The progress and promise of RNA medicine—an arsenal of targeted treatments

Janet M. Sasso[†1], Barbara J. B. Ambrose[†1], Rumiana Tenchov[†1], Ruchira S. Datta[1], Matthew T. Basel[2], Robert K. DeLong*[3] and Qiongqiong Angela Zhou*[1]

[†]Authors J.M.S., B.J.B.A., and R.T. contributed equally to this paper.

*Corresponding authors:

Robert K. DeLong robertdelong@vet.k-state.edu

Qiongqiong Angela Zhou qzhou@cas.org

Affiliations:

[1] CAS, a division of the American Chemical Society

2540 Olentangy River Rd, Columbus, OH 43202, USA

[2] College of Veterinary Medicine, Kansas State University, Manhattan, KS 66506, USA

[3] Nanotechnology Innovation Center Kansas State, Kansas State University, Manhattan, KS 66506, USA

ABSTRACT

In the last decade, there has been a shift in research, clinical development, and commercial activity to exploit the many roles of RNA in physiology for use in medicine. With the rapid success in the development of lipid-RNA nanoparticles for mRNA vaccines against COVID-19 and with several approved RNA-based drugs, RNA has catapulted to the forefront of drug research. With diverse functions beyond the role of mRNA in producing antigens or therapeutic proteins, many classes of RNA serve a regulatory role in cells and tissues. These RNAs have potential as new therapeutics, with the RNA itself serving as either a drug or a target. These new types of RNA drugs require a plethora of modification chemistries to improve their therapeutic benefit. We describe the current state of the art for RNA medicine. Using the CAS Content Collection data, we examine the publication trends covering the roles of RNA in the cell, the application of RNA in medicine, and the use of chemical modifications and nanotechnology to deliver effective RNA pharmaceuticals to their cellular targets. This review reveals the sustained global effort that propelled this field to the cusp of realization for novel medical applications of RNA in many diseases. It serves as an easy-to-understand overview so that scientists from many different disciplines can appreciate the current state of the field of RNA medicine and join in solving the remaining challenges for fulfilling its potential.

Introduction

Recent advances in RNA design and delivery have enabled the development of RNA-based medicine for a broad range of applications, including therapeutics, vaccines, and diagnostics. While human RNA medicine has faced many challenges in terms of efficacy and immunogenicity, the recent success of mRNA vaccines against COVID-19 and the approval of new RNA-based drugs provide new momentum to the field. Many classes of RNA play important regulatory roles in cells and tissues, beyond the obvious role of mRNA in protein synthesis. Scientific research, clinical development, and commercial production now focus on exploiting the many roles of RNA for use in biotechnology and medicine. Advances in understanding RNA structure and function are combined with a robust production pipeline to develop clinically effective RNA-related applications. [1-12]

Many key discoveries have contributed to the advancing of RNA medicines we have today. Early research in the 1950s on nucleic acids led to the discovery of mRNA. [13] In the next decade, the 5'-cap on mRNA was discovered [14, 15], the first liposome-entrapped RNA was delivered into cells [16, 17], and antisense oligomers (ASOs) were used to inhibit respiratory syncytial virus (RSV) [18]. In the 1980s, *in vitro* transcription from engineered DNA templates using a bacteriophage SP6 promoter and RNA polymerase [19] allowed the manufacture of mRNA and expression of other types of RNA in cell-free systems. Later in the 1980s, the first cationic-lipid mediated mRNA delivery was achieved [20, 21]. The discovery of RNA interference (RNAi) [22] and the approval of the first antisense RNA drug in the late 1990s [23] were key to the development of RNA therapeutics. For their pioneering work on RNAi and the RNA-induced silencing complex (RISC) [24], Fire and Mello were awarded the Nobel Prize in Physiology and Medicine in 2006. During the 2000s, the discovery of the importance of pseudouridine modification [25] and further research on mRNA led to the first human trial of an mRNA vaccine against melanoma in 2008 [26]. In 2010, a pivotal human clinical trial showed that siRNA could target specific human genes[10], and subsequent pre-clinical research and development led to the approval of the first siRNA drug in 2018 [27]. Most recently, Doudna and Charpentier were awarded the Nobel Prize in 2020 for CRISPR/Cas9 gene editing. Two human mRNA vaccines against COVID-19 received Emergency Use Authorization in 2020 and one of them was finally approved in 2021 [28-30]. These key milestones and achievements are captured in Figure 1.

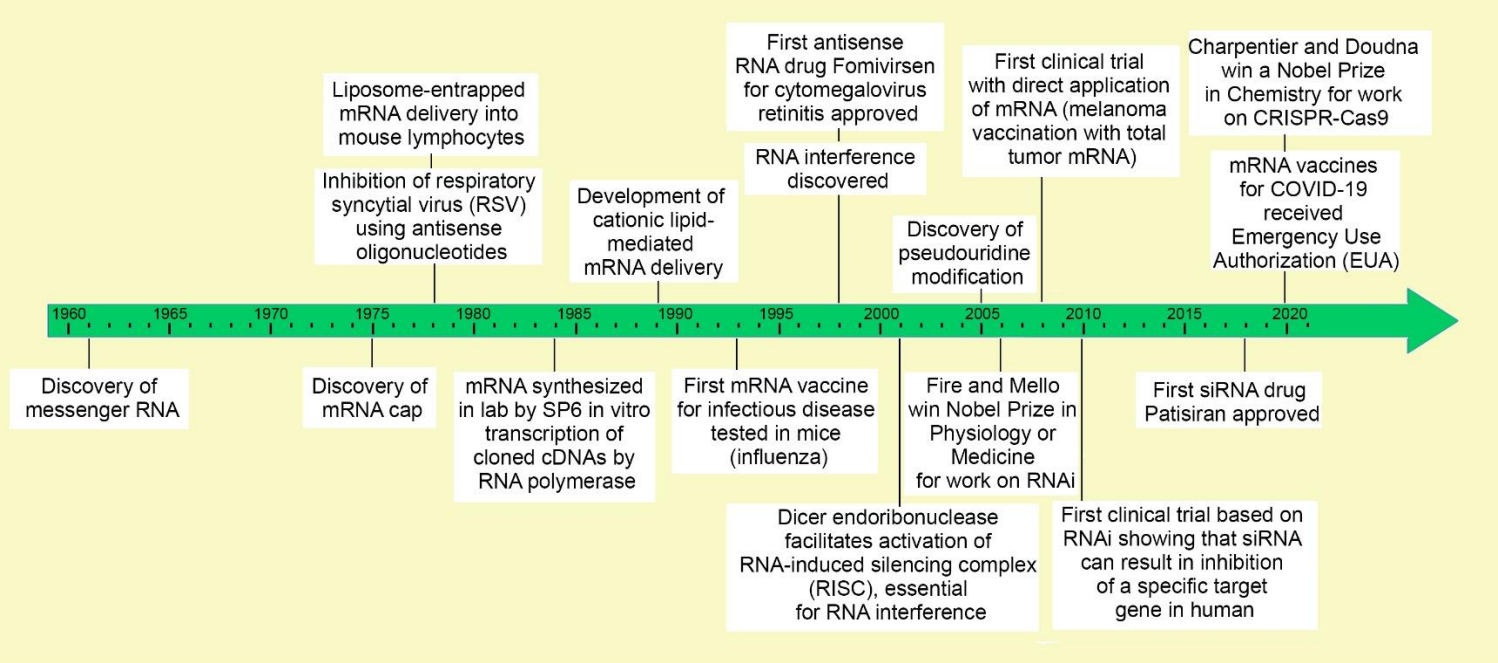


Figure 1: Timeline of major RNA research and development milestones. A more detailed timeline table complete with references is provided as Supplemental Table S1.

RNA technology provides an innovative approach for developing new drugs for rare or difficult-to-treat diseases. Since 2014, several drugs been approved to treat macular degeneration, Duchenne muscular dystrophy (DMD), polyneuropathy, and amyotrophic lateral sclerosis. [31] Drugs to treat many other diseases, including cancer, hepatic and renal diseases, cardiac diseases, metabolic diseases, blood disorders, respiratory diseases, and autoimmune diseases are currently in various stages of clinical studies, some showing promising results.

Compared to other biomolecules, RNA molecules are unstable and transient. Foreign RNA molecules, when introduced to human body, have limited protein expression level in cell and often trigger immunogenicity in the body. However, these practical problems can often be mitigated by various optimizations on RNA molecules, including chemical modifications, mRNA Cap/codon/tail optimization, etc. Leveraging the unique aspect with both chemical and biological information of the CAS Content Collection, this paper focuses on chemical modifications to the base, backbone, sugar, and 5’ or 3’ ligations to other molecules. While chemical modifications increase RNA stability, complexes of RNA within nanoparticles provide further protection. Except for aptamers that bind to a cell surface target, RNA must be delivered into the cell by a carrier. After the RNA is internalized, it must be released from the membrane-bound vesicle or endosome to the cytosol. Without a doubt, the delivery system has been an important research topic for RNA medicine.

In this paper, we reviewed naturally occurring RNAs with their cellular functions and the associated research trends based on the analysis of CAS Content Collection. We then appraised different types of RNA in medical applications: their advantages, challenges, and research trends. Subsequently, we assessed the development pipelines of RNA therapeutics and vaccines with company research focuses, disease categories, development stages and publication trends. Finally, we discussed

RNA chemical modifications and delivery systems in detail, as they are critical to the success of RNA medicine. We hope this review can serve as an easy-to-understand overview so that scientists from many different disciplines can appreciate the current state of the field of RNA medicine and join in solving the remaining challenges for fulfilling its potential.

Types of naturally occurring RNA and their functions in biological systems

RNA, a versatile macromolecule that is specialized for many functions, can be broadly defined as coding or messenger RNA (mRNA) and non-coding RNA (ncRNA). There are several different types of ncRNA including ribosomal RNA (rRNA) [32, 33], transfer RNA (tRNA) [32, 33], small nuclear RNA (snRNA) [34-36],, small nucleolar RNA (snoRNA) [35, 37-44], long non-coding RNA (lncRNA) [7, 45-51], short hairpin RNA (shRNA), micro-RNA (miRNA) [52-58], transfer messenger RNA (tmRNA), small interfering RNA (siRNA), small activating RNA (saRNA), piwi-interacting RNA (piRNA) [3, 4, 59-77], circular RNA (circRNA), ribozymes, and exosomal RNA [78-82]. The cellular localizations of different types of RNA are illustrated in Figure 2 and an overview of their functions is provided in the later section.

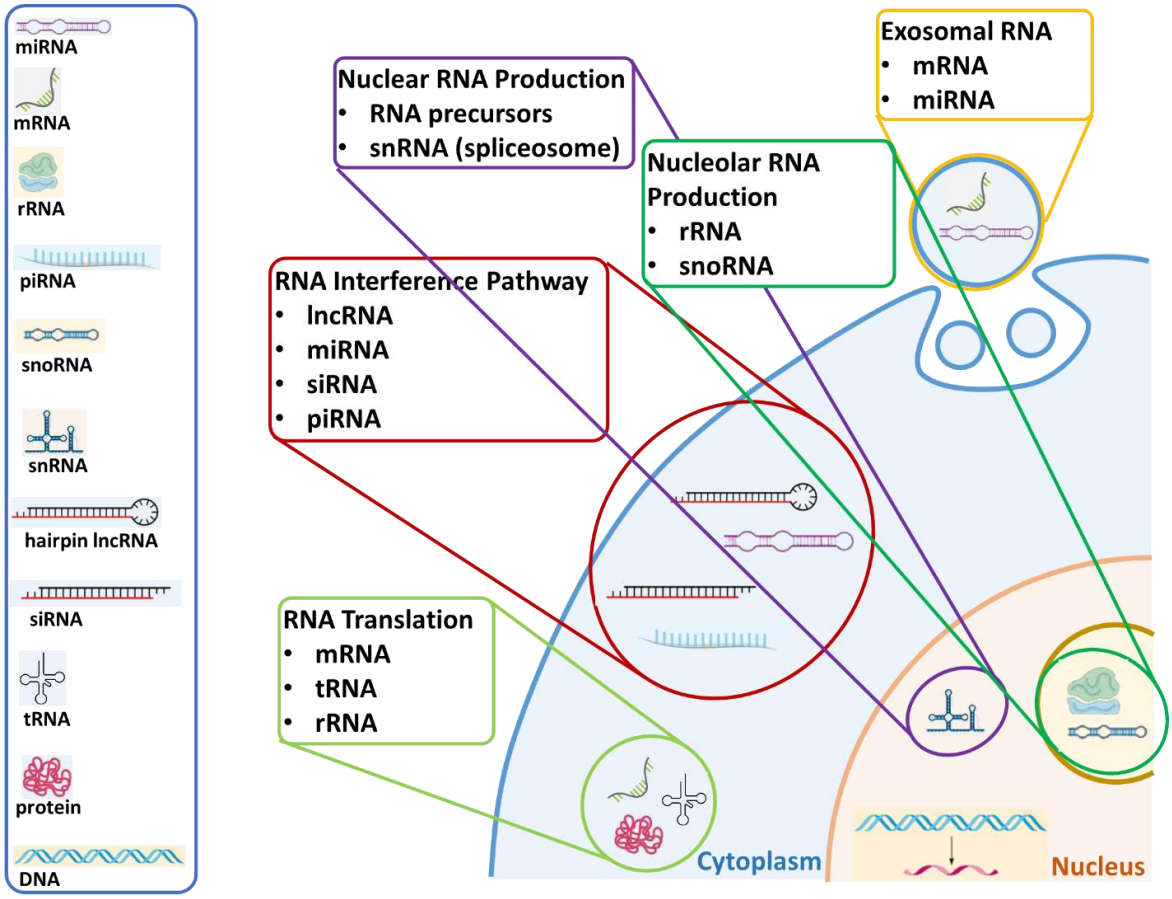


Figure 2. Types of naturally occurring RNA and their cellular functions and localizations

