RNA therapeutics

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ABSTRACT

"RNA therapeutics" refers to a disease treatment or drug that utilizes RNA as a component. In this context, RNA may be the direct target of a small-molecule drug or RNA itself may be the drug, designed to bind to a protein, or to mimic or target another RNA. RNA has gained attention in the drug-development world, as recent clinical successes and breakthrough technologies have revolutionized the drug-like qualities of the molecule or its usefulness as a drug target. In this special issue of *RNA*, we gathered expert perspectives on the past, present, and future of the field, to serve as a primer and also a challenge to the broad scientific community to incorporate RNA into their experimental design and problem-solving process, and to imagine and realize the potential of RNA as a therapeutic drug or target.

We have assembled this special issue of the *RNA* journal on the topic of RNA therapeutics, in response to the success of nucleic acid–based drugs in the clinic and the growing number of scientists working to translate their RNA research into treatments. Over the course of just a few years, RNA therapeutics have advanced from a largely hypothetical concept to clinical reality. In 2015, on the occasion of the 20th anniversary of *RNA*, we and more than 200 other RNA researchers contributed short reflections on the field, including what we believed the future held for RNA. Very few conjured RNA therapeutics as a major future advance. In our own separate reflections, even as we both worked on RNA-based disease-modifying approaches, we only briefly mentioned the RNA-targeted therapeutics that were undergoing clinical trials at the time, and one of us cautiously stated the belief that "RNA targeting … has tremendous potential to alter gene expression for research and practical applications." Little did we anticipate the speed at which the RNA therapeutics field would grow in the years to follow. Shortly after those circumspect views were published, breakthrough RNA-based medicines for spinal muscular atrophy and Duchenne muscular dystrophy were approved for clinical use and caught the attention of the public as a new treatment modality for rare genetic diseases. And COVID mRNA vaccines were developed in record time, with billions of doses administered under emergency use authorization. Today, there are already 18 clinically approved RNA-based therapeutics, including two vaccines that made mRNA a household word during the COVID-19 pandemic.

From these vaccines to siRNAs and antisense oligonucleotides, from treating global pandemics to the rarest of diseases in single patients, RNA therapeutics is no longer a niche field, but a mainstream approach to drug discovery and development with a pipeline of hundreds of new RNA-based therapeutics in preclinical and clinical development for a wide range of diseases. These achievements were made possible by decades of advances in basic research in the field of RNA. In this special issue of *RNA*, we asked experts in different areas of RNA therapeutics to contribute perspectives that balance the promise of RNA technology with the conceptual and technical challenges that still need to be solved. In addition, we asked them to complete their work within a short time frame. We are very grateful to the authors that rose to this challenge, and we acknowledge that this accelerated time frame made it impossible to cover all relevant topics. What emerged as the final product of this project is a compendium of original perspectives that broadly share the theme of therapeutic approaches for targeting RNA, including editors, small molecules, antisense oligonucleotides, and siRNAs. We anticipate future special issues that specifically consider other related and rapidly advancing topics, such as mRNA therapeutics and CRISPR-mediated gene editing. In the perspectives that follow, the authors draw on their experiences and highlight important findings that open the door to therapeutic targeting and development, as

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well as the obstacles that must be surmounted to reach such goals.

Although the potential of RNA technology is enormous, the challenges of developing novel therapeutics require thoughtful consideration. There are many hurdles to overcome. Dowdy, in his perspective, delves into a key requirement for any medicine: effective delivery. For RNA therapeutics, this is a nuanced topic with many different obstacles that, in some cases, are dependent on the type of RNA being delivered. Many of the drugs currently in the clinic do not require formulation or delivery modalities, instead relying on the chemical nature of modifications and conjugates to the RNA that stabilize and/or facilitate internalization of the molecules, allowing for their uptake from circulating biofluids. Other RNAs or RNA-targeting strategies require packaged delivery to escape immune detection and/or degradation or other barriers and allow for cellular uptake. Irrespective of how an RNA therapeutic is delivered to a cell, most known entry paths involve endosomal trafficking, and release from the endosome is imperative for the RNA therapeutic to carry out its intended activity. Dowdy elaborates on this endosomal escape problem, evaluating current strategies in use or in development, and providing a comprehensive perspective on the requirements for developing an endosomal-escape domain for RNA therapeutics to increase their potency and efficacy, and thus hasten RNA drug development.

