high blood flow, intricate structure, and complex functions pose substantial challenges for the targeted delivery of oligonucleotide therapeutics.[118] Recent studies indicate that receptors with high expression level in the kidneys, is promising for the effective delivery.[119]

The Renal Tubule-Targeting Carbohydrate (RENTAC) platform, developed by researchers at the University of Connecticut, employs chemically modified carbohydrate ligands that specifically bind to the megalin receptor, a protein that is abundantly expressed on the apical membrane of proximal tubule epithelial cells.[120] Preclinical evidence demonstrates that RENTAC platform significantly enhances the accumulation of oligonucleotides in the kidney, offering a novel therapeutic approach for conditions such as renal fibrosis.[121] Similarly, JUDO Bio has established STRIKE™ platform which focuses on exploration of megalin receptor family for siRNA delivery to proximal tubule. The conjugate consists of a megalin ligand, a cleavable linker, and siRNA.[122]

Table 2. Clinical progress of extrahepatic oligonucleotide drugs

Drug name	Indication	Target	Phase	Delivery system	Delivery routes	Organization	Target tissue
AOC-1001	DM1	DMPK	Phase III	Antibody (TfR1)	IV	Avidity	Muscle
AOC-1020	FSHD1	DUX4	Phase III	Antibody (TfR1)	IV	Avidity	
AOC-1044	DMD	Exon44	Phase II	Antibody (TfR1)	IV	Avidity	
SRP-5051	DMD	Exon51	Phase II	CPP	IV	Sarepta	
DYNE-101	DM1	DMPK	Phase II	Antibody (TfR1)	IV	Dyne	
DYNE-251	DMD	Exon51	Phase I/II	Antibody (TfR1)	IV	Dyne	
PGN-EDODM1	DM1	DMPK	Phase II	CPP	IV	PepGen	
ARO-DUX4	FSHD1	DUX4	Phase I	Peptide (αvβ6)	IV	Arrowhead	
ARO-DM1	DM1	DMPK	Phase I	Peptide (αvβ6)	IV/SC	Arrowhead	
Obudanersen	AS	UBE3A	Phase III	N.A.	IT	Ionis	CNS
STK-001	DS	SCN1A	Phase III	N.A.	IT	Stoke	
ARO-ATXN2	SCA2	ATXN2	Phase I	Lipid	IT	Arrowhead	
ARO-MAPT	AD	MAPT	Phase I/II	Antibody (TfR1)	SC	Arrowhead	
ALN-APP	CAA/AD	APP	Phase II	C16	IT	Alnyalm	
RAG-17	ALS	SOD1	Phase II	ACO	IT	Ractigen	
ALN-HTT02	HD	HTT	Phase I	C16	IT	Alnyalm	
ALN-SOD	ALS	SOD1	Phase I	Lipid	IT	Alnyalm	
ALN-5288	AD	MAPT	Phase I	N.A.	IT	Alnyalm	
TRK-250	IPF	TGF-β1	Phase I	N.A.	INH	Bonac	Lung
ARO-MUC5AC	COPD	MUC5AC	Phase I	Peptide (αvβ6)	INH	Arrowhead	
ARO-RAGE	IPD	RAGE	Phase I	Peptide (αvβ6)	INH	Arrowhead	
ARO-MMP7	IPF	MMP7	Phase I	Peptide (αvβ6)	INH	Arrowhead	
ARO-ALK7	OB	ALK7	Phase I	Dual lipid	SC	Arrowhead	Adipose
SGB-ALK7	OB	ALK7	Preclinical	N.A.	SC	Sanegene	
ALN-2232	OB	ALK7	Preclinical	Lipid	SC	Alnylam	

