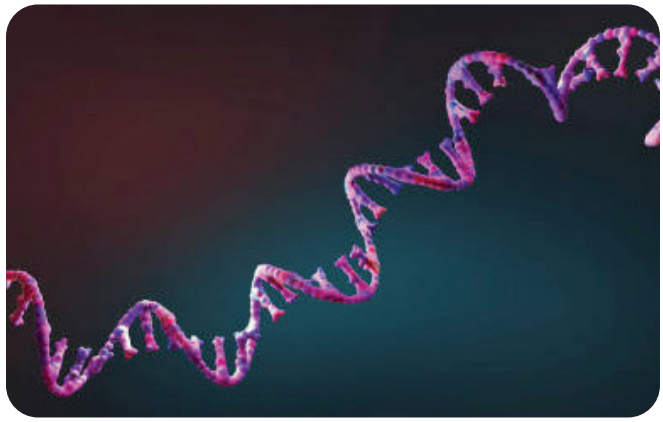
An emerging therapeutic modality: RNA carves its path out of the paradigm

In 1990 when Wolff et al. injected mRNA into mice muscle *in vivo* and triggered protein expression they laid the foundations for the use of RNA molecules for therapeutic applications.[1] Since then, interest in RNA has grown immensely and researchers have identified several types of RNAs involved in cell functional and regulatory pathways. There are also tools and technologies available that enable *in vitro* or chemical synthesis of RNAs. Considering the growing interest in RNA therapies, this article will highlight several promising types of RNA for therapeutics, including RNAi, CRISPR, base editing, and mRNA vaccines, as well as the potential to combine RNA technologies with CAR-T.

Gene silencing by RNA interference

RNA interference (RNAi) is an endogenous cellular process whereby double-stranded RNA molecules known as small interfering RNAs (siRNA) trigger the degradation of a particular RNA target. This gene silencing method has opened exciting possibilities for gene therapy, allowing researchers to utilize RNAi to study the function of genes within biological pathways. RNAi is also used therapeutically to inhibit the expression of genes linked to disease-specific phenotypes.

Gene expression modulation is achieved by transfecting cells with synthetic siRNA oligos, or by transducing cells with lentiviral particles encoding for small hairpin RNAs (shRNA) in the case of difficult-to-transfect cells. One of the main concerns with siRNA transfection has been the instability of small RNA molecules *in vivo*. Various researchers have addressed this challenge by either testing molecules that encapsulate and protect the siRNA oligos,[2] making chemical modifications that allow the passive internalization of siRNA *in vivo*,[3] or by increasing the stability of the siRNA oligos.



Another challenge when working with siRNAs is the potential of off-target effects or down-regulation of unintended targets. Such issues have been reduced by adding 2′-O-methyl modifications to specific positions within the siRNA seed region[4] or using algorithms to design the siRNAs.[5]

To date, the FDA has approved four siRNA therapeutics for managing rare metabolic disorders, such as hereditary transthyretin amyloidosis (hATTR), acute hepatic porphyria (AHP), primary hyperoxaluria type 1 (PH1), and heterozygous familial hypercholesterolemia (HeFH).[6] Clinical trials are also evaluating the ability of RNAi to treat other diseases, including infectious and rare genetic[7] diseases, as well as certain cancers.

Further advancing gene function studies with CRISPR

While RNAi has proven to be an effective tool for gene silencing, the discovery of CRISPR has also revolutionized the field of gene editing. The CRISPR system relies on two components to precisely edit a target sequence: a designed guide RNA (gRNA) which recognizes a specific target region,

and a nuclease (Cas9) which cleaves the genomic DNA. The CRISPR-Cas9 system can be used in multiple ways for various gene editing applications, such as site-specific deletions and insertions. CRISPR can also be used to validate gene function data obtained using RNAi approaches. In addition, the availability of CRISPR screening libraries has enabled whole genome studies to be performed, generating data on entire gene families or biological pathways.