Chemical modifications and targeting of RNA therapeutics are other critical aspects of drug success. Most of the perspectives in this issue consider the development of therapies that use RNA as the primary drug modality. Many RNA therapies take advantage of the fundamental principles of complementary base-pairing of nucleic acids, relying on such antisense base-pairing to target a synthetic oligonucleotide to a specific RNA. The exact chemical modifications, length, structure, and other features of the synthetic RNA determine the outcome of its base-pairing to its intended target. Small interfering RNA (siRNA) drugs are chemically modified double-stranded RNAs that, following delivery to the cell, are incorporated into the endogenous RNA-interference silencing complex (RISC), which enables binding to the target mRNA sequence and subsequent degradation of the transcript. Single-stranded RNA drugs, such as steric blocking/splice-switching and RNase-H targeting/gapmer ASOs work by different mechanisms from siRNAs. Gapmers are composed of a central segment of DNA nucleotides flanked on either end by RNA-like nucleotides. Upon base-pairing to its target RNA, the DNA/RNA hybrid sequence becomes a substrate for cellular RNase H, which cleaves the RNA strand, thereby down-regulating gene expression. Steric-blocking ASOs, unlike siRNAs and gapmers, do not recruit cellular proteins as mediators, but rather function by blocking recognition of RNA by RNA-binding proteins or RNPs, such as pre-mRNA-splicing factors, thereby modulating RNA processing and altering the expression or function of the targeted RNA. Irrespective of their mechanism of action, all of these types of therapeutics require modifications to improve their drug-like qualities. For this reason, research and development on chemical modifications has been a major area of advancement.

Egli, Schlegel, and Manoharan provide a detailed anthology of the chemistry of siRNA drugs and modifications that have been key to the clinical success, and those on the horizon that will likely lead to better drugs in the future. As the chemistry of siRNAs as drugs is further optimized, the door opens for expanding the repertoire of targets and for better understanding the cellular process of RNAi to identify novel uses for siRNAs in the clinic. Along these lines, Johnson and Corey consider RNAi activity in the nucleus of the cell, and provide a provocative potential intervention point for RNA therapeutics based on these mechanisms. Hall, in his perspective, delivers an expansive outlook on ASO and siRNA therapeutics, reviewing the clinical successes of these molecules to date, and the path taken to get to those successful end points, including the chemical and delivery-design hurdles that were surmounted. Finally, he provides an outlook for the future in this rapidly evolving and growing area of RNA therapeutics. The search for new RNA-based therapeutics with enhanced stability, specificity, and deliverability is ongoing; Pradeep, Malik, Slack, and Bahal explore the utility of one particular type of modified antisense oligomers called peptide nucleic acids (PNAs) as a therapeutic modality, outlining the advantages they might have as drugs and the development challenges they have faced.

One attractive quality of ASOs is their inherent target specificity and mechanism of action, which enables rapid design and lead-compound designation, opening the door for personalized drugs tailored to specific mutations. Indeed, in the last several years, n-of-1 ASO treatments have been successfully implemented in remarkably short time frames. These successes have led to the emergence of new regulatory guidance for the development of RNA-based therapeutics, creating an environment that is favorable for growth of the field. Aartsma-Rus and colleagues share their experience in working to develop personalized splice-switching ASOs for ultrarare diseases. The authors describe and discuss recent recommendations and guidelines from European regulatory and advisory groups for designing and implementing such n-of-1 human studies.

The potential of oligonucleotide therapeutics extends beyond those that function by virtue of complementary base-pairing with a specific target RNA. RNA aptamers are RNAs that are selected to form a stable three-dimensional structure that binds to a specific target, usually a protein, and to modify its activity in a therapeutic manner. Aptamers might be designed to block or modify target activity, detect targets for diagnostic purposes, or even enable targeted delivery of other therapeutics, such as

ASOs. The aptamer Macugen/pegaptanib was one of the first FDA-approved RNA-based drugs, approved by the FDA in 2004 to target VEGF and treat age-related macular degeneration. Despite this success story, limitations in the pharmacokinetic properties of aptamers have delayed further commercial development so far. Nonetheless, optimism for this platform persists. Yu, Frederiksen, and Sullenger provide a past and future perspective on the use of RNA aptamers as anticoagulants, an important need in the clinic for which RNA may have some unique advantages to ideally address the shortcomings of current drugs.