Research trends on different types of RNA as reflected by number of publications

The CAS Content Collection [83] is the largest human-curated collection of published scientific knowledge, used for quantitative analysis of global scientific publications against variables such as time, research area, formulation, application, disease association, and chemical composition. We searched the title, abstract, or CAS-indexed terms using RNA-related keywords and their synonyms to identify relevant published documents. Figure 3 shows trends in the number of publications for specific types of RNA. In the past 25 years, the research areas became more diverse as new types of RNA were discovered, and this is reflected in both journal and patent publications, particularly in the areas of siRNA, miRNA, lncRNA, and CRISPR-related research. CRISPR technology has recently increased rapidly in volume of patent publications, and it accounted for 20% of the RNA-related patent applications in 2020.

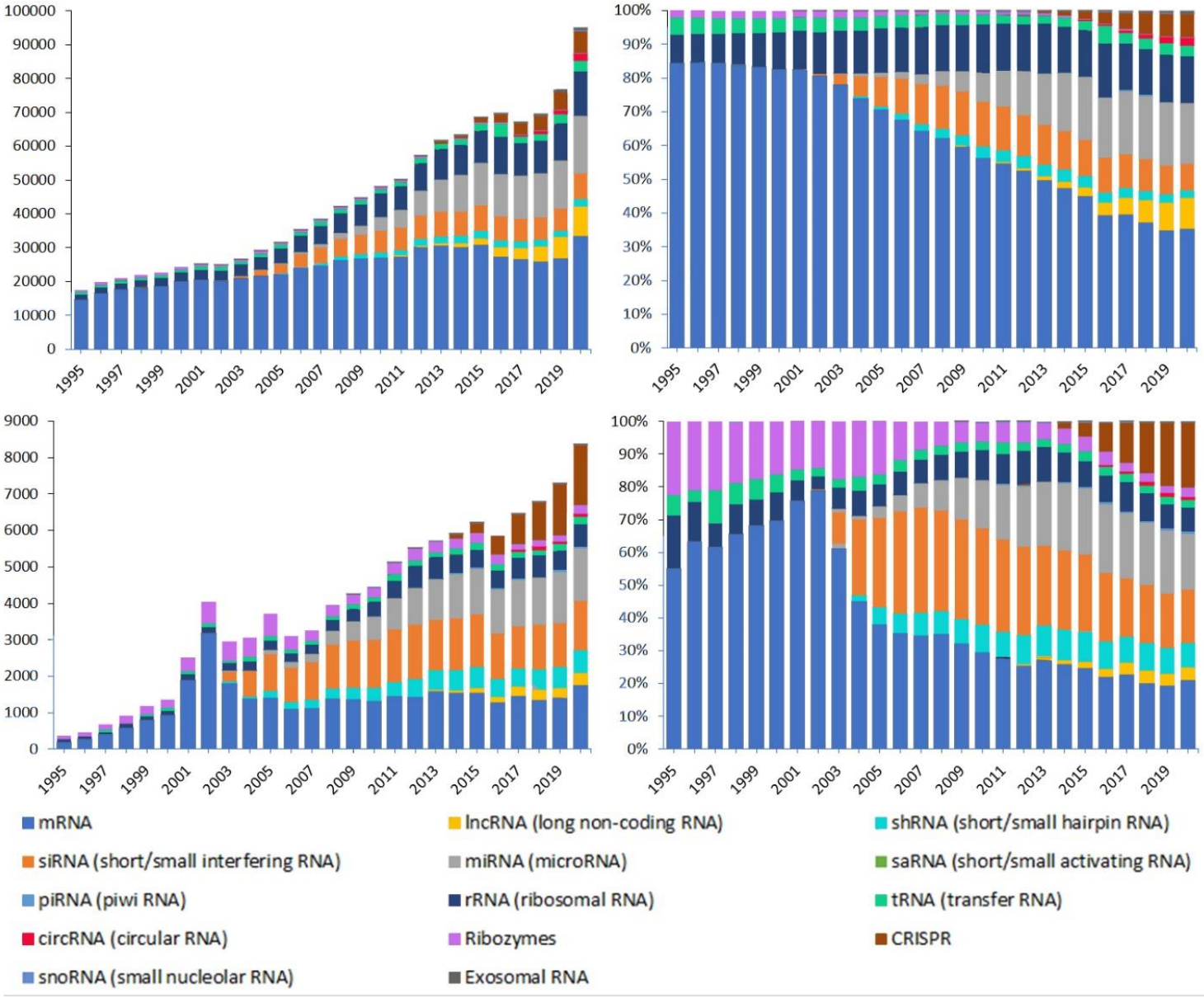


Figure 3. Document publication trends for different types of RNA from 1995-2020. Top two panels: journal publications in absolute numbers and given year percentages. Bottom two panels: patent publications (counted once per patent family) in absolute numbers and given year percentages.

To better reveal the rising trends of those recently emerged types of RNA, the percentage of document publications of a specific year was calculated within the given type of RNA over the time (Figure 4). Although the cumulative publication numbers for circRNA, exosome RNA, lncRNA, and CRISPR, are relatively small compared with others (Figure 3), their rates of increase are much faster.

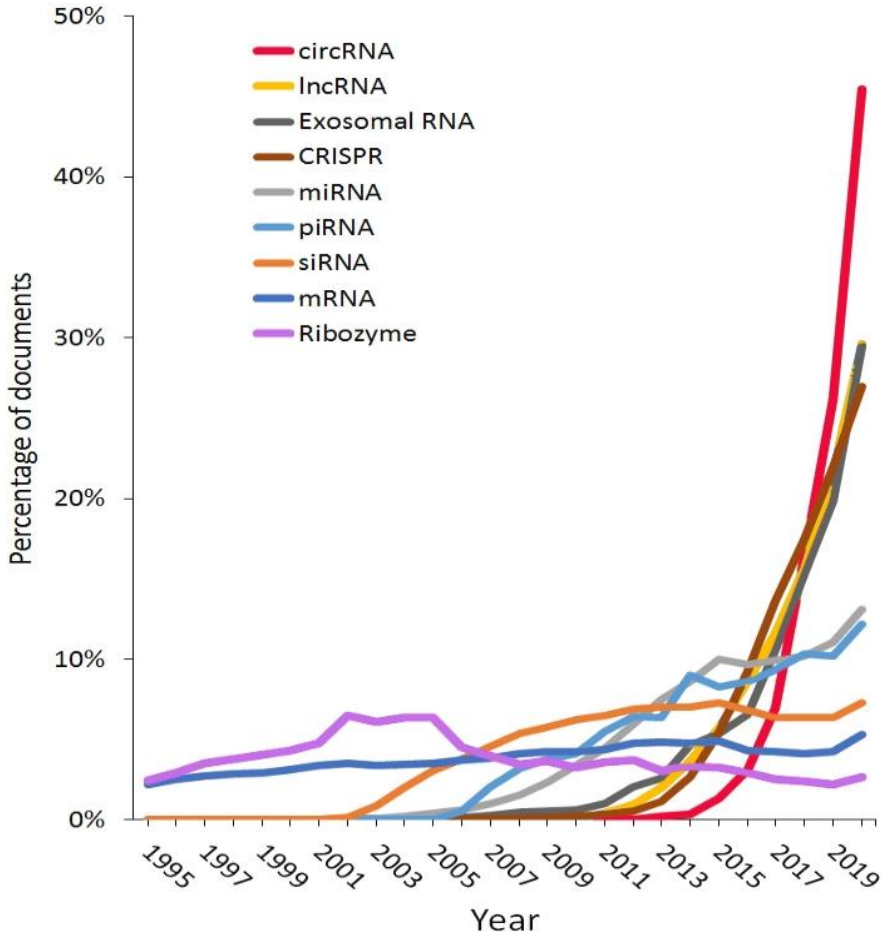


Figure 4. Trends in publication volume for different RNA types in the years 1995-2020. Percentages are calculated with yearly publication numbers normalized by total publications in the years 1995-2020 for each RNA type.

Functions of the naturally occurring RNA types

mRNA, which was the first RNA to be characterized, is the initial transcription product of a protein-coding gene and includes both protein-coding exons and non-coding introns. The mature, translatable mRNA must be spliced to remove the introns and, in transcripts that can go through alternative splicing, one or more potential exons. The mature RNA has a 5'-7-methylguanosine cap, a 5'-

untranslated region, a start codon (unique sequence of 3 bases) for the translated region of the gene, a stop codon that ends the translated region of the gene and starts the 3'-untranslated region, and a 3'-polyadenosine tail. Gene expression, which is often regulated by the amount of mRNA for the gene, is controlled by the balance between synthesis and degradation of mRNA. Although mRNA is a critically important RNA, it makes up only 1-5% of the RNA in a cell. [32, 33]

ncRNAs, in contrast to mRNA, are the final functional gene products. Although it was thought initially that ncRNAs were nonfunctional junk RNA, in the 1950s, in the same paper that introduced the phrase "Central Dogma", Francis Crick correctly hypothesized that the ncRNA might function in the translation of mRNA into protein. [84] In 1955, George Palade identified ribosomes as a small particulate component of the cytoplasm that contains RNA, and in 1965, Robert Holley purified a tRNA from yeast and determined the structure. [85, 86] In the last half-century, many types of ncRNA with various functions have been identified; many are involved in regulating transcription and protein expression in the cell. [87-94]

rRNA, which constitutes up to 80% of the RNA in an active cell, comprises three rRNAs (the 5S, 5.8S, and 28S) complexed with many proteins to form the large subunit of the ribosome and one rRNA (the 18S) complexed with proteins to form the small subunit of the ribosome. There are also two mitochondrial rRNA genes (the 12S and 16S) which, along with many proteins, form the mitochondrial ribosome. rRNA in the ribosome, acting as a ribozyme, catalyzes peptide bond formation between two amino acids. Synthesis of the large amount of rRNA occurs in the nucleolus, a heterochromatic region found in most nuclei. [32, 33]

tRNA, which makes up 10–15% of the RNA in the cell, translates the mRNA codon sequence for each amino acid. The many different tRNAs, which are usually 75–95 nucleotides, all fold into very similar three-dimensional structures. The 3D structure exposes three unpaired nucleotides that serve as the anti-codon to base pair with the mRNA. Specific amino acids are covalently bound to a tRNA by aminoacyl tRNA synthetases. The specificity between the anti-codon and the bound amino acid is the basis of translation. Each tRNA anticodon can bind to several different mRNA codons. This pairing is based on the wobble rules for the third nucleotide position of the anti-codon, which is often post-transcriptionally modified to allow for wobble-pairing. Common modifications at the third position include 5-methyl-2-thiouridine, 5-methyl-2'-O-methyluridine, 2'-O-methyluridine, 5-methyluridine, 5-hydroxyuridine, hypoxanthine, and lysidine.[32, 33]