DM1: myotonic dystrophy type 1; DMPK: dystrophia myotonica protein kinase gene; FSHD1: facioscapulohumeral muscular dystrophy 1; DUX4: double homeobox 4; CPP: cell-penetrating peptide; TfR1: transferrin receptor 1; αvβ6: integrin alphav beta6; AS: angelman syndrome; UBE3A: ubiquitin protein ligase E3A; DS: down syndrome; SCN1A: sodium voltage-gated channel alpha subunit 1; SCA2: spinocerebellar ataxia type 2; ATXN2: taxin 2; AD: alzheimer's disease; MAPT: microtubule-associated protein tau; CAA: cerebral amyloid angiopathy; APP: amyloid beta precursor protein; ACO: auxiliary oligonucleotide; HD: huntington's disease; HTT: huntingtin protein; IPF: idiopathic pulmonary fibrosis; MiR-29: microRNA 29; TGF-β1: transforming growth factor beta 1; COPD: chronic obstructive pulmonary disease; MUC5AC: mucin 5AC; IPD: interstitial lung disease; RAGE: receptor for advanced glycation end products; matrix metalloproteinase 7; OB: obesity; ALK7: activin receptor-like kinase 7; IV: intravenous administration; IT: intrathecal administration; SC: subcutaneous administration; INH: inhalation administration.

In mouse models, subcutaneous administration results in approximately 70% mRNA knockdown of the target gene.[123] In NHPs, this conjugate selectively accumulates in renal tubular epithelial cells and induces functional outcomes, such as increased urinary solute excretion.[124] Rigerna Therapeutics exploits the high renal expression of the natriuretic peptide receptor (NPR3) for oligonucleotide delivery. Their targeting ligand is an 11-amino acid peptide linked to a bivalent linker via click chemistry (LD110, Figure 4).[125] In NHP models, this system achieves over 70% target gene silencing efficiency.[126] Furthermore, Alnylam recently revealed mouse data for its kidney-targeted siRNA conjugate: a single subcutaneous injection (1 mg/kg) achieved 75% mRNA and 85% protein silencing of the target gene, further validating the feasibility of receptor-mediated strategies for renal delivery.[127]

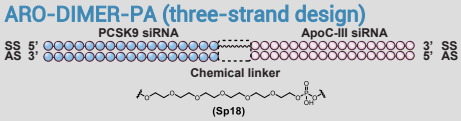
DUAL-TARGETING OLIGONUCLEOTIDE THERAPEUTICS

After years of development, the applications for oligonucleotide therapeutics have expanded from rare diseases to chronic conditions.[128] Chronic diseases often involve intricate pathological mechanisms, affecting multiple signaling pathways, diverse cell types, and interactions across various organ systems.[129] Targeting a single pathway usually results in limited efficacy or can easily lead to drug resistance. Dual-targeting oligonucleotide therapeutics, capable of simultaneously silencing two disease-associated genes, offer a novel strategy for synergistically regulating complex pathogenic networks.[130] In recent years, dual-targeting oligonucleotides have emerged as a crucial focus in the innovative development of drugs for chronic diseases. This section systematically reviews design strategies and clinical research advancements in dual-targeting oligonucleotides, aiming to provide new insights into the development of this therapeutic class.

Design of dual-targeting oligonucleotide therapeutics

Dual-targeting oligonucleotide therapeutics are diverse in terms of their molecular architecture: single-strand design (e.g., two distinct antisense oligonucleotides covalently linked), two-strand design, and three-strand one

