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# Progress and Remaining Translational Challenges for Antisense Oligonucleotides, siRNAs, miRNAs, and Aptamers as Nucleic Acid Drugs

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Dear Editor,

The combination of high-throughput sequencing, bioinformatics, RNA biology, genome editing, nanotechnology, and molecular diagnostics is revolutionizing our understanding and treatment of diseases. Consequently, nucleic acid science has evolved from a specialized discipline into an essential component of modern medicine, emphasizing the study of nucleic acid molecules rather than proteins. Approved nucleic acid therapies include antisense oligonucleotides, small interfering RNAs, and aptamers (1). Other nucleic acid therapeutics, such as messenger RNAs (mRNAs), which can transiently provide instructions for protein production, are also important. Chemical modification, sequence engineering, and lipid nanoparticle delivery have shown promise for their use in treating infectious diseases and cancer (2).

Despite rapid advances and growing clinical momentum, a key translational gap remains for antisense oligonucleotides (ASOs), small interfering RNAs, microRNAs (miRNAs), and aptamers: the transition from successful target engagement in silico and in vitro to predictable, safe therapeutic exposure in vivo. This letter highlights how structure-informed computational methods could support the rational design of aptamers, ASOs, siRNAs, and miRNAs or improve their target selectivity before experimental validation.

Antisense oligonucleotides, the first approved group of nucleic acid drugs, use complementary base pairing to recognize RNA. They can modify pre-mRNA splicing, promote RNase H-dependent degradation, or interfere with RNA function. Their therapeutic maturity has therefore brought a more demanding medicinal chemistry challenge: increasing target engagement without increasing nonspecific interactions, tissue accumulation, or toxicity. The BROTHERS (BRace On a THERapeutic aSo) architecture provides a useful example of how this problem is being reframed. In this design, a gapmer ASO is paired with a partially complementary peptide nucleic acid strand. The peptide nucleic acid "brace" masks interaction-prone features of the ASO and creates a toehold-mediated strand-displacement mechanism that favors recognition of the intended RNA target. The primary experimental study reported that optimized BROTHERS reduced nonspecific interactions and substantially mitigated hepatotoxicity while retaining on-target knockdown in vivo (3). A recent discussion of BROTHERS further emphasized the concept as a nonequilibrium strategy for separating therapeutic activity from off-target toxicity (4). The key implication for ASO development is that sequence complementarity should not be the sole determinant of selectivity. Future ASO development should integrate computational approaches to predict protein binding, off-target hybridization, tissue exposure, and intracellular trafficking, thereby prioritizing candidates for experimental evaluation.

Second, the siRNA field has provided the clearest recent example of regulatory translation. Small interfering RNAs are double-stranded RNA molecules, usually 21 - 23 nucleotides long, designed to be complementary to a target mRNA. They are based on RNA interference (RNAi), a natural process discovered by Fire and Mello that inhibits

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gene expression at the posttranscriptional level. Early RNAi applications were limited because longer dsRNAs triggered an interferon response and nonspecific mRNA reduction. This limitation was overcome by using dsRNA fragments with two-nucleotide 3′ overhangs, which can induce sequence-specific mRNA degradation without triggering interferon production (5). On November 18, 2025, the United States Food and Drug Administration approved plozasiran, an apolipoprotein C3 (APOC3)-directed siRNA, as an adjunct to diet to reduce triglycerides in adults with familial chylomicronemia syndrome. Redemplo (plozasiran) is an N-acetylgalactosamine (GalNAc)-conjugated siRNA targeting hepatic apolipoprotein C-III and effectively reduces triglyceride-rich chylomicrons and lowers the risk of acute pancreatitis in this rare genetic disorder (6). Another new approach involves lipid-layered core RNA-assembled nanomodules, introduced as a next-generation siRNA delivery platform that achieved ultrahigh RNA loading, efficient fusion-based cellular uptake, and durable in vivo gene silencing, thereby addressing key limitations of conventional lipid nanoparticles in nucleic acid therapeutics (7). These findings suggest that the next stage of siRNA development will depend increasingly on organ-specific delivery, controlled intracellular release, and repeat-dose safety rather than sequence design alone. Computational frameworks that integrate siRNA sequence features, target accessibility, off-target risk, chemical modification, and pharmacokinetic–pharmacodynamic modeling can prioritize clinically viable candidates and guide dose optimization, thereby accelerating the translation of siRNA therapeutics into clinical use (8).

Third, miRNAs present a unique therapeutic opportunity because a single RNA molecule can affect several transcripts and signaling pathways simultaneously. However, this capability also poses challenges because of its broader biological implications, which require careful consideration in therapeutic applications. Recent advances include engineered delivery systems such as BiRDS26, a tetrahedral DNA-based bioswitch for miR-26a-5p in idiopathic pulmonary fibrosis. This system enhances miRNA stability, cellular uptake, and airway delivery, leading to improved outcomes in models, including reduced pulmonary fibrosis and effects on the Hippo and Wnt pathways (9). Another bioswitchable RNA delivery system (BiRDS) was reported for Tri-miR-22. It used a tetrahedral framework DNA nanostructure for extraocular, transscleral delivery of miRNA mimics, achieving efficient choroidal and retinal accumulation and suppressing pathological neovascularization while preserving retinal neuronal integrity. These findings illustrate the potential of multifunctional RNA nanocarriers for the minimally invasive treatment of ocular neovascular and neurodegenerative diseases (10). In addition, multiplexing has emerged as a key feature of miRNA nanomedicine. A 2025/2026 pharmacology review highlighted cationic polymer-based nanocomplexes for the codelivery of multiple tumor-suppressive miRNAs and addressed challenges such as immunogenicity, scalability, reproducibility, and regulation of combinatorial products (11). Another recent study showed that miR-340, miR-382, and miR-17, delivered via brain-penetrating nanoparticles and MRI-guided focused ultrasound, inhibited glioblastoma growth and extended survival in mice (12). Recent studies suggest that computational methods can strengthen the clinical translation of miRNA therapeutics by enabling network-level intervention design, supporting quantitative dose–exposure–response modeling, and providing systematic off-target risk assessment before in vivo evaluation (11).

Fourth, research on aptamer design and development has consistently shown that chemical flexibility and synthetic accessibility make aptamers attractive for diagnostics, but their biological activity must be rigorously characterized before therapeutic applications are considered (13). The recent Auto-SELEX (systematic evolution of ligands by exponential enrichment) microfluidic platform has greatly accelerated aptamer discovery. By integrating rapid electrokinetic target–aptamer separation with plasmonic bead-based PCR, it enabled the identification of high-affinity, target-specific single-stranded DNA aptamers in just two to three selection rounds. Compared with conventional SELEX, this approach can reduce the time, labor, and resources required (14). Moreover, hybrid machine-learning and genetic-algorithm approaches could enhance the in silico design of novel aptamers (15). These methods support the view that computational prioritization should be a starting point in aptamer development rather than a final conclusion. Ultimately, the value of in silico studies lies in generating testable hypotheses, and their clinical relevance depends on whether these hypotheses withstand rigorous biochemical and in vivo validation.

Molecular diagnostics based on RNA/DNA biomarkers are essential to transforming healthcare. Sequencing and nucleic acid tests can identify genetic markers that aid disease classification, treatment decisions, and patient monitoring. Researchers are investigating circulating RNAs as potential biomarkers detectable in serum and other fluids. These diagnostic approaches help identify specific molecular characteristics, categorize patients by treatment response, measure residual disease, and detect resistance (16, 17). Beyond traditional approaches, recent advances have demonstrated the diagnostic potential of miRNA-based molecular computation. One example is a

modular multivalent cascaded DNA logic platform that simultaneously detects miR-21, miR-587, and miR-210 and converts their expression patterns into three-bit fluorescent codes, enabling discrimination of non-tumorigenic, malignant (Luminal/HER2+), and metastatic (triple-negative) breast cancer cells in intracellular and clinical plasma samples (18).

Finally, bioinformatics provides crucial enabling infrastructure in this field. Modern sequencing generates information at a scale that cannot be reliably interpreted through manual inspection alone (19). Computational pipelines identify variants, quantify transcripts, and reconstruct regulatory networks, all of which are crucial to noncoding RNA biology. MicroRNAs and long noncoding RNAs play roles in gene expression and signaling networks, offering potential for biomarker discovery and therapeutic intervention in disease (16, 17). Rather than representing a single breakthrough, advances in bioinformatics have created an ecosystem that links sequence regulation, off-target prediction, and the integration of multi-omics and clinical data (19, 20) with gene-regulatory biology, which elucidates how coding and noncoding RNAs shape cellular states (17), and therapeutic chemistry and delivery engineering, which translate these insights into medicines (21).

Nevertheless, a major challenge in computational nucleic acid research is translating in silico predictions into experimentally validated mechanisms. Associations between microRNAs and tumor phenotypes do not establish causality or clinical relevance. Integrating computational predictions with rigorous molecular, cellular, and in vivo validation is essential for meaningful translation (20). Although machine-learning models can enhance variant interpretation and biomarker classification, their reliability may be affected by small datasets, technical variation, and batch effects. Clinical bioinformatics studies should report model accuracy, calibration, and the potential impacts of false-positive and false-negative results (15).

In conclusion, advancing nucleic acid-based therapeutics and diagnostics requires combining experimental and computational approaches, with a focus on four key priorities: clinically validating bioinformatics pipelines for reproducibility and interpretability; shifting delivery research from general potency claims to tissue-specific targeting and feasible repeat dosing; incorporating molecular diagnostics into clinical trials with prespecified biomarker hypotheses; and ensuring that long-term follow-up is proportionate to biological persistence, distinguishing transient RNA exposure from permanent genome editing.

Nucleic acid innovation should be viewed as a foundation for a more informative approach to medicine rather than as a replacement for existing therapies. Its potential lies in targeting disease mechanisms and linking treatments to molecular diagnostics, but challenges remain in delivery, off-target effects, manufacturing, accessibility, and long-term safety (22). Future progress will come from integrating these disciplines rather than celebrating them separately. If bioinformatics, gene regulation, therapeutics, and diagnostics are developed as a single translational system, nucleic acids may deliver a more precise and adaptive model of clinical care.

## Footnotes

**AI Use Disclosure:** The authors declare that no generative artificial intelligence tools were used in the creation of this article.

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