snRNAs (~150 nucleotides) are components of the small nuclear ribonucleoproteins (snRNPs) of the spliceosome. They act as catalysts that splice mRNA into its mature form and they are important in the selection of alternative splicing sequences. [34-36]

snoRNAs (60-300 nucleotides) are bound to four core proteins and act as guides to correctly target modifications for the maturation of rRNA. They comprise two classes of RNA. C/D snoRNAs participate in the 2-methylation of targeted nucleotides, while H/ACA snoRNAs participate in the modification of uridine to pseudouridine. They help guide the protein to the specific target, rather than catalyzing the reaction directly. As participants in rRNA maturation, snoRNAs are found in the nucleolus. [35, 37-44]

siRNAs are products of double-stranded lncRNAs (e.g., hairpin lncRNA) and are central to RNA interference, which negatively regulates gene expression. Double-stranded RNA (dsRNA), either from genomic lncRNA or dsRNA viruses, is recognized and cleaved by the endonuclease Dicer into 20–24 base-pair sections with short overhangs on both ends. These siRNAs bind the Argonaute protein to form the pre-RISC (RNA-induced silencing complex). Argonaute selects the less thermodynamically stable strand of the siRNA and releases the other strand to form the mature RISC. RISC recognizes mRNA complementary to the single-stranded siRNA, and the Argonaute endonuclease cuts this targeted mRNA, thereby downregulating the gene product. The binding of a RISC to a target mRNA also prevents efficient ribosome binding and translation, further downregulating the gene product. Active RISCs may also affect the transcription of target genes by inducing chromatin reorganization through epigenetic modifications. This can be a defense mechanism against dsRNA viruses or an endogenous gene-regulatory mechanism. [59-70]

miRNAs are closely related to siRNAs but are formed from pri-miRNAs, which are long, imperfectly paired hairpin RNA transcripts. The pri-miRNA is processed first by Drosha nuclease into a~70-nucleotide imperfectly-paired hairpin pre-miRNA that is then, like siRNA, processed by Dicer to produce the 21–23 bp, mature, double-stranded miRNA that binds to Argonaute to form the RISC. Alternatively, some miRNAs are made from introns in mRNAs. After splicing, that intron is a pre-miRNA that is processed by Dicer to form a RISC. miRNAs form negative gene regulatory networks and intronic miRNAs may regulate and balance potentially competing pathways. [52-58]

piRNAs, like siRNA and miRNA, negatively regulate gene expression, but they interact with the Piwi class of Argonaute proteins. Unlike siRNA and miRNA, piRNAs (24–31 nucleotides) are produced from long, single-stranded RNA transcripts through an uncharacterized Dicer-independent mechanism. Mature piRNAs bind to Piwi proteins to form RISCs that act primarily as epigenetic regulators of transposons (genetic elements that move around the genome) but may also regulate transposons post-transcriptionally through the ping-pong pathway. [3, 4, 71-77]

saRNA, like siRNA, is a ~21-bp dsRNA long that interacts with Argonaute proteins to form a RISC. Unlike siRNA, saRNA upregulates target gene expression by an unknown mechanism, perhaps activating transcription by targeting the promoter region of the gene. saRNA may be produced endogenously or artificially to strongly activate the target gene. [5, 95-101]

lncRNA comprises a mixed group of RNAs >200 bp, which differentiates them from short ncRNAs such as snoRNA, siRNA, miRNA, piRNA, etc. lncRNAs have a wide variety of functions including regulation of chromosome architecture and interactions, chromatin remodeling, and positive or negative regulation of transcription, nuclear body architecture, and mRNA stability and turnover. [7, 45-51]

circRNAs are lncRNAs with 5' and 3' ends linked covalently to form a continuous circle. circRNAs are broadly expressed in mammalian cells and have shown cell-type and tissue-specific expression patterns. [102] Neither the mechanisms leading to circularization of the RNA nor the function of circRNA is known, but the leading hypothesis is that they may serve as miRNA sponges. Many circRNAs contain large numbers of miRNA target sites that may competitively antagonize the ability of miRNA to silence its target genes.

Exosomal RNAs are mRNAs, miRNAs, siRNAs, and lncRNAs that are packaged and exported from the cell through the exosomal pathway. Although they are poorly understood, exosomal RNAs may serve as signaling molecules to regulate gene expression in target cells. These circulating RNAs, especially miRNAs, may serve as diagnostic and/or prognostic targets for various diseases such as cancers. [78-82]

Types of RNA used in medical applications, their advantages, and challenges

Publication trends for RNAs used in medical applications

The CAS Content Collection [83] shows a steady increase in the number of journal articles and patents related to RNA applications in medicine (Figure 5). The peak in patents in 2001–2002 may correlate with the first clinical trials using dendritic cells transfected with mRNA encoding tumor antigens (a therapeutic mRNA cancer vaccine) in 2001. [103, 104] The spike in journal article numbers in 2020 likely resulted from interest in the COVID-19 mRNA vaccines. The increase in journal articles and patents on therapeutic RNA from 2011–2016 can be attributed to initial interest in siRNA and miRNA, which decreased temporarily with the discovery of their off-target effects. Interest in mRNA also increased from 2011–2016, then decreased and only recovered once mRNA vaccines took center stage in the fight against COVID-19.

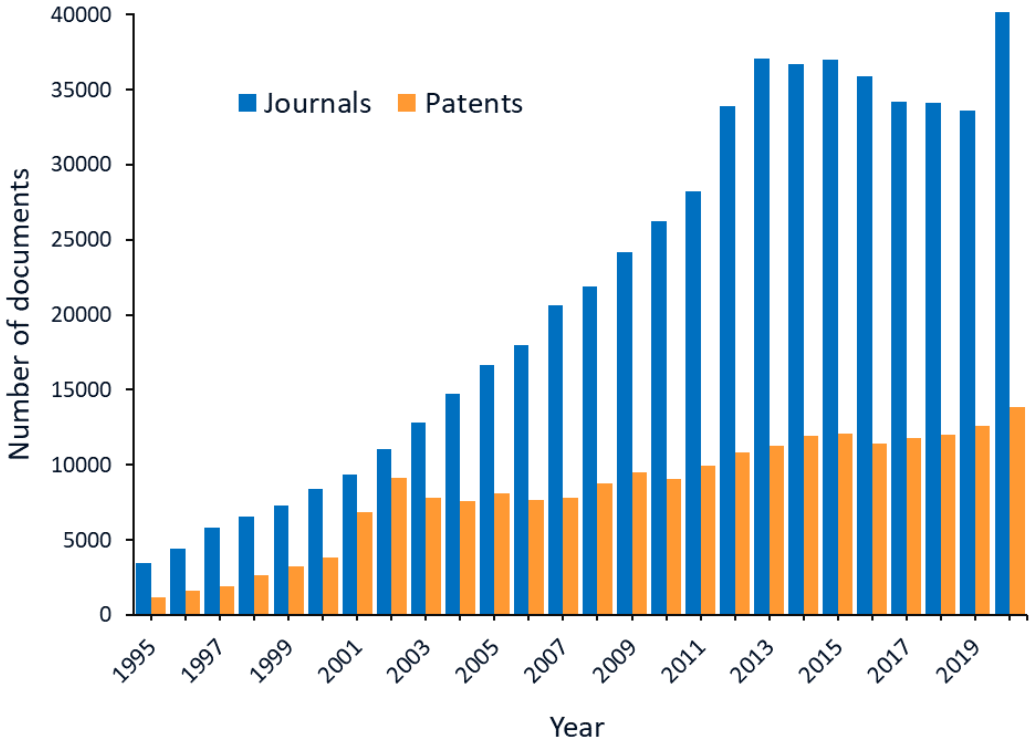


Figure 5. Numbers of journal documents and patents related to RNAs for medical use by year.

Types of RNAs and their applications in medicine

mRNA transcripts can act as therapeutic RNAs, diagnostic biomarkers, or therapeutic targets. Translation of an mRNA in the cell can produce a therapeutic protein to replace a defective or missing protein. In the case of vaccines, mRNA translation can generate antigenic targets for the immune system, such as the spike glycoprotein of SARS-CoV-2 in the COVID-19 mRNA vaccines. mRNA may also serve as a therapeutic target for ASOs, siRNA, miRNA, aptamers, and suppressor tRNAs.

In the cell, miRNAs bind to the 3'-untranslated region of mRNAs and target them for degradation by the RISCs. [105] Because a single miRNA binds to multiple mRNAs, miRNAs serve as regulatory check points. The cellular processes regulated by miRNAs include those involved in many diseases, such as cardiovascular disease, cancer, and disease-related metabolic pathways. [106, 107] Thus, they can serve as biomarkers for disease diagnosis, as potential drugs, or as attractive targets for other regulatory RNAs.

Although siRNAs, like miRNAs, use the RISC to degrade their target mRNAs, siRNAs bind to specific areas in the mRNA coding region. This target specificity makes them attractive as potential drugs, but off-target effects can negate this advantage. In order to minimize off-target effects, siRNAs are modified to decrease their thermal stability, increase their target specificity, and decrease the stability of their binding of the siRNA to mRNAs that are not an exact match to the intended target mRNA. These usually include 2'-O-methyl and 2'-MOE ribose modifications that introduce steric hindrance to decrease binding affinity (more discussion in chemical modification section below). [105]

One of the earliest therapeutic RNAs, ASOs, recognize and bind to complementary DNA or RNA sequences, including mutated sequences that may lead to disease. Upon binding to the mutated sequences, ASOs may facilitate proper mRNA splicing, prevent translation of a defective protein, or target RNAs for degradation. [105]

In therapeutic antibody-oligonucleotide conjugates (AOCs), the antibody targets the site of interest while carrying an ASO or a siRNA that acts on the targeted region. [108] Conjugation of an ASO to an antibody to create an AOC improves the pharmacokinetics of the ASO *in vivo* by increasing tissue distribution and prolonging gene silencing in multiple tissues. [109]

The CRISPR-Cas system uses a guide RNA that is either a combination of a trans-activating CRISPR RNA (tracrRNA) and a CRISPR RNA (crRNA) or a joined single guide RNA (sgRNA). The guide RNA directs the CRISPR complex containing a Cas endonuclease to a specific site in the genome for cleavage. [6] The ability of the CRISPR-Cas system to create directed double-stranded breaks in DNA allows the repair of genetic mutations. Changing the endonuclease activity of Cas can convert CRISPR-Cas to a system that nicks a single strand of the DNA or that deaminates a specific nucleotide. [110] If the Cas endonuclease is inactivated, the system can simply bind to DNA to regulate transcription. [105]

Aptamers are structure-based rather than sequence-based ligands that neither hybridize with other nucleic acids nor produce proteins. They can be RNA, DNA, RNA/DNA combinations, or even proteins. *In vitro* systematic evolution of ligands by exponential enrichment (SELEX) is used to identify single-stranded RNA or DNA oligonucleotides with a high affinity for a target. Because their binding depends on their 3D structure, aptamers can bind a wide range of targets, including proteins, cells, microorganisms, chemical compounds, and other nucleic acids. [111] Aptamers may also serve as delivery agents for siRNA in nanoparticles for cancer therapy. [112]

To measure the distribution of research effort using different types of RNA as therapeutics, vaccines, or diagnostics, the percentages of journal publications and patents for each type of RNA was determined from data in the CAS Content Collection (Figure 6). [83] miRNA and mRNA, the two most popular therapeutic RNAs in the journal and patent literature, can serve as drugs, disease biomarkers, and drug targets. Together with siRNA, they represent most of the therapeutic RNA patent activity. Approved RNA drugs include mRNAs, siRNAs, ASOs, and aptamers; these RNAs along with CRISPR RNAs and AOCs comprise most of the clinical candidates.