RNA therapeutics comprise more than medicines that are made of RNA or RNA-like molecules. A major area of development in RNA therapeutics is on targeting endogenous RNAs not with synthetic RNAs, but rather with small molecules. Most traditional pharmaceutical drugs are small-molecule compounds, and most of them target proteins. However, the FDA approval of risdiplam in 2020, a small-molecule drug that works by targeting RNA to treat spinal muscular atrophy, has triggered new interest in RNA as a target for small-molecule drug discovery. The ability to identify compounds that can bind to RNA and alter its function or expression broadens the landscape of druggable targets. However, many unique challenges currently limit the rational design of RNA-targeting molecules. These challenges and potential solutions are discussed in two perspectives: Nickbarg, Spencer, Mortison, and Lee draw on their experience in discovering small molecules that target the long noncoding RNA Xist in their perspective on designing, testing, and identifying drug-like compounds that specifically bind to RNA using direct RNA-binding screens; Bagnolini, Luu, and Hargrove also consider the possibilities of small molecules that target RNA for clinical applications, championing computational methods that can be used to facilitate identification and optimization of specific RNA-binding compounds.

Another type of RNA-focused therapeutic approach that is showing promise arose, in part, from the field of DNA editing. Direct editing of DNA has gained much attention as a potentially game-changing treatment for disease, though off-target effects with the potential to introduce unintended deleterious mutations remain a concern. Editing DNA might be considered an optimal therapy or even a cure for a disease, because it would have a lasting effect through the life of the patient. However, targeting mRNA, though not as long-lasting, may be less risky in terms of off-target changes, given the short half-life of RNA and the reversibility of its effects. In their perspective, Morelli, Smargon, and Yeo take on the topic of nucleic-acid editing, focusing on the promising possibilities of RNA editing for treating disease. Unlike DNA editing, direct modification of RNA sequences is a naturally occurring mechanism in eukaryotic cells that can be specifically directed to an RNA target using engineered editing enzymes. Quiroz, Siskel, and Rosenthal also consider RNA editing as a therapeutic tool, providing an insightful perspective on manipulating natural cellular pathways via RNA editing as novel treatment approaches that can fine-tune gene expression in a transient manner to achieve a clinically beneficial outcome.

When considering RNA therapeutics and different applications and targets for development, it is important to remember that the most appropriate approach will depend on many factors, including the specific target, mutation, site of action, intended outcome, etc. To highlight this concept, Bashari, Siegfried, and Karni describe RNA-targeting and RNA-based strategies that are being explored for cancer treatment. Cancer may be uniquely suitable for RNA therapeutics, as RNA processing is often deregulated in tumors, and cancer cells may be more sensitized to directed modulation than non-cancer cells. With this rationale in mind, a number of RNA-targeting approaches are being pursued, and considerable potential for additional strategies exists.

As we venture further into the world of RNA therapeutics, unexpected challenges and exciting advances undoubtedly await us. One common theme of the perspectives in this issue is the barriers that are generally faced when trying to make a drug for clinical use, some of which are unique to RNA. For example, many aspects of RNA therapeutics rely on very specific knowledge about the identity and mechanism of action of disease mutations. Thus, genetic testing/genomic sequencing information and analysis are important for identifying targets for RNA therapies. Along these same lines, growing biobanks to increase individual sample/data archiving and retrieval helps facilitate assay design and hence RNA-targeted therapeutics development. In many cases, artificial means can be used (gene editing, cell-specific differentiation of iPSCs, etc.), but individual patient-derived samples are still invaluable in many cases. Preclinical testing in animal models is still a standard of many drug-development programs. Having models with the equivalent human mutation or humanized animal models with knocked-in human sequences for testing drugs in vivo helps in preclinical drug studies, as well as in determining the mechanism of action of any given mutation. Many of the perspectives present a historical review of RNA-based drug development, which highlights the importance of engaging a team of stakeholders with diverse expertise, from academic and industry researchers to physicians, patients, and regulatory agencies in order to achieve success in clinical development. Finally, creativity, basic science discoveries, and the courage to challenge dogma have been ingredients for the advancement of RNA therapeutics and will likely drive forward what promises to be a revolutionizing tool for medicine and science.

Last but not least, we are grateful to Tim Nilsen for having the vision for this special issue, and to Ann Marie Micenmacher, our executive producer, for helping us to make this vision a reality.