Therapeutically, CRISPR gene editing has opened new possibilities in precision medicine. Trials are currently underway to treat blood disorders, cancers, inherited eye disease, diabetes, infectious and inflammatory diseases, as well as protein-folding disorders. A critical step with CRISPR editing is the design of the gRNA to minimize off-target effects, which has led to the development of algorithms[8] that help avoid non-specific editing of the genomic DNA.

A versatile therapeutic modality

Advances in CRISPR gene editing have also enabled new developments in chimeric antigen receptor (CAR) T cell therapies. One of the benefits of CRISPR-Cas9 is the precision of gene edits, meaning fewer off-target effects. By combing both approaches, researchers hope to improve T cell effector function and persistence and reduce treatment-related toxicities. mRNA technologies are also enhancing the capabilities of CAR-T therapies. For example, scientists have generated disease-specific CAR-Ts *in vivo* using modified mRNA contained in T cell-targeted lipid nanoparticles.[9] This combined approach successfully reduced fibrosis and restored cardiac function in a mouse model of heart disease.

Gene editing technologies have also opened up new avenues for chemically modified synthetic gRNA to recruit a deaminase to a DNA locus of interest, facilitating highly efficient and precise nucleotide or base conversion, known as base editing. Compared to Cas9-mediated knockout, base editing enables higher cell viability, less gRNA-dependent off-target editing, and reduced frequency of chromosomal translocations. Importantly, edited T cells retain their proliferative and cytotoxic ability *in vitro*.

In other applications, researchers are exploring the role of epigenetic modifications of RNA and their implications in antiviral immunity, tumorigenesis, and cancer progression. For example, one of the most common and abundant transcriptional modifications of RNA is N^6-methyladenosine (m^6A) RNA methylation. A recent review article suggests that m^6A RNA methylation can regulate malignant cancer phenotypes and treatment resistance through its impact on mRNA stability and translation efficiency, suggesting that such modification hold potential for treating certain cancers.[10]

Unlocking the potential of mRNA vaccines

One of the most well-documented therapeutic applications of RNA in recent years is its use in vaccine development. mRNA vaccines possess several beneficial features that make them attractive alternatives to traditional vaccines. These include the flexible nature of the mRNA platform and the ability to efficiently deliver mRNA *in vivo* using lipid-based vehicles, allowing rapid uptake and expression in the cytoplasm without any genome integration risk and the generation of subsequent mutagenesis. mRNA can also be manufactured in a cell-free manner, allowing rapid, scalable, and cost-effective production.

Interest in mRNA-based vaccines spiked in 2020 when two leading and successful SARS-CoV-2 candidates from Moderna and Pfizer-BioNTech used the RNA encoding the virus spike protein as an immunogen. However, mRNA-based vaccine studies have been conducted for decades, albeit with limited success. The first mRNA vaccine was developed in 1995 for the treatment of cancer.[11] Unfortunately, it took many years for such experiments to yield sufficient data for this approach to move to human clinical trials.

The first clinical trial for an mRNA-based vaccine was launched in 2013, which tested the safety and immunogenicity of an mRNA rabies vaccine in healthy adults.[12] Two years later, the first lipid nanoparticle (LNP)–formulated mRNA vaccine was evaluated against two highly pathogenic avian influenza strains.[13] However, it wasn't until the COVID-19 pandemic that an mRNA-based vaccine received emergency use authorization or regulatory approval. Now, mRNA-based vaccines continue to be the focus of many SARS-CoV-2 vaccine strategies, as well as other infectious diseases and cancer. In the oncology field, mRNA cancer vaccines are being evaluated in combination with drugs that enhance the body's immune response to tumors. Researchers are also exploring personalized vaccines that stimulate an antitumor response based on the mutational signature of a patient's tumor.[14]

Heading for therapeutic success

Given the success of the COVID-19 vaccines, it is not surprising that RNA-based therapeutics have gained significant attention, with the potential to grow considerably. But this was only achievable due to decades of research, failures, and perseverance. Efficient and safe delivery remains an important hurdle to overcome, while manufacturing and scale-up challenges also need to be addressed. In addition to the science itself, companies must also consider process development optimization to address supply chain issues and effective "chain of custody" for mRNA-based vaccine delivery – closing the gap between mass production and precision medicine.

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