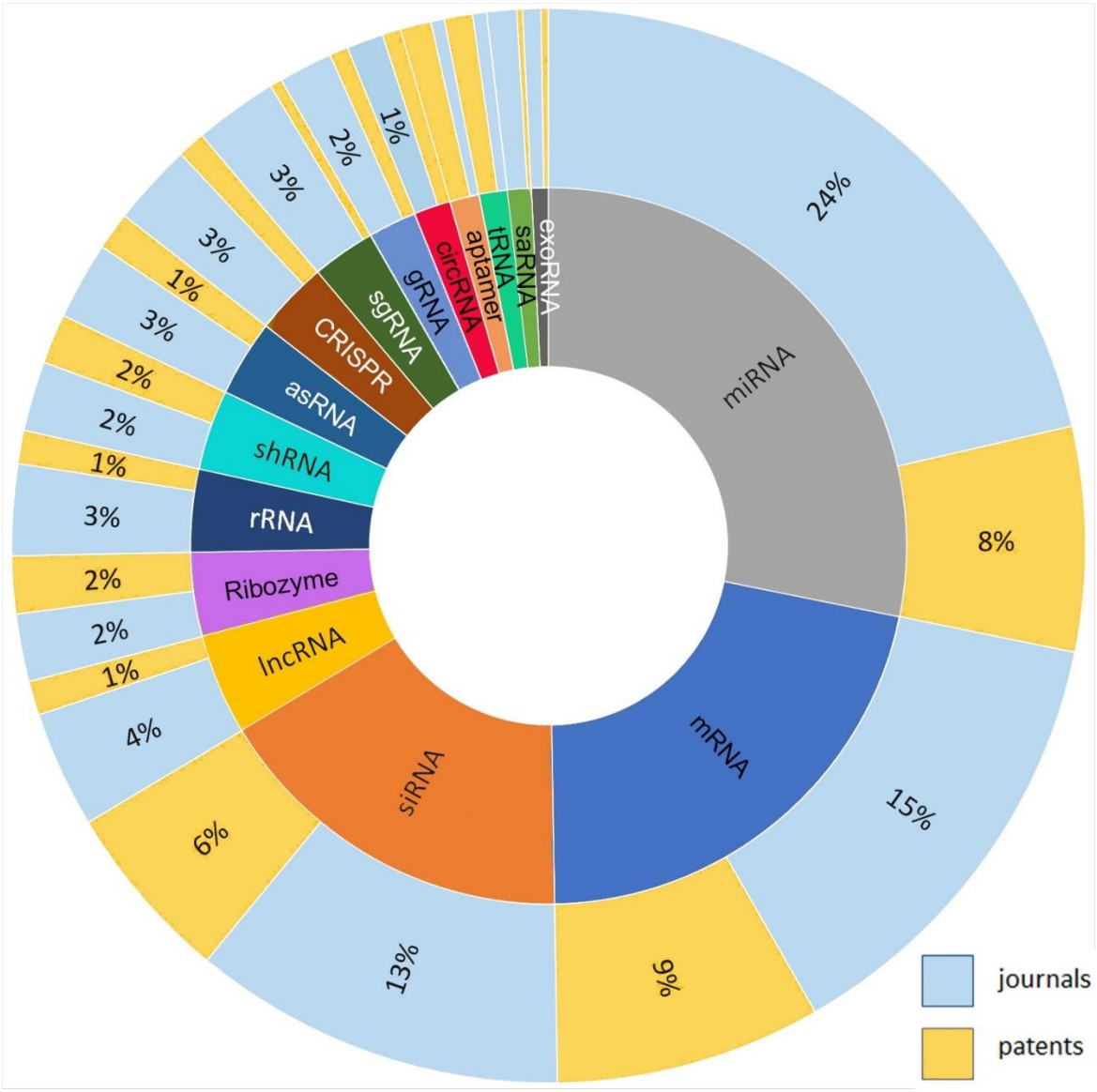


Figure 6. Percentage of journal documents and patents for various types of RNA used in medical studies including therapeutics, vaccines, and diagnostics.

However, other types of RNA are potential therapeutics or targets. These RNAs include (a) shRNA; (b) lncRNA, an RNA whose role in gene regulation is generating increasing interest; (c) circRNA, once

thought to be a by-product of RNA splicing that may have a role in regulation as a miRNA sponge; (d) saRNA, which regulates gene transcription; and (e) exosomal RNA, which is contained in naturally occurring lipid vesicles called exosomes, which can cross the blood-brain barrier and appear to be vital for cell-to-cell communication, and which transport mainly miRNA. [113]

Based on the CAS Content Collection data for CRISPR RNA, miRNA, and mRNA used in vaccines, diagnostics, and therapeutics [83], only mRNA has substantial applications in vaccines (Figure 7). However, both miRNA and mRNA have demonstrated their potential as therapeutics and diagnostics.

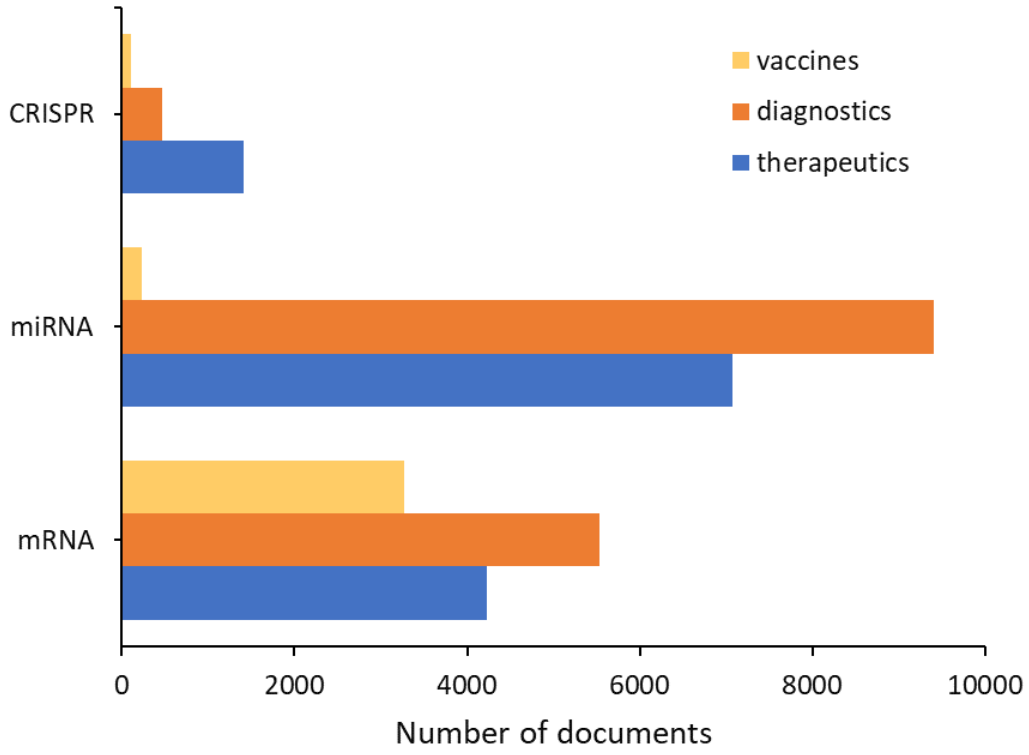


Figure 7. The number of journal publications and patents for mRNA, miRNA, and CRISPR with applications in therapeutics, vaccines, and diagnostics.

The large body of work on miRNA and mRNA in diagnostics may be surprising since these molecules are much more susceptible to nuclease degradation than DNA. However, the essential roles of mRNA and miRNA in cellular metabolism make them excellent biomarkers for the study of normal cellular processes and the diagnosis of disease. Metabolic diseases such as cancer as well as infectious diseases can be diagnosed via miRNA biomarkers by reverse transcriptase-quantitative polymerase chain reaction (RT-qPCR) and lateral flow immunoassays. [114, 115] High-throughput sequencing of total cellular mRNA pinpoints changes in gene expression that can be used in diagnosis. [116] The specificity of the CRISPR system for its target DNA makes it a potential tool with both diagnostic and therapeutic purposes. [117] The number of journal publications and patents using RNA for diagnosis demonstrates its power (Figure 7); however, a comprehensive review of RNA as a diagnostic biomarker or, in the case of CRISPR RNA, as a diagnostic agent, is beyond the scope of this review. Here we focus on RNAs as therapeutic agents.

The advantages and challenges of RNAs as therapeutics

There are several important advantages to RNA therapeutics: (a) target specific, (b) modular with easy-to-switch sequences, (c) predictable in terms of pharmacokinetics and pharmacodynamics, (d) economical in comparison to antibodies or protein drugs since they are synthesized from widely available synthons on an automated synthesizer, and (e) relatively safe, as most of them do not alter the genome.

Proteins must be designed for synthesis from genes in plasmids, then optimized for expression and purification, and these processes may be different for each protein and challenging to be optimized. In contrast, RNA can be easily synthesized and purified by established methods using commercially available reagents and equipment. Small RNAs, such as aptamers, siRNAs, miRNAs, and ASOs can be synthesized using solid-support chemistry in commercial oligonucleotide synthesizers. [118, 119] *In vitro* transcription using commercial kits [120] produces longer RNAs, i.e., mRNAs and lncRNAs. The sequence of the RNA can be changed easily, providing custom molecules targeting different proteins or genes. Thus, the development of RNA therapeutics and vaccines that target disease-specific genes or proteins is relatively fast and straightforward. This was demonstrated by the development, testing, and administration of the COVID-19 mRNA vaccines within a year of the isolation and sequencing of the SARS-CoV-2 viral genome. [109]

Most RNAs regulate transcription, post-transcriptional processing, and translation, but they do not alter the genome. The exception is the CRISPR-Cas system in which the guide RNA and Cas endonuclease can edit the genome. RNA aptamers, which mimic ligands, regulate post-translational protein activity. The rapid degradation and predictable pharmacokinetics of RNAs provide them with a safety advantage over gene therapies. [105]

Despite the attractiveness of the plug-and-play concept of RNA therapeutic drug design, they require testing to determine their efficacy and safety, and cell delivery is difficult because RNA is easily degraded. The therapeutic RNA must penetrate the cell membrane and escape endosomal entrapment. [105] Although designed for specific targets, therapeutic RNAs can have off-target effects, limiting their usefulness as drugs. Several of these limitations can be mitigated by chemically modifying the RNA to increase target specificity, lower nuclease susceptibility, and improve cellular uptake. [105, 109]

The types of RNA in medicine in the development pipeline and their targeted diseases

The distribution of diseases associated with RNA medicine in publications and patents

Since the first approved ASO RNA therapeutic in 1998, the research and development of RNA in medical applications have increased. We analyzed data from the CAS Content Collection [83] for journal publications and patents on RNAs as therapeutics, vaccines, or diagnostic agents for diseases and found that 50% of the publications are associated with cancer diagnosis or treatment, although lung, liver, and metabolic diseases are also highly represented (Figure 8). There was little correlation between the type of RNA and a targeted disease, indicating that different types of RNA have been explored for many kinds of diseases in the research phase (Supplement Figure S1). Infectious diseases and cancer have shown

the greatest growth and are the most frequent diseases treated by RNA, followed by eye and cardiovascular diseases, which grew in the first decade of this century and remained relatively stable in the second decade (Figure 9). Specifically, the association of RNA medicine with pancreatic neoplasm, melanoma, non-small cell lung cancer, hepatitis B, and influenza has increased quickly in the past 20 years. Patent publications for RNA therapeutics for hepatitis C have decreased in recent years, most likely due to the approval of several effective small molecule drugs for hepatitis C. RNA therapeutics for atherosclerosis, hypertension, glaucoma, and age-related macular degeneration research have remained relatively stable. The top 20 patent assignees for patent publications on RNA therapeutics, vaccines, or diagnostics are mostly in the US or China, although a few are in Germany, Korea, Japan, Switzerland, or Israel (Figure 11).

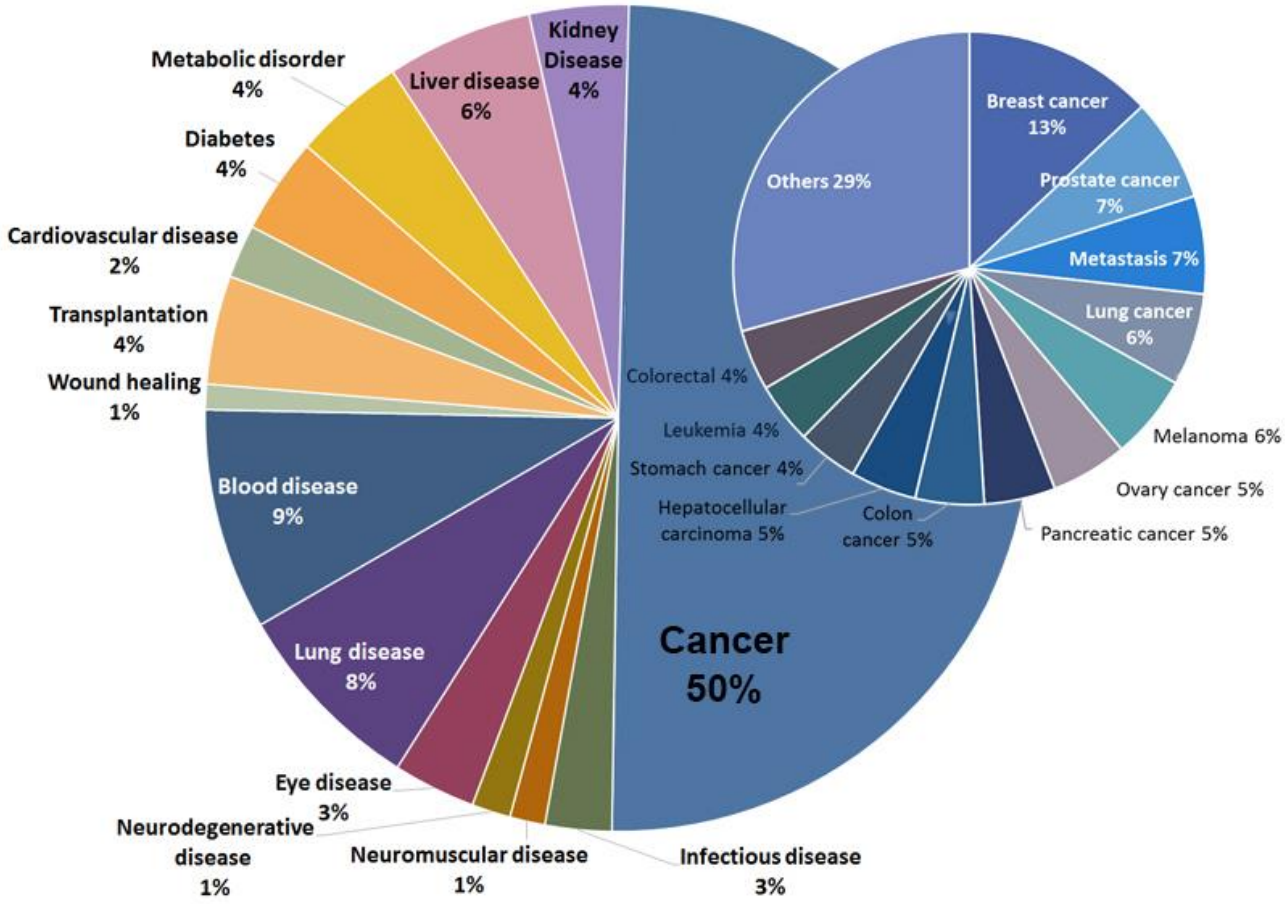


Figure 8. Percentage of publications associated with RNAs in medical applications

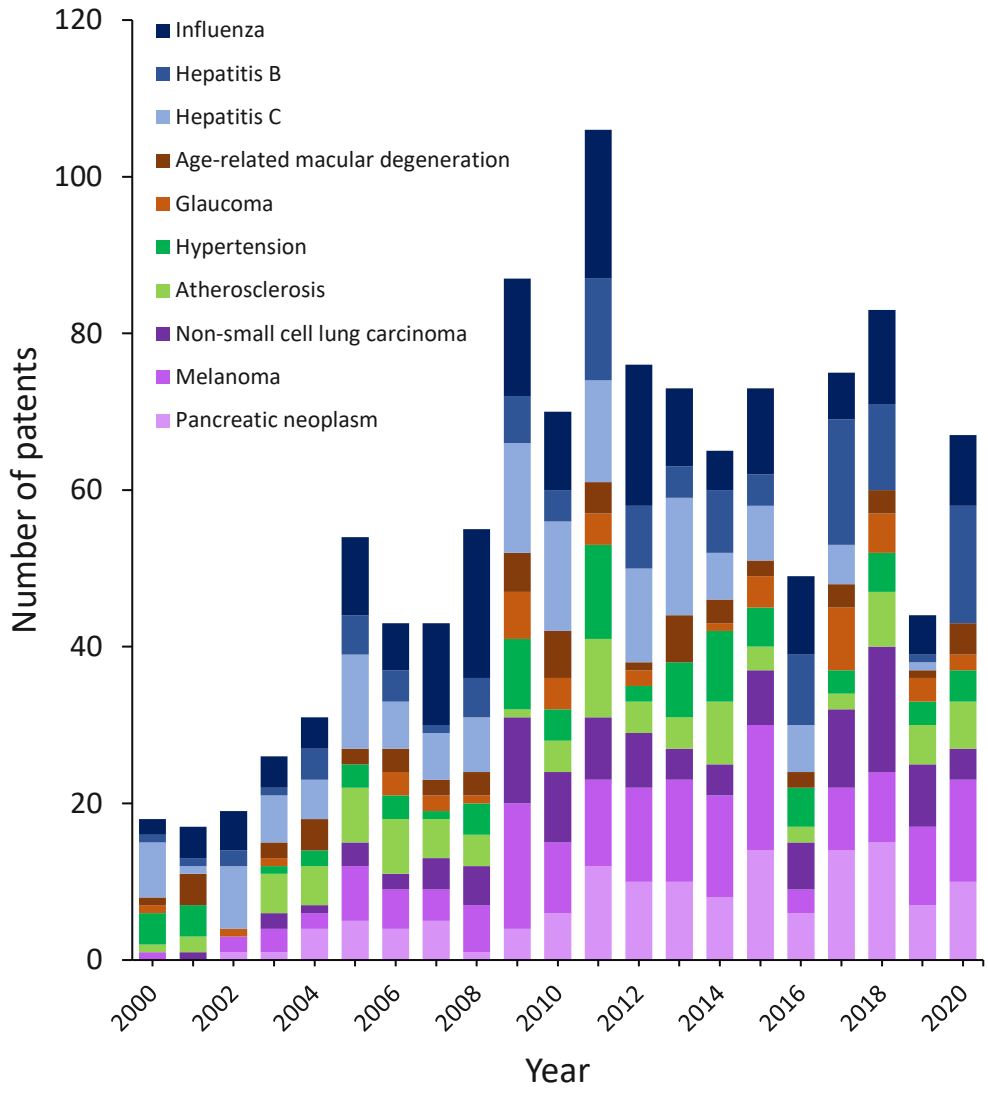


Figure 9. The yearly number of patent publications on specific diseases targeted by RNA therapeutics, vaccines, and diagnostics.

The pipeline dynamics of RNAs for therapeutics and vaccines

After decades of extensive research, the therapeutic potential of RNAs has led to the development of over 250 therapeutics that are approved or in development (Supplement Table S2). Among the top 15 RNA therapeutics companies (Figure 10), which are located worldwide (Supplement Figure S2), each mostly focuses on one type of RNA to develop novel RNA therapeutics for treating diseases that range from very rare to common. mRNA and siRNA are the most common RNAs used by the top 15 companies, followed by ASO, CRISPR, and aptamers. Many of these companies are among the top patent assignees for RNA therapeutics, vaccines, and diagnostics in the commercial sector (Figure 11).

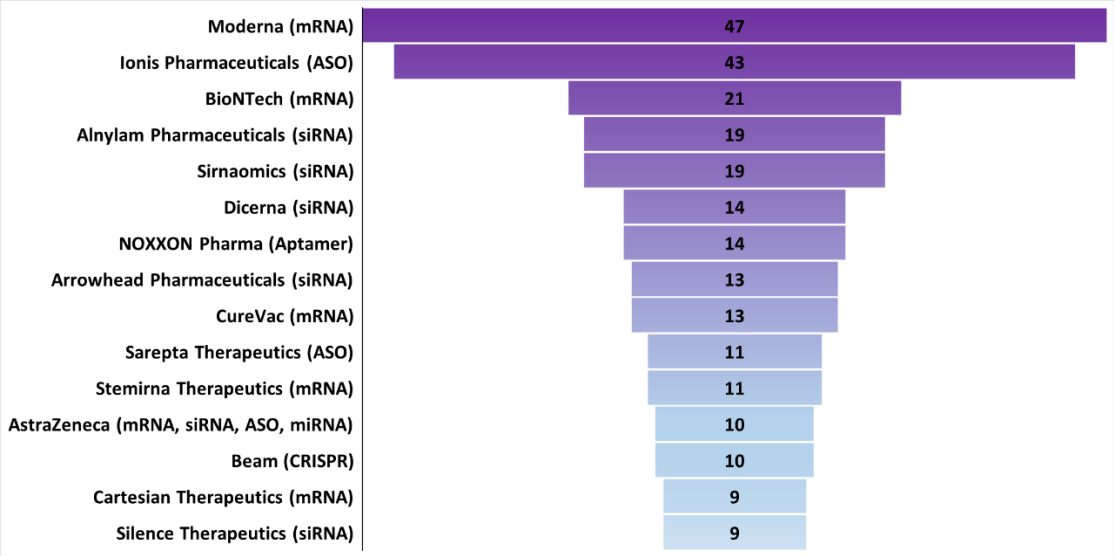


Figure 10. Top pharmaceutical companies ranked by the number of RNA therapeutic and vaccine agents in the development pipeline. Counts include RNA agents in company-announced preclinical development, in clinical trials, or approved. A single RNA agent can be counted multiple times when applied to multiple diseases.

Patent Count	Assignee (commercial)	Patent Count	Assignee (university/hospital/government)
245	Bayer Healthcare AG, Germany	128	University of California, USA
142	Beijing Yangshen Bioinformation Technology, China	114	United States Dept. of Health and Human Services, USA
131	Moderna TX, USA	93	University of Texas, USA
115	Isis Pharmaceuticals, USA	89	Central South University, China
97	Novartis AG, Switzerland	85	Johns Hopkins University, USA
77	Alnylam Pharmaceuticals, USA	83	Military Medical Universities, China
76	CureVac GmbH, Germany	80	Nanjing University, China
75	SNU R&DB Foundation, S. Korea	77	Sun Yat-Sen University, China
66	OncoTherapy Science, Inc., Japan	70	University of Pennsylvania, USA
56	Millennium Pharmaceuticals, USA	64	General Hospital Corporation, USA
55	F. Hoffmann-La Roche AG, Switzerland	62	Brigham and Women's Hospital, USA
54	Bode Gene Development Co., Shanghai, China	61	Harvard College, USA
47	Genentech, Inc., USA	58	Dana-Farber Cancer Institute, USA
46	aTyr Pharma, USA	57	Ohio State University, USA
45	Ruiqu Biotechnology, Shanghai, China	55	University of Massachusetts, USA
44	Pangu Biopharma, China	52	Leland Stanford Junior University, USA
42	Rosetta Genomics Ltd., Israel	52	Massachusetts Institute of Technology, USA
40	Wyeth, John, and Brother Ltd., USA	52	Shandong University, China
38	Incyte Pharmaceuticals, Inc., USA	51	Zhejiang University, China
30	Merck Sharp & Dohme Corp., USA	47	Korea Research Institute of Bioscience and Biotechnology, S. Korea

Figure 11. Top patent assignees for RNA therapeutics, vaccines, and diagnostics.

Moderna, BioNTech, CureVac, Stemirna Therapeutics, and Cartesian Therapeutics specialize in mRNA related therapeutics or vaccines. Moderna, headquartered in Massachusetts, USA [121], leads this group with over 45 therapeutics in its pipeline (Figure 10) and 131 RNA therapeutic/diagnostic patents (Figure 11). BioNTech and CureVac are both headquartered in Germany. [122] [123] BioNTech has in its pipeline 20 therapeutics that leverage the immune system to treat cancer and infectious diseases (Figure 10). [121] CureVac has 13 therapeutics (Figure 10) along with 76 RNA therapeutic/diagnostic patents (Figure 11) for mRNA medicines. [123] Stemirna Therapeutics headquartered in China, [124] has a pipeline of 11 mRNA-based therapeutics (Figure 10). Cartesian Therapeutics, headquartered in Maryland, USA [125], has 9 therapeutics and is a pioneer in using mRNA for cell therapies within and beyond oncology, with products in development for autoimmune and respiratory disorders (Figure 10).

Alnylam, Sirnaomics, Arrowhead Pharmaceuticals, Silence Therapeutics, and Dicerna develop siRNAs for their RNA therapeutics. (Effective December 28, 2021, Dicerna is a wholly owned subsidiary of Novo Nordisk. In this paper Dicerna is considered separately.) Alnylam Pharmaceuticals and Dicerna are headquartered in Massachusetts, USA. [126] [127] Alnylam is the leader in siRNA therapy with over 19 therapeutics in their pipeline and 77 therapeutic/diagnostic patents (Figure 10). Dicerna treats both rare and common diseases with its 14 therapeutics (Figure 10). Sirnaomics, headquartered in Maryland, USA, has developed over 19 therapies for human diseases that have no treatments. [128] Arrowhead Pharmaceuticals, headquartered in California, USA [129], treats previously intractable diseases with their 13 therapeutics (Figure 10). Silence Therapeutics, headquartered in London, UK [130], has 9 therapeutics (Figure 10).

Ionis Pharmaceuticals and Sarepta Therapeutics use ASOs for their RNA therapeutics. Ionis Pharmaceuticals is headquartered in California, USA [131], and Sarepta Therapeutics in Massachusetts, USA. [132] Ionis has developed over 40 therapeutics (Figure 10) and 115 therapeutic/diagnostic patents (Figure 11). Sarepta uses antisense technology to target neurological diseases including neuromuscular and neurodegenerative disease with their 11 therapeutics (Figure 10).

NOXXON Pharma, headquartered in Germany, uses RNA aptamers for their 14 therapeutics (Figure 10). [133] Their current pipeline is focused only on oncology, but previously they researched treatments for metabolic, blood, autoimmune, and kidney diseases (Supplemental Table S2). Beam Therapeutics, headquartered in Massachusetts, USA [134], is pioneering the use of base editing with CRISPR/Cas. Beam has 10 therapeutics in development (Figure 10). AstraZeneca supports multiple RNA platforms through partnerships with many of the top RNA companies. [135]

All but two of the top 15 RNA medicine companies specialize in one type of RNA (Figure 12). AstraZeneca supports multiple RNA platforms and CureVac is partnering with CRISPR Therapeutics for their current preclinical CRISPR RNA therapy [136] along with their mRNA therapy. Companies typically specialize in one type of RNA but treat multiple diseases. All but two of the top 15 RNA medicine companies cover multiple diseases. Sarepta specializes in neurological and neuromuscular diseases and NOXXON has supported multiple diseases in the past but is now dedicated exclusively to the treatment of cancer.

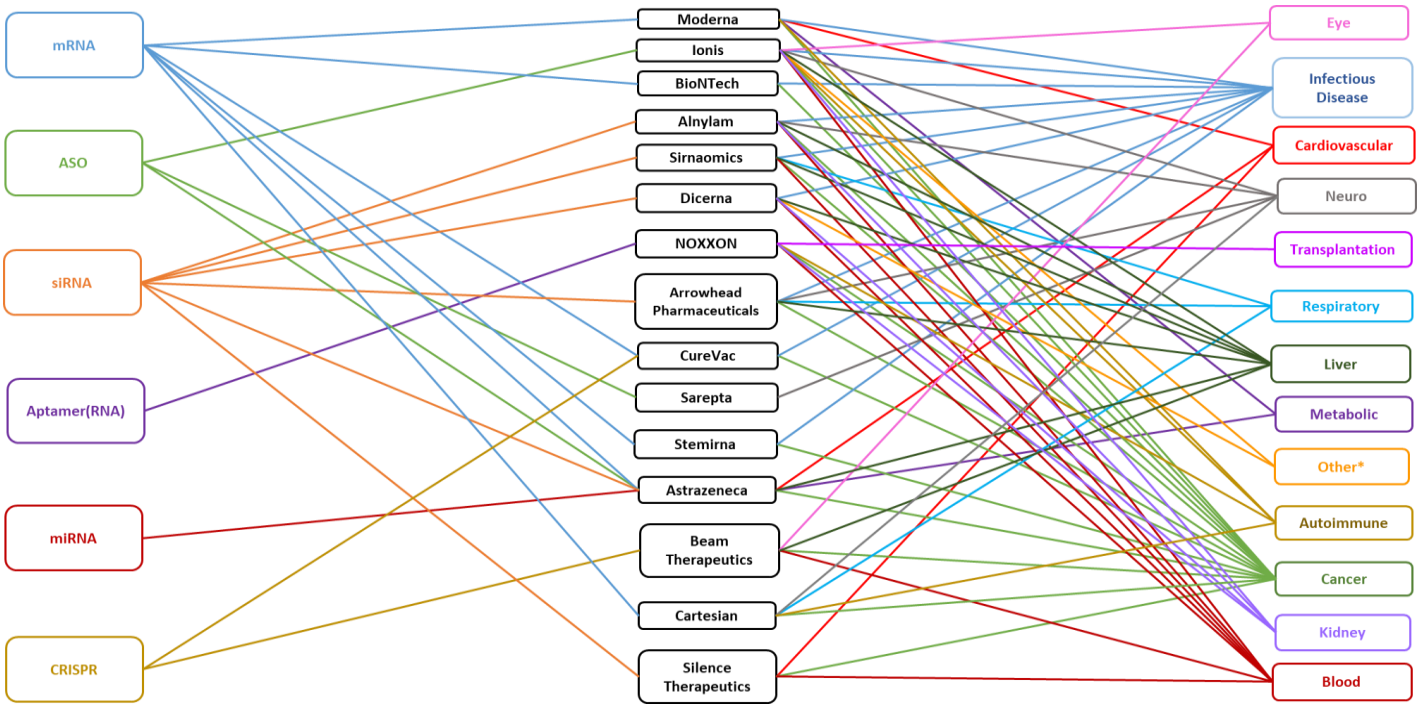


Figure 12. Types of RNA by company and targeted diseases. *Other: Acromegaly, Hereditary Angioedema, and Alcohol Use Disorder.

To investigate the research trends among different types of RNA therapeutics, the collected RNA therapeutics were further grouped by their types and development stages (Figure 13 and 14). The newer types of RNA therapeutics, such as AOCs and CRISPR, often have higher numbers of preclinical trials, indicating a great potential for future drug approval. Whereas, those more established types of RNA therapeutics, such as ASO and siRNA, often have a higher percentage of therapeutics on the market and a higher percentage of active and completed clinical trials, suggesting a shifting of research focus on the early development pipeline.

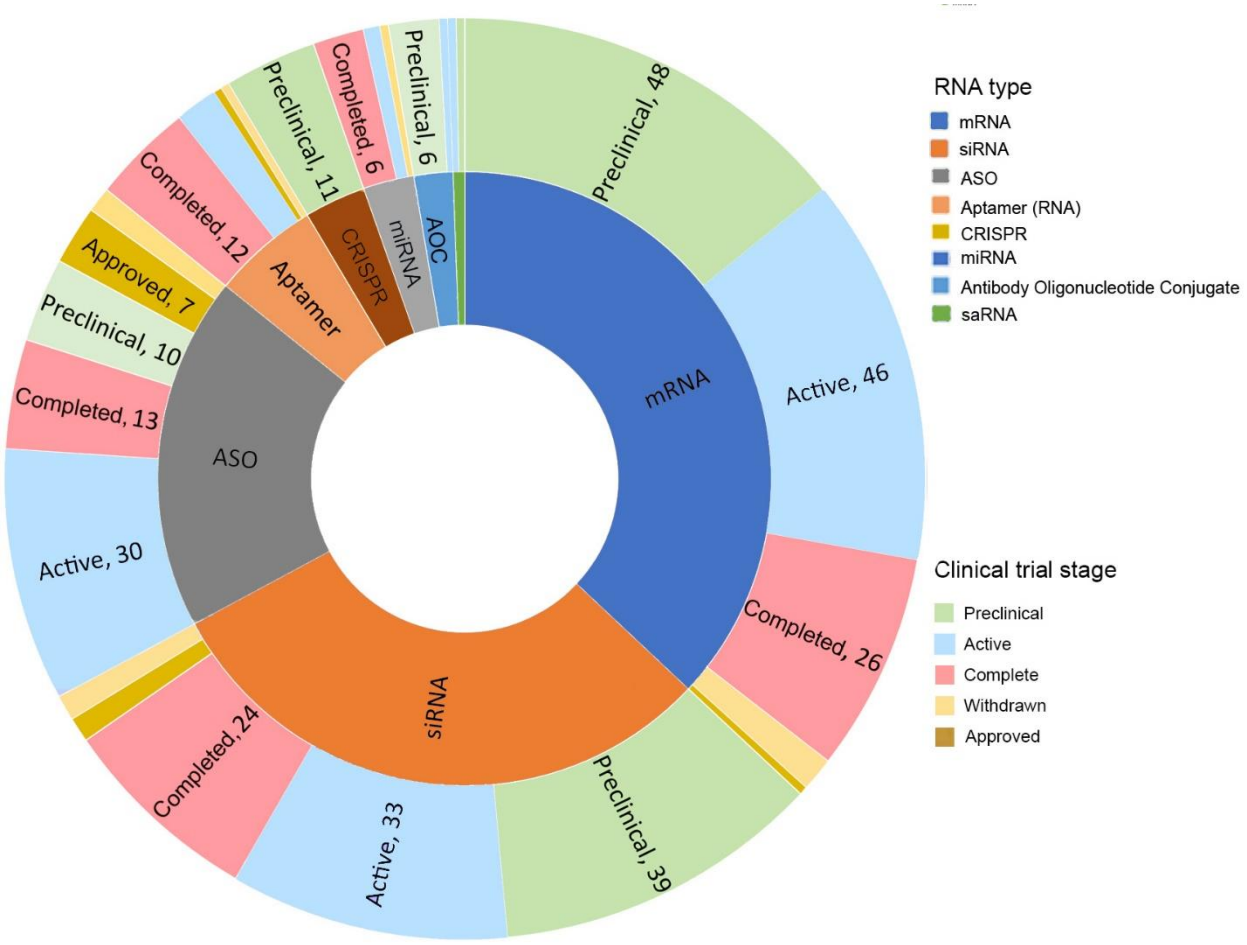


Figure 13. Counts of potential therapeutics and vaccines in different stages of development (preclinical, clinical, completed, withdrawn, and approved) for the various types of RNA. A full list of clinical trials is provided in Supplemental Table S2.

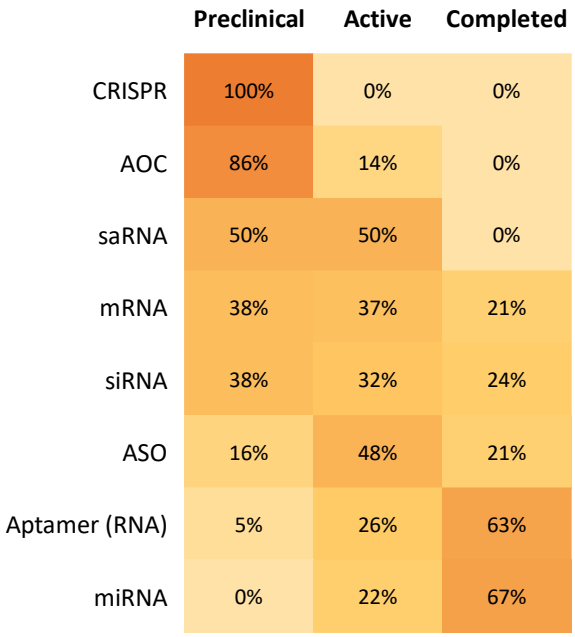


Figure 14. Percentage of preclinical, active, and completed clinical trials by RNA type. Figure rows may not sum to 100% because approved and withdrawn clinical trials are not included in this figure.

Disease-specific RNA therapeutics and vaccines

To further assess the pipeline dynamics, the above collected RNA therapeutics (Supplement Table S2) were then categorized based on their targeting diseases and development status (Figure 15). Cancer has attracted the highest number of therapeutics and vaccines in the research phase, with infectious diseases at the second. Neurological and neuromuscular diseases have the most approved treatments on the market, followed by cardiovascular and infectious diseases. The COVID-19 pandemic quickly catapulted RNA therapeutics for infectious diseases in both the research phase and approved vaccines to the forefront. While diseases such as familial hypercholesterolemia and DMD have multiple approved RNA therapeutics, blood diseases, cancers, and respiratory diseases currently do not have any approved RNA treatments. Respiratory disease, autoimmune disease, and blood diseases have the highest percentage of therapeutics in preclinical trials but so far have no approved treatments. Figure 16 shows the percentages of RNA therapeutics in various development stages based on disease type, revealing places with high activities, as well as places needing more attention.

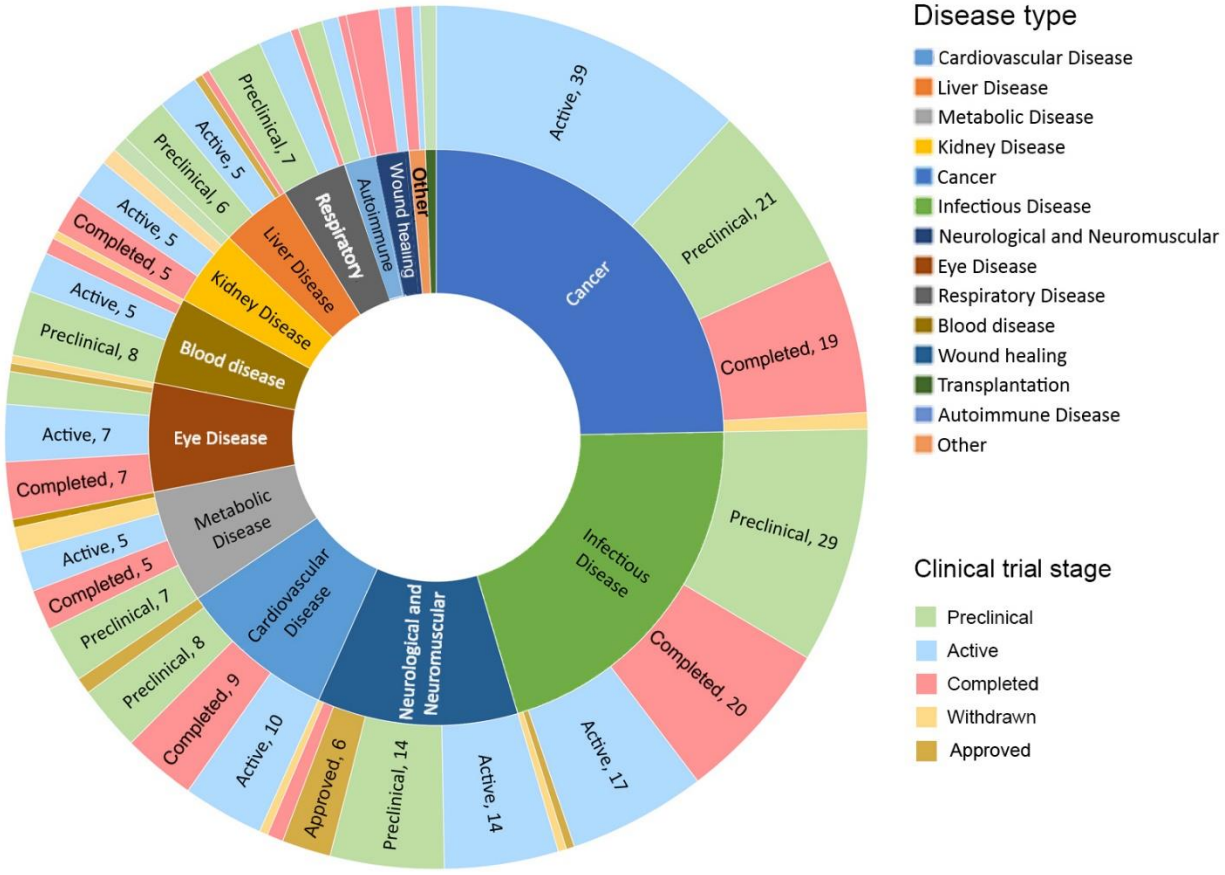


Figure 15. Counts of potential therapeutics and vaccines in different development stages (preclinical, clinical, completed, withdrawn, and approved) for various disease types. A full list of clinical trials is provided in Supplemental Table S2.

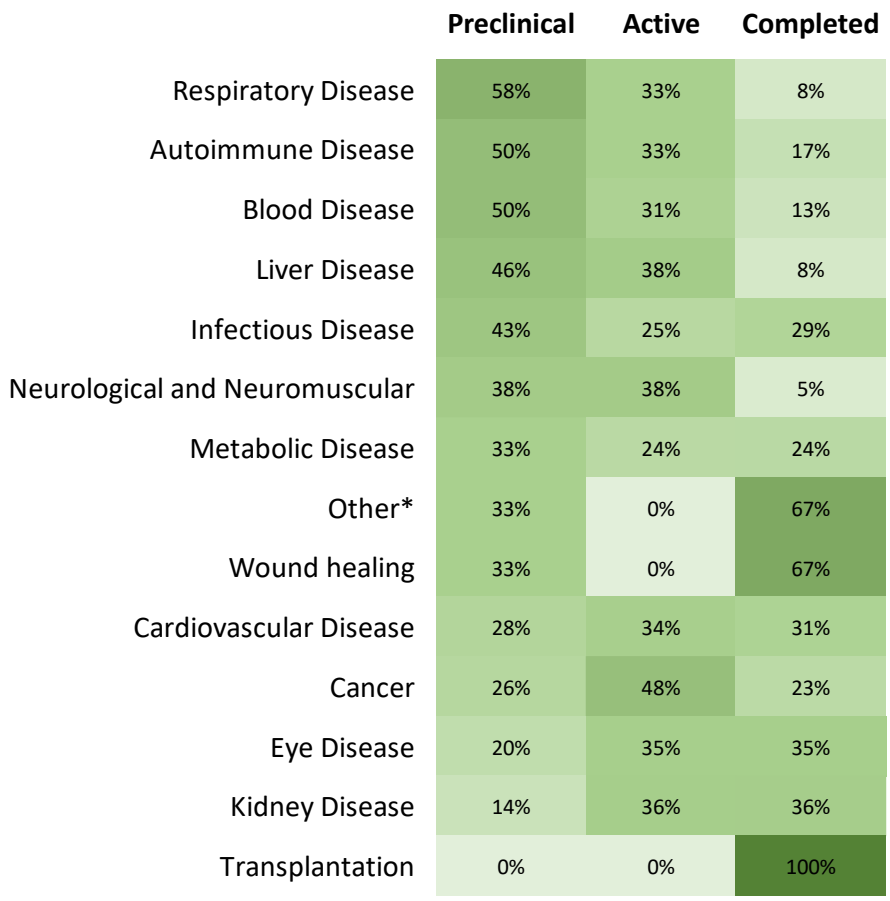


Figure 16. Percentage of preclinical, active, and completed clinical trials by disease type. Figure rows may not sum to 100% because approved and withdrawn clinical trials are not included in this figure. *Other: Acromegaly, Alcohol Use Disorder, and Hereditary Angioedema

Cardiovascular diseases, which account for 32% of all deaths, are the leading cause of death worldwide, taking an estimated 17.9 million lives each year. [137] Cardiovascular diseases include disorders of the heart and blood vessels. Over 80% of cardiovascular disease deaths are due to heart attacks and strokes, and one-third of these deaths occur prematurely in people under 70 years of age. [137]

There are two current drugs, Kynamro and Leqvio, approved for the treatment of the cardiovascular disease, familial hypercholesterolemia. In 2013, Ionis Pharmaceuticals received US FDA approval for their ASO drug Kynamro that targets apolipoprotein B. [9] However, Kynamro has serious hepatotoxicity risk and patients prescribed the drug are monitored closely. [138] The Novartis siRNA therapy drug Leqvio (developed by Alnylam) requires only two doses per year, and it received US FDA approval very recently in December 2021. [139] Leqvio targets proprotein convertase subtilisin kexin type 9 (PCSK9) to lower LDL cholesterol. Leqvio is a trivalent *N*-acetylgalactosamine (GalNAc)-conjugated siRNA with three GalNAc molecules clustered and conjugated to one siRNA molecule (Figure 23). [140]

In addition to those approved ones, there are several more RNA therapeutics for cardiovascular diseases currently in the clinical trial stages. Silence Therapeutics is evaluating the siRNA product SLN360 in a phase I clinical trial for its safety, tolerance, and pharmacodynamic and pharmacokinetic response in

individuals with high lipoprotein A (LpA) cardiovascular disease. [141] By targeting the LPA gene, SLN360 lowers the levels of LpA, decreasing the risk of heart disease, heart attacks, and strokes. [130] Alnylam is also evaluating the siRNA product Zilebesiran that targets angiotensinogen for sustained reduction of hypertension. [142] Interim phase I results show >90% reduction in serum angiotensinogen (AGT) for 12 weeks at a dosage of 100 mg or greater given quarterly or biannually. [143] This dosing regimen and the continued efficacy and safety of the drug are being evaluated in a phase II study initiated in June 2021 (NCT04936035). [143] Zilebesiran also uses Alnylam's trivalent GalNAc-conjugated siRNA delivery platform (Figure 23). [140] AstraZeneca and Moderna are collaborating on the mRNA drug AZD8601 to treat ischemic heart disease. [144] AZD8601 targets vascular endothelial growth factor-A (VEGF-A). [145] When AZD8601 is injected into the epicardium, VEGF-A is produced close to the damaged heart muscle, allowing cardiac regeneration. [146]

Table 1. RNA therapies for cardiovascular diseases

Cardiovascular Disease	**Drug name/lab code**	**Type of RNA**	**Target**	**Company**	**Development stage**	**Clinical trial number**
Familial hypercholesterolemia	Kynamro	ASO	Apolipoprotein B (ApoB) mRNA	Ionis Pharmaceuticals	FDA approval in 2013 [147]	
Familial hypercholesterolemia	Leqvio	siRNA	Proprotein convertase subtilisin/ kexin type 9	Alnylam/ Novartis	FDA approval in 2021 [139]	
Cardiovascular disease with high LpA	SLN360	siRNA	Lipoprotein(a) (LpA)	Silence Therapeutics	Phase I	NCT04606602 [141]
Ischemic heart Disease	AZD8601	mRNA	Vascular endothelial growth factor-A (VEGF-A)	Moderna/ AstraZeneca	Phase II	NCT03370887 [148]
Hypertension	Zilebesiran	siRNA	Angiotensinogen (AGT)	Alnylam	Phase I	NCT03934307 [149]

Metabolic diseases affect over a billion people worldwide by causing too much or too little of essential substances in the body. [150] Diabetes alone affects 422 million people worldwide, causing 1.5 million deaths annually. [151] Waylivra, an ASO product targeting apolipoprotein C-III (apoC-III) by Ionis Pharmaceuticals, received European Union (EU) approval in 2019 as a treatment for familial chylomicronemia syndrome (FCS). [152] FCS is a disease that prevents the body from breaking down consumed triglycerides. ApoC-III protein, which is produced in the liver, regulates plasma triglyceride levels in FCS patients, and Waylivra (volanesorsen) reduces its mean plasma levels. [153] IONIS-GCGR$_{Rx}$ is another ASO product developed by Ionis Pharmaceuticals and treats diabetes by reducing the production of the glucagon receptor (GCGR). [154] Glucagon is a hormone that opposes the action of insulin and stimulates the liver to produce glucose, particularly in patients with type-2 diabetes. [155]

Alpha-1 antitrypsin deficiency (AATD) is a hereditary metabolic disease. Alpha-1 antitrypsin (AAT) is a glycoprotein produced in the liver that travels through the bloodstream to protect the lungs from inflammation. [156] Mutations in the *SERPINA1* gene cause a deficiency of AAT in the blood, leading to toxic effects in the lungs and the accumulation of high levels of AAT in the liver that cause liver damage. [156] ARO-ATT, a siRNA product developed by Arrowhead Pharmaceutical, targets the *SERPINA1* mutation, and in preclinical studies has shown promise in reducing AAT liver disease. [157] Beam Therapeutics is developing a CRISPR base editing drug to treat AATD by correcting the E342K mutation in the *SERPINA1* gene. [158] Beam's base editor has two main components, a CRISPR protein bound to a guide RNA and a base editing enzyme, which are fused to form a single protein. [159] This fusion allows the precise targeting and editing of a single base pair of DNA, which has not been previously achieved. [159] Repairing the mutation would restore normal gene function and eliminate abnormal AAT production. This editing system uses a non-viral lipid nanoparticle delivery system. [158]

Methylmalonic acidemia (MMA) is a hereditary metabolic disease in which the body is unable to metabolize certain proteins and lipids correctly. [160] MMA is caused by mutations in the *MMUT*, *MMAA*, *MMAB*, *MMADHC*, and *MCEE* genes. The mutation in the *MMUT* gene accounts for about 60% of MMA cases. Moderna has developed an mRNA therapeutic mRNA-3705 that targets the *MMUT* mutation. [144] mRNA-3705 instructs the cell to restore the missing or dysfunctional proteins that cause MMA. mRNA-3705 entered clinical trials with the first patient treated in August 2021. [161]

Table 2. RNA therapies for metabolic diseases

Metabolic Disease	**Drug name/lab code**	**Type of RNA**	**Target**	**Company**	**Development stage**	**Clinical trial number**
Familial chylomicronemia syndrome	Waylivra	ASO	Apolipoprotein C-III (APOCIII)	Ionis Pharmaceuticals	EU approval in 2019 [152]	
Diabetes	IONIS-GCGRRx	ASO	Glucagon receptor GCGR	Ionis Pharmaceuticals	Phase II	NCT01885260 [162]
Methylmalonic acidemia (MMA)	mRNA-3705	mRNA	Mitochondrial enzyme methylmalonic-CoA mutase (MUT)	Moderna	Phase I/II	NCT04899310 [163]
Alpha-1 antitrypsin deficiency	ARO-AAT	siRNA	Mutant of α1-antitrypsin (Z-AAT)	Arrowhead Pharmaceuticals	Phase II	NCT03946449 [164]
Alpha-1 antitrypsin deficiency	Unnamed	CRISPR	Precise correction of E342K mutation	Beam Therapeutics	Preclinical [158]	

Liver diseases affect more than 1.5 billion people worldwide [165] and account for over 2 million deaths per year. [166] The siRNA therapeutic GIVLAARI, developed by Alnylam, received FDA approval in 2019 for the treatment of acute hepatic porphyria. [142] Acute hepatic porphyria is a genetic disease characterized by life-threatening acute attacks and chronic pain. [167] GIVLAARI is the first approved GalNAc-conjugated RNA therapeutic. [168]

Non-alcoholic steatohepatitis (NASH) is an accumulation of fat in the liver that causes liver damage. The antisense therapeutic ION839/AZD2693 from Ionis/AstraZeneca entered phase I trials in 2020 in patients with NASH and fibrosis. [169] AZD2693/ION839 targets patatin-like phospholipase domain-containing 3 (PNPLA3), reducing its expression. [154] Mutation of *PNPLA3*, which produces a protein that accumulates on the surface of intracellular lipid droplets, is strongly associated with an increased risk for NASH. [170]

Table 3. RNA therapies for liver diseases

Liver Disease	**Drug name/lab code**	**Type of RNA**	**Target**	**Company**	**Development stage**	**Clinical trial number**
Acute hepatic porphyria	GIVLAARI	siRNA	5-aminolevulinic acid synthase 1 (ALAS1) mRNA	Alnylam	FDA approval in 2019 [171]	
Nonalcoholic fatty liver disease	ION839/AZD2693	ASO	Patatin-like phospholipase domain-containing protein 3(PNPLA3)	Ionis/AstraZeneca	Phase I	NCT04483947 [169]

Cancer includes a large group of diseases that are characterized by abnormal cell growth in various parts of the body. It is the second leading cause of death globally, accounting for an estimated 10 million deaths per year with over 19 million new cases diagnosed in 2020. [172] Currently there are no approved RNA therapeutics for cancer treatment. NOXXON Pharma's lead aptamer candidate NOX-A12 is in development as a combination therapy for multiple types of cancers. [173] It is intended to enhance other anti-cancer treatments without side effects. A phase I/II trial of NOX-A12 in combination with radiotherapy in newly diagnosed brain cancer patients who would not benefit from standard chemotherapy is ongoing. [173] Interim data from the study in June 2021 showed tumor reduction in five of six patients consistent with an anti-cancer immune response, and there were no unexpected adverse events. [173] NOXXON is collaborating with Merck in their pancreatic cancer program. [173] NOXXON, in a

phase I/II combination trial with Merck's Keytruda, reported success in treating metastatic pancreatic and colorectal cancer and entered a second collaboration with Merck to conduct a phase II study in pancreatic cancer patients. [174] NOX-A12 targets C-X-C motif chemokine ligand 12 (CXCL12)[173], which, with its receptors, acts as a link between tumor cells and their environment, promotes tumor proliferation, new blood vessel formation, and metastases, and inhibits cell death. [175]

The anti-cancer siRNA product, STP707 by Sirnaomics[176], targets TGF-β1 and COX-2 mRNAs. A preclinical study demonstrated that knocking down *TGF*-β1 and *COX-2* gene expression simultaneously in the tumor microenvironment increases active T cell infiltration and combining the two siRNAs produces a synergistic effect that diminishes pro-inflammatory factors. [177]Descartes-11 developed by Cartesian is a CAR T-cell therapy for treating multiple myeloma, a white blood cell cancer that affects plasma cells.[178] It is currently in phase II studies with newly diagnosed patients. [179] Descartes-11 contains autologous $CD8^+$ T cells engineered with RNA chimeric antigen receptors (CARs) that bind to B-cell maturation antigen (BCMA). [178] BCMA is highly expressed in all myeloma cells, and Descartes-11 binds and kills BCMA-positive myeloma cells. [178]

Table 4. RNA therapies for cancers

Cancer	**Drug name/lab code**	**Type of RNA**	**Target**	**Company**	**Development stage**	**Clinical trial number**
Brain cancer/glioblastoma	NOX-A12	Aptamer (RNA)	C-X-C motif chemokine ligand 12 (CXCL12)	NOXXON Pharma	Phase I/II	NCT04121455 [180]
Pancreatic cancer	NOX-A12	Aptamer (RNA)	C-X-C motif chemokine ligand 12 (CXCL12)	NOXXON Pharma	Phase II	NCT04901741 [181]
Solid tumor	STP707	siRNA	Transforming growth factor beta (TGF-β), cyclooxygenase-2 (Cox-2)	Sirnaomics	Phase I	NCT05037149 [182]
Multiple myeloma	Descartes-11	mRNA	B-cell maturation antigen	Cartesian Therapeutics	Phase I/II	NCT03994705 [183]

Infectious diseases are caused by bacteria, viruses, fungi, or parasites. The first antisense antiviral, Vitravene, approved for use in 1998, treats cytomegalovirus (CMV) retinitis. [184] It was developed to treat the large number of cases of CMV retinitis in patients with acquired immunodeficiency syndrome (AIDS). [185] After the introduction of highly active antiretroviral therapy (HAART), the incidence of CMV retinitis decreased and Vitravene was withdrawn in the US in 2001. [184] However, it paved the way for other drugs in its class, proving the clinical efficacy of ASO therapeutics.

SARS-CoV-2 has infected over 280 million people, causing over 5.4 million deaths worldwide. [186] The COVID-19 pandemic brought the first approved mRNA vaccine to market. BioNTech/Pfizer's COVID-19 BNT162/Comirnaty vaccine was given US FDA emergency use authorization in 2020 and approved by the FDA in 2021. [187] Moderna's COVID-19 mRNA-1273/Spikevax vaccine was also given US FDA emergency use authorization in 2020. [188] Globally over 9 billion COVID-19 vaccine doses have been administered. [189]

Arbutus Biopharma Corporation developed AB-729, an RNA interference (RNAi) therapeutic specifically designed to reduce all hepatitis B virus (HBV) antigens, including hepatitis B surface antigen (HBsAg). [190] HBsAg interferes with host immune response [191], and preliminary data indicate that long-term suppression of HBsAg with AB-729 results in an increased HBV-specific immune response. [190]

Moderna's first quadrivalent seasonal influenza mRNA vaccine candidate mRNA-1010 is in phase I/II trials. [144] mRNA-1010 targets influenza lineages recommended by the World Health Organization (WHO) for the prevention of influenza, including seasonal influenza A H1N1 and H3N2 and influenza B Yamagata and Victoria. [192] Moderna has also developed a vaccine for RSV [144], a common respiratory virus that can cause serious illness in infants and older adults. Moderna's mRNA-1345 is currently in a phase I trial to evaluate its tolerance and reactogenicity in children, younger adults, and older adults. [193, 194] CureVac also has an mRNA prophylactic vaccine for RSV in their pipeline [136] that is in pre-clinical development. [136]

Table 5. RNA therapies and vaccines for infectious diseases

Infectious Disease	**Drug name/lab code**	**Type of RNA**	**Target**	**Company**	**Development stage**	**Year (first posted)**	**Clinical trial number**
COVID-19	BNT162/ Comirnaty	mRNA	SARS-CoV-2 spike protein	BioNTech/Pfizer	FDA approval in 2021 [187]		
COVID-19	mRNA-1273/ Spikevax	mRNA	SARS-CoV-2 spike protein	Moderna	FDA authorization for emergency use in 2020 [188]		
CMV retinitis	Vitravene	ASO	Immediate early 2 (IE2) mRNA	Isis Pharmaceuticals/ Novartis	FDA approval in 1998 [184]		
Hepatitis B viral (HBV)	AB-729	siRNA	Hepatitis B viral surface antigen (HBsAg)	Arbutus Biopharma Corporation	Phase I	2021	NCT04775797 [195]
Influenza	mRNA-1010	mRNA		Moderna	Phase I/II	2021	NCT04956575 [196]

Respiratory syncytial virus (RSV)	Unnamed	mRNA		CureVac	Preclinical [136]		
Respiratory syncytial virus (RSV)	mRNA-1345	mRNA		Moderna	Phase I	2021	NCT04528719 [197]

Neuromuscular diseases affect the function of muscles and nerves that communicate sensory information to the brain. [198] They affect the brain as well as the nerves found throughout the body and the spinal cord. [199] These types of diseases have the greatest number of approved RNA therapeutics. There are two approved treatments for DMD: Sarepta's ASO therapeutics Exondys 51, which targets exon 51 of the dystrophin gene, was FDA approved in 2016 [200] and Vyondys 53, which targets exon 53 of the dystrophin gene, was FDA approved in 2019. [201] Ionis Pharmaceuticals received FDA approval in 2016 for ASO therapeutic Spinraza targeting survival of motor neuron 2 (SMN2) for the treatment of spinal muscular atrophy. [202] Their second therapeutic approved by the FDA in 2018 was the ASO neurological therapeutic Tegsedi, which targets hepatic production of transthyretin (TTR) and is used for the treatment of hATTR amyloidosis-polyneuropathy a disease due to mutations in the gene encoding TTR that leads to abnormal amyloid deposits on nerves. [203] Onpattro by Alnylam is another approved RNA drug for the treatment of hATTR amyloidosis-polyneuropathy by targeting hepatic production of transthyretin. [204] Onpattro, which was FDA approved in 2018, was the first siRNA drug. [27] Sarepta Therapeutics has two other ASO therapeutics for the treatment of DMD, SRP-5044 and SRP-5050 (targeting exon 44 and exon 50 of the dystrophin gene, respectively) that are in preclinical studies. [205]

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease that affects nerve cells in the brain and spinal cord. [206] Ionis Pharmaceuticals in partnership with Biogen has an ASO investigational drug Tofersen in phase III clinical trials. [154] Tofersen targets superoxide dismutase 1 (SOD1), the second most common and best understood genetic cause of ALS.

Avidity Biosciences has a pipeline of AOC therapeutics focused on neuromuscular diseases. Their leading candidate, AOC 1001, is a siRNA conjugated with a monoclonal antibody (mAb). [207] AOC 1001 has an ongoing phase I/II trial in adults with myotonic dystrophy type 1 (DM1). DM1 is a progressive neuromuscular disease that impacts skeletal and cardiac muscle. DM1 is caused by an abnormal number of CUG triplet repeats in the myotonic dystrophy protein kinase gene (*DMPK*), reducing muscle blind-like protein (MBNL) activity and disrupting muscle development. [208] AOC 1001 is designed to reduce DMPK levels and CUG triplet repeats so that MBNL can perform normally. [207] Avidity Biosciences has also developed an AOC to treat DMD. [207] The oligonucleotides are designed to promote skipping of specific exons to produce dystrophin in patients with DMD. Their leading DMD drