

Minding the gap: Unlocking the therapeutic potential of aptamers and making up for lost time

Aptamers are RNAs that can bind proteins directly and modulate protein-protein interactions. Given their therapeutic potential, aptamers would be expected to capture the interest of both scientists and investors. However, concerns regarding safety, efficacy, and delivery have delayed aptamer development and dampened investor support. Herein, we discuss the major hurdles stalling the translational application of aptamers over recent years and focus on approaches to overcome current barriers and attract the scientific community and investors to the aptamer field.

THE BLOSSOMING OF RNA DRUGS

The unprecedented success of the recently developed mRNA-based vaccine platform for coronavirus disease 2019 (COVID-19) has drawn global attention to the advantages of RNA-based therapeutics, i.e., precision targeting, high efficacy, and low toxicity.

The earliest publications reporting the ability of RNA to bind proteins and other biomolecules date back to the 90s. Since then, the development of RNA platforms has accelerated in an attempt to unlock their broad translational potential. To this end, understanding the roles played by numerous classes of non-coding RNAs (ncRNAs) has been the critical first step, while overcoming the challenges of delivering negatively charged RNA molecules has been the second. Chemical modifications of synthetic RNAs (coding or non-coding) may facilitate the development of RNA therapeutics. These modifications can enhance RNA stability and prevent immune activation, as well as lay the groundwork for lipid-based carriers.¹⁻³

One major advantage of RNAs is their ability to assume structured conformations enabling functional interactions with proteins and other RNAs. Indeed, RNA-binding proteins (RBPs) in functional complexes regulate key intracellular processes, e.g., RNA interference and gene expression, with precise targeting. Hence, RNA-based drugs have the potential for rapid, cost-effective development and manufacturing. They may also be able to reach targets otherwise “undruggable,” namely, factors controlling nuclear functions or gene and protein expression.

RNA therapeutics include short-interfering RNAs (siRNAs), microRNAs (miRNAs), messenger RNAs (mRNAs), and aptamers that can serve as a versatile platform to address unmet clinical needs. Although only a small number of RNA therapeutics and vaccines have been approved for clinical applications, the number in preclinical development and clinical trials is growing rapidly.⁴ The compounds under investigation are expected to change the standard of

care for many diseases in the next few years. Many studies are also underway to improve the distribution, toxicity, functional delivery, and intracellular uptake of RNA molecules *in vivo*.

MECHANISMS OF ACTION

The most well-studied RNA mechanism of action pertains to siRNAs and miRNAs. Through the RNA interference (RNAi) pathway, siRNAs and miRNAs are incorporated into the RNA-induced silencing multiprotein complex (RISC). RNAs within the RISC recognize complementary sequences in target RNA transcripts and modulate their expression by post-transcriptional gene silencing.⁵

RNA aptamers, also known as nucleic-acid monoclonal antibodies (mAbs), are artificial, single-stranded short RNAs that can bind proteins and biomolecules with high specificity and affinity. Instead of binding to the RISC, following cellular uptake, RNA aptamers assume a folded structure to interact with the disease-associated target directly. Like mAbs, RNA aptamers bind to proteins, but some pharmacokinetic and biodistribution properties remain to be elucidated.

FIRST RNA DRUGS APPROVED

Pegaptanib (Macugen) was the first RNA biotherapeutic approved by the US Food and Drug Administration (FDA) in 2004 for the clinical treatment (via intravitreal injection) of age-related macular degeneration (AMD). Pegaptanib is a pegylated RNA-modified aptamer, originally raised against isoform 165 of vascular endothelial growth factor A (VEGF-A) as a 2'-fluoropyrimidine RNA-based aptamer.⁶ Nearly 14 years later, Patisiran (Onpattro, Alnylam Pharmaceuticals) became the first clinically available RNAi drug for the treatment of hereditary transthyretin amyloidosis (hATTR) with polyneuropathy. In 2019, another siRNA drug, Givosiran (Givlaari, Alnylam Pharmaceuticals), was approved for the treatment of acute hepatic porphyria.³

THE APTAMER PARADOX

Even though the first FDA-approved RNA drug was an aptamer, no other aptamer drugs have arrived on the market in 20 years.⁶ A few aptamer-based molecules are currently in clinical trials or at various stages of development,^{6,7} but enthusiasm for clinical development of aptamers seems to lag behind that of siRNAs and mRNAs. The origin of this paradox could be attributed to the premature termination of a phase 3 clinical trial of Pegnivacogin following severe cases of allergic reactions, even though these reactions were not directly related to the aptamer itself.⁸ Similarly, commercial enthusiasm for Macugen, the RNA aptamer targeting the isoform 165 of VEGF, waned when competitor mAbs (Ranibizumab, Lucentis) targeting

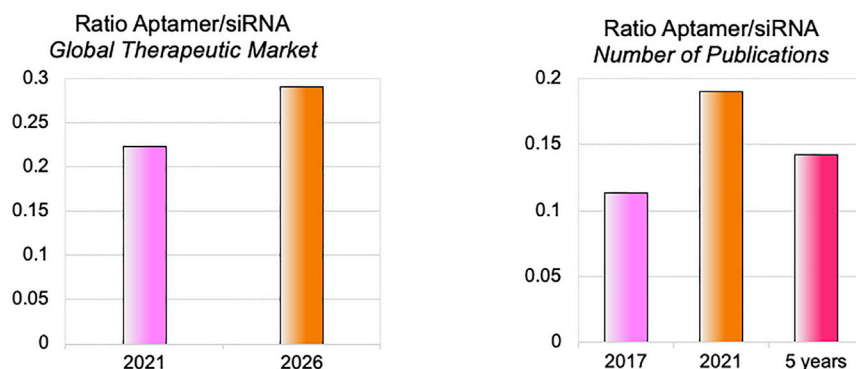


Figure 1. The gap between aptamers and siRNAs, measured as the ratio of aptamer/siRNA expected investments and publications

Market ratios (left panel) were extrapolated from the expected values of markets using data from *Research and Markets- Reports 2022* – “Global Aptamers Market” and “Global RNAi Therapeutics Market.” Publication ratios (right panel) were obtained using “therapeutic aptamer” or “therapeutic siRNA” as keywords in PubMed.

multiple isoforms of VEGF with a broader range of action proved to be more effective against AMD. These setbacks fueled hesitation among investors and companies and drove the perception that the aptamer field was too immature for the clinic.⁷

Still, RNA aptamers hold key advantages with respect to other biotherapeutics, many of which are shared with other therapeutic RNAs: (1) low batch-to-batch variability (due to fully synthetic production), (2) cost-effective manufacturing, and (3) the ability to introduce chemical modifications that increase stability and reduce immunogenicity. Additionally, RNA aptamers can be designed as chiral ligands, namely Spiegelmers, that are not substrates for RNAses. NOXXON Pharma AG has developed the Spiegelmer platform, and one candidate, NOX-A12, is in a phase 2 study for cancer patients. RNA aptamers can also be designed as aptamer moieties, enabling cell-specific delivery via nanoparticles or naked RNAs that bind cell-surface receptors expressed on target cells. This latter approach has led to bifunctional and bispecific aptamer conjugates being developed to act as both therapeutics and therapeutic carriers.⁹

HURDLES TO THERAPY

The main obstacles to the development of RNA aptamers and other RNA therapeutics are the poor stability and rapid renal clearing rate of RNAs in circulating fluids. These obstacles have been addressed by the introduction of chemical modifications to protect RNA molecules, administered as naked conjugates (Alnylam: Givosiran, Lumasiran) or loaded into lipid nanoparticles (Alnylam: Patisiran, Onpatro, or COVID-19 vaccines mRNA-1273 and BNT162b21).⁵

The strange case of Pegnivacogin

Polyethylene glycol (PEG) is a family of molecules of different lengths and branching that are considered biologically inert and present in the formulation of various drugs. Pegnivacogin (Regado Biosciences) is a modified, rapidly reversible anticoagulant RNA aptamer against factor IXa (FIXa). To enhance the pharmacokinetics properties of Pegnivacogin, the naked aptamer was conjugated to a 40-kD

branched PEG. Unfortunately, two phase 3 clinical trials were terminated prematurely due to the onset of severe allergic reactions in 0.6% of patients receiving Pegnivacogin. Interestingly, these adverse events were observed exclusively in patients with pre-existing anti-PEG antibodies. Whether the patients’ reactions were caused solely by the specific size and branching of the PEG or in combination with a response elicited by the FIXa aptamer remains unclear.⁸ This case has also spread uncertainty among investors about the translational application of aptamers.

A larger community matters

In stark contrast to the miRNA field,¹⁰ the aptamer field has lagged behind and remains the prerogative of only a few pioneering laboratories. Consequently, overall investments and resources devoted to aptamer research have been surpassed by those supporting mRNA and siRNA research. This is well reflected by the trend in the expected size of market investments (Figure 1). While “the global RNAi therapeutics market is expected to record a value of US\$ 1.15 billion in 2026, progressing at a Compound Annual Growth Rate (CAGR) of 10.19%, over the period 2022–2026, [...] the Global Aptamers Market size is expected to reach \$392.7 million by 2027, rising at a market growth of 17.6% CAGR during the forecast period” (Research and Markets Reports). Remarkably, most big pharma companies are absent in the aptamer market. Instead, this market is dominated by small and medium companies, including SomaLogic (listed on Nasdaq since September 2021), Aptagen, Aptamer Group, Aptamer Sciences, NOXXON Pharma, Ophthotech, Duet Therapeutics, and Aptadel Tx.

CHANGE IS POSSIBLE

Given the unique advantages of aptamers, significant efforts should be made to exploit their full clinical potential. In addition to the administration of aptamer-loaded lipid nanoparticles that have been effective for local treatments, the use of naked aptamers for targeted delivery is coming to fruition. Aptamer selection remains a laborious process, but several companies, such as Aptagen and the Aptamer Group, provide a custom development service that bypasses

the challenges of systematic evolution of ligands by exponential enrichment (SELEX). Likewise, publicly available databases will facilitate development of the field (<https://www.aptagen.com/apta-index/>; <https://scicrunch.org>).

CONCLUDING REMARKS

The increasing number of RNA therapeutics in clinic and advanced clinical trials provides a blueprint for the development of precise tools to modulate the expression of target genes. As described herein, aptamers combine the functional binding features of antibodies with the conformational flexibility of RNAs. Moreover, the ability to conjugate RNA aptamer molecules with other aptamers and/or therapeutic RNAs enables precise targeting to cells and disease-associated targets.

Thanks to these unique traits, RNA aptamers are now poised to rapidly emerge as a class of precise RNA-based drugs for undruggable or hard-to-reach targets. Therefore, a revival of joint efforts by the scientific community and investors is expected to translate the therapeutic potential of RNA aptamer-based platforms from bench to bedside.

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AUTHOR CONTRIBUTIONS

A.D.R. and V.d.F. conceived and wrote the editorial.

DECLARATION OF INTERESTS

The authors declare no conflict of interests.

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