Table 3. The design strategies of dual-targeting oligonucleotide

Modality	Design	Efficacy	Ref.
Brogidirsen (single-strand design) 	• Two directly linked 12-mer PMOs targeting exon 44 at 64-75 nt and 92-103 nt	• Exon 44-skipping EC_{50}: 0.63 μmol/L	131
bs YW-61 (two-strand design) 	• Modify sequences to ≤ 2 mismatches per gene • Add G/C to the 5′ end, and A/U to the 3′ end • Fully complementary double strands with 3′ TT overhangs	• YAP1 KD (in vitro): >80% • WWTR1 KD (in vitro): >85%	135
dsiLamin(19)-TIG3(19) (two-strand design) 	• dsiRNA designed with both antisense 5′ ends facing outward	• Parent mixture (in vitro): Lamin KD: ~85%; TIG3 KD: >90% • dsiLamin(19)-TIG3(19) (in vitro): Lamin KD: ~85%; TIG3 KD: ~90%	132
Bis-siRNA-1/2 (two-strand design) 	• Link the 3′- and 5′-ends of the two sense strands or antisense strands via complementary or non-complementary nucleotide linkers	• Parent mixture (in mice): Target 1 KD>90%; Target 2 KD>85% • Bis-siRNA-1/2 (in mice): Target 1 KD>90%; Target 2 KD>85%	134
Bis-siRNA-14 (three-strand design) 	• Link the 3′- and 5′-ends of the two sense strands via cleavable nucleotide linkers	• Parent mixture (in mice): Target 1 KD>95%; Target 2 KD>90%; • Bis-siRNA-14 (in mice): Target 1 KD>90%; Target 2 KD>95%;	
ARO-DIMER-PA (three-strand design) 	• Link the 3′- and 5′-ends of the two sense strands via chemical linkers • The delivery ligand is located at the end of the sense chain	• Parent mixture (in NHP): PCSK9 KD~80%; ApoC-Ⅲ KD~80% • ARO-DIMER-PA (in NHP): PCSK9 KD~80%; ApoC-Ⅲ KD~75%	139

PMOs: phosphorodiamidate morpholino oligomers; EC_{50}: half maximal effective concentration; KD: knock down; YAP1: yes 1 associated transcriptional regulator; WWTR1: ww domain containing transcription regulator 1; TIG3: tazarotene-induced gene 3; Parent mixture: mix siRNA1 (targeting target1) and siRNA2 (targeting target2) in equal amounts.

(Table 3). This section addresses the design principles, advantages, and limitation of each category.

Single-strand design is well exemplified by dual-targeting antisense oligonucleotide.[131] With a sequence of "TTGTGTCTTTCTTCTGTTAGCCAC", Brogidirsen comprises two 12-mer sequences linked directly, which can bind, by complementary base pairing, to sites 64-75 and 92-103 of Duchenne muscular dystrophy (DMD) gene exon 44, leading to the exon skipping. In vitro studies using myotubes derived from DMD patients have shown that Brogidirsen demonstrates impressive activity, with a maximum half-maximal effective concentration (EC_{50}) of 0.63 μmol/L. Importantly, the production process for these dual-targeting single-strand antisense oligonucleotides (ASOs) is akin to that of single-target ASOs, incurring no additional manufacturing costs.

Two-strand design strategy is widely employed in dual-targeting siRNA. Chan Il Chang et al. developed various dsiRNA structures that effectively silence both target genes simultaneously.[132] In all these designs, the 5′ end of the antisense strand, oriented outward from the RNA core structure, exhibited stronger gene silencing activity. This orientation suggests that the seed region of the antisense strand can be effectively loaded into Ago2 after Dicer processing, which is essential for its activity.[133]

Alnylam has developed a design strategy for double-strand, dual-targeting siRNAs by covalently linking the 5′- and 3′-ends of the sense and antisense strands of two distinct siRNAs with 3 to 4 complementary or partially complementary nucleotides. An in vivo study revealed that these dual-targeting constructs, whether linked via complementary or non-complementary bonds, showed slightly reduced activity compared to a direct mixture of single-target siRNAs.[134] It is speculated that, because this design does not orient the 5′ ends of the antisense strands outward, the nucleotide linkers need to be cleaved by nucleases upon entry into the body.[132] This cleavage would release individual siRNAs, allowing them to exert their intended effects.

Kaito Ueda and colleagues proposed a strategy to silence two target genes using a conventional 21/21-siRNA duplex.[135] By targeting at a common sequence found in the transcripts of the YAP1 and WWTR1, they successfully suppressed the expression of both genes simultaneously. The sequence design process involved identifying a 17-nucleotide sequence shared across all YAP1 and WWTR1 transcript variants, wherein at least 13 bases are identical (allowing for a maximum of 4 mismatches). This 17-nucleotide sequence was further refined by focusing the selection on the sense strand to ensure neither YAP1 nor WWTR1 exceeded two mismatches. The sequence was then extended at the 5′ end to 19 nucleotides, with a dTdT overhang added at the 3′ end of both strands to create dual-targeting siRNA. In vitro tests showed that this approach could achieve over 80% simultaneous knockdown of both YAP1 and WWTR1. The primary advantage of this design is its efficiency, as the double-stranded oligonucleotide functions immediately upon entering the cell. Furthermore, the candidate sequences are cost-effective to produce and impose minimal delivery demands. However, this approach requires a high degree of sequence homology with the target, limiting its applicability to specific targets only.

Three-strand dual-targeting oligonucleotides are engineered by covalently linking sense and antisense strands using nucleotides or chemical linkers. These complementary strands anneal with a longer strand, with the delivery molecule typically attached to the 3′ end of the sense strand.[136] This design is widely adopted due to its simplicity. For ease of synthesis, researchers often connect the 3′ end of one sense strand of siRNA to the 5′ end of another using a linker. However, this method positions the 5′ end of one antisense strand inward, potentially affecting gene silencing efficiency. To enhance in vivo silencing efficacy of dual-targeting siRNAs, extensive studies have optimized these linkers. For instance, Alnylam employs a covalent linkage via three nucleotides.[134] In vivo mouse studies demonstrated that dual-targeting oligonucleotides with a cleavable linker (GAU) can achieve silencing effects on two target proteins comparable to separate, mixed siRNAs. Beyond nucleotide linkers, chemical linkers have also been extensively explored. Arrowhead Pharmaceuticals utilizes chemical linkers to covalently bond the 5′ and 3′ ends of siRNA sense strands, optimizing the linker's length and stability to regulate activity both in vitro and in vivo.[137] A study in macaque revealed that the linker-optimized dual-targeting drug (ARO-DIMER-PA) achieved the PCSK9 gene silencing comparable to the mixture, whereas its effect on the ApoC-III gene was slightly reduced.[138] These studies indicate

Table 4. Representative dual-targeting siRNA drugs under clinical development

Drug name	Indication	Target	Phase	Delivery system	Organization
STP705	isSCC/BCC	TGF-β1 & COX-2	Phase II	PNP	Sirnaomics
STP707	ST	TGF-β1 & COX-2	Phase I	PNP	Sirnaomics
ARO-DIMER-PA	DL	ApoC-III & PCSK9	Phase I	GalNAc	Arrowhead
SA1211	HBV	PD-L1 & HBx	Phase I	GalNAc	Sirnabio
RN5681	DL	PCSK9 & LPA	Phase I	GalNAc	Rona
SR122	DL	N.A.	IND	N.A.	Ribo
STP251G	HCh/HH	ApoC-III & TMPRSS6	IND	GalNAc	Sirnaomics
STP237G	HCh/HTN	ApoC-III & AGT	IND	GalNAc	Sirnaomics
X17	DL	PCSK9 & ANGPTL3	Preclinical	GalNAc	Salubris

isSCC: in situ squamous cell carcinoma; BCC: basal cell carcinoma; COX-2: cyclooxygenase-2; PNP: peptide nanoparticle; ST: solid tumor; DL: Dyslipidemia; HBV: hepatitis b virus; PD-L1: programmed death-ligand 1; HBx: hepatitis b virus x protein; LPA: lipoprotein a; HCh: Hypercholesterolemia; HH: Hereditary Hemochromatosis; HTN: Hypertension; AGT: angiotensinogen; TMPRSS6: transmembrane serine protease 6; ANGPTL3: angiopoietin-like 3.

that the type and length of the linker can affect bis-siRNA efficacy, underscoring the necessity for systematic optimization. Next, we will review recent research progress on dual-targeting nucleotide drugs.

Preclinical and clinical studies of bis-siRNA

To date, several dual-targeting oligonucleotide drugs have entered clinical development. Arrowhead Pharmaceuticals' ARO-DIMER-PA (Table 4) targets both PCSK9 and ApoC-III for the treatment of atherosclerotic cardiovascular disease. Preclinical studies suggest that a single dose significantly and persistently improves lipid profiles, reducing serum low-density lipoprotein cholesterol (LDL-C), other atherogenic lipoproteins, and triglyceride levels by approximately 50%. The company submitted a formal application for a Phase I/IIa clinical trial of this drug in October 2025.[139] Rona Therapeutics' RN5681 targets PCSK9 and LPA, and its clinical trial application has been accepted, with Phase I dosing anticipated to begin in first quarter of 2026.[140] SiranBio has independently developed SA1211, a bis-siRNA drug that targets the hepatitis B virus (HBV) and PD-L1 for the treatment of chronic HBV infection. Preclinical data indicate that a single administration achieves combination therapy efficacy, with HBsAg clearance and HBV DNA seroconversion rates both reaching 80%, and no rebound observed. By directly eliminating the virus and lifting immune suppression, this drug may restore patients' adaptive immune response to HBV, addressing the significant clinical challenge of viral recurrence after treatment discontinuation.[141] SA1211 is currently in Phase I clinical trials, with the first patient dosing completed.[142] STP705, developed by Sirnaomics, inhibits TGF-β1 and COX-2 simultaneously, and has demonstrated significant efficacy in local tumor treatment. Phase II clinical data show that 76% of patients achieved complete histological clearance of tumor cells, with this rate reaching 90% in the two optimal dose groups.[143]

Overall, bis-siRNA therapeutics exhibit remarkable clinical potential in the fields of cardiovascular disease, infectious diseases, and oncology. Their dual-targeting strategy not only enhances therapeutic efficacy but also effectively addresses critical clinical challenges that single-target approaches have yet to resolve.

CRITICAL CONSIDERATIONS AND FUTURE DIRECTIONS

Safety

Despite significant advancements in the development of oligonucleotide drugs, safety of this new modality remains a critical consideration. Safety concerns primarily revolve around nephrotoxicity, hepatotoxicity, thrombocytopenia, immunogenicity, and neurotoxicity, which can vary depending on the route of administration.[144] The occurrence of these toxic reactions is closely linked to the drug sequences, their chemical modifications, and their distribution and accumulation in the body. The kidneys and liver, due to their high exposure to these drugs, are the main organs affected. Thrombocytopenia and immune activation are mainly associated with the phosphorothioate backbone and certain sequences.[145] In contrast, the off-target toxicity of siRNA is largely due to its unique mechanism of RNA interference. Its "seed region", which partially complements non-target mRNA, can trigger microRNA-like regulation, potentially leading to adverse effects such as hepatotoxicity.[146]

Lack of efficient extrahepatic delivery system

The extrahepatic delivery of oligonucleotide therapeutics remains a significant challenge. Due to the inherent negative charge, oligonucleotides tend to have poor membrane permeability. They are also susceptible to nonspecific uptake and clearance by reticuloendothelial system-rich organs such as the liver and kidneys, hindering their ability to reach and enter target tissues outside the liver. Although there has been notable progress in using receptor-mediated delivery for oligonucleotides, identifying the right target receptors is still a major hurdle. Ideally, the target receptor should be highly expressed in the intended organ and have low expression in non-target tissues. Additionally, the repeated binding and endocytosis of the receptor by the delivery ligand can lead to receptor downregulation, reducing cellular entry, causing drug resistance, and diminishing long-term efficacy. Furthermore, the developed delivery ligands should not compete with endogenous ligands to prevent interference with the receptor's normal physiological functions. Since most delivery targets are membrane receptors, isolating and purifying these proteins is challenging and complicates subsequent ligand discovery. Currently, delivery ligands are largely limited to a limited set of modalities, such as macromolecules targeting TfR1, which lead to increased dosing requirements and higher production costs.

Challenges in dual-targeting oligonucleotide

While dual-targeting oligonucleotides offer synergistic benefits, they also introduce new challenges. First, their increased sequences complexity expands the potential for unintended interactions with off-target molecules, thereby heightening the risk of off-target effects through cross-reactivity. Second, the pharmacological complexity of simultaneously inhibiting two genes exceeds that of single-target agents. In addition to target-specific toxicities, unforeseen crosstalk between the signaling pathways of the two targets, may occur, potentially leading to unpredictable cellular dysfunction or toxicity. Lastly, compared to single-target oligonucleotides, delivering dual-targeting nucleotides poses systemic challenges as the therapeutic paradigm shifts from "precision" to "synergy". Currently, the liver is the primary target organ for most dual-targeting oligonucleotide therapeutics, and development of delivery systems capable of mediating extrahepatic activity poses considerable difficulties.

Manufacturing complexity

While novel delivery systems overcome the in vivo limitations of oligonucleotide therapeutics, they may introduce significant complexity into the

manufacturing process, demanding rigorous precision in chemistry, manufacturing, and control (CMC). For instance, the production of antibody-oligonucleotide conjugates (AOCs) presents formidable challenges in chemical conjugation and biomolecular manufacturing. These challenges include optimizing linker technology, precisely controlling the drug-to-antibody ratio (DAR), as a DAR greater than 4 may pose risks, and overcoming analytical bottlenecks stemming from the limited resolution of techniques such as mass spectrometry. In the context of dual-targeting agents, the synthesis of oligonucleotide sequences longer than 40 nucleotides with both high purity and yield, coupled with effective separation of these products from impurities generated by incomplete reactions, present substantial challenges in process development.

Future developments in chemistry for advancing oligonucleotide

Over the past decade, advances in nucleic acid chemistry have greatly accelerated the transition of oligonucleotide therapeutics from bench to bedside. Techniques such as Enhanced Stability Chemistry (ESC) and the improved ESC+ design have significantly enhanced the metabolic stability of oligonucleotides, optimized pharmacokinetic properties, and reduced off-target effects caused by seed regions.[147] Second-generation ASOs exhibit increased target affinity and improved safety profiles.[148] Despite widespread research into nucleotide monomers and the synthesis and assessment of various chemically modified monomers in vitro, only a few have progressed to clinical application. Efficient delivery beyond hepatic tissues remains a major challenge, underscoring the need for novel modifications like EVP and its analogues to enhance metabolic stability, safety, and therapeutic efficacy.[149] Primary safety concerns for ASOs include drug accumulation-induced hepatotoxicity and nephrotoxicity, underscoring the need for innovative chemical modifications.[150] In addition to structural optimization of monomers, the vast combination of modification site arrangements makes traditional sequential synthesis and validation impractical. Therefore, future efforts should focus on developing micro-scale synthesis, high-throughput screening, and AI-assisted exploration of modification patterns.[151] While current manufacturing relies on chemical synthesis, next-generation production will increasingly adopt efficient and sustainable biocatalytic methods.[152]

Exploration and discovery of novel membrane receptors for extrahepatic delivery of oligonucleotides

Delivery is crucial for the success of oligonucleotide therapeutics. Receptor-mediated delivery strategies have recently gained attention over nanoliposome systems due to their simple structure, single-component composition, and low production costs.[153] An ideal receptor for delivery should be tissue or cell-specific, efficiently internalized, and quickly recycled. Many receptors now enable targeted oligonucleotide delivery to various tissues and organs. The asialoglycoprotein receptor (ASGPR) is notably successful, efficiently delivering oligonucleotides to the liver.[154] TfR1 shows promise in targeting both muscle and the CNS, with several TfR1-mediated therapeutics in clinical trials.[155] Integrin αvβ6-targeted strategies aim for both delivery to lungs and muscles and are at the proof-of-concept stage.[156] For kidney targeting, research focuses on megalin and natriuretic peptide receptors. Despite these advances, receptor expression heterogeneity within tissues limits broader disease applications.[157] There's an urgent need to discover more suitable receptors for oligonucleotide delivery. Integrating multi-omics data with deep learning models, alongside traditional research methods, offers hope for faster prediction and screening of effective delivery receptors. Validation of new receptors typically involves: (i) In vitro assessments, such as confocal microscopy-based colocalization analysis and gene silencing efficacy testing; (ii) In vivo pharmacokinetic and therapeutic efficacy trials in animals; (iii) Comprehensive safety evaluations to ensure receptor-mediated delivery doesn't disrupt essential physiological functions.

Selecting the optimal delivery ligand for oligonucleotides

Delivery ligands play a crucial role in the effectiveness of oligonucleotide therapeutics. Currently, the main types of delivery ligands include antibodies, peptides (notably cyclic peptides), and small molecules.[158] For example, antibodies are the primary targeting ligands for TfR1, facilitating delivery to both skeletal muscle and the CNS.[159] The distribution of these antibodies is significantly influenced by their affinity and kinetic properties: high affinity and slow dissociation are essential for muscle targeting, while moderate affinity with fast binding and dissociation is better suited for CNS delivery.[65] In AOCs, a drug-antibody ratio (DAR) of 1 generally shows better PK properties and in vivo efficacy compared to DAR2.[160] Optimizing AOCs for therapeutic effectiveness involves careful adjustments of the antibody structure, conjugation site, linker design, conjugation chemistry, and receptor affinity. Small molecules and cyclic peptide ligands are attracting more interest due to their low molecular weight, ease of synthesis, reduced dosing needs, and cost-effective production.[66] Bicyclic peptides targeting TfR1 and peptidomimetic ligands targeting integrin αvβ6 are notable examples demonstrating potential. High-throughput screening methods like DNA-encoded libraries (DEL), phage display, and mRNA display offer efficient means to discover these ligands.[161] Nevertheless, small molecules and cyclic peptides often exhibit low affinity, necessitating multivalent linkers or designs to achieve effective gene silencing.[162] Additionally, incorporating segments that modulate pharmacokinetics, such as saturated fatty acid chains or PEG, can extend circulation time in vivo and improve oligonucleotide accumulation in target tissues.[163]

Enhancing synergy at the biological pathway level: moving beyond simple siRNA target combination

Dual-targeting oligonucleotide therapeutics are attracting increasing interest due to their synergistic effects, reduced dosing frequency and amount, minimized resistance, and lower risk of drug interactions.[164] Key areas for advancing these therapies include: (i) Systematic investigation of linkers' impact on efficacy and stability to inform the design of diverse and structurally sound linking strategies. (ii) Exploration of synergistic mechanisms between both targets to identify potential antagonistic effects and risks of signaling pathway crosstalk while extending therapeutic duration and addressing target-specific toxicity. This involves modulating the metabolic half-lives of oligonucleotide components and establishing quantitative methods for assessing the cumulative toxicity. From a clinical translation perspective, challenges such as optimizing dual-component dosing and developing patient stratification strategies based on biomarkers must be tackled. Clarification of regulatory pathways is essential. Furthermore, overcoming manufacturing bottlenecks related to purity and stability at scale, as well as addressing biological uncertainties such as resistance evolution, demands interdisciplinary collaboration across nucleic acid chemistry, bioinformatics, and clinical research to accelerate clinical realization.

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DATA AND CODE AVAILABILITY

Not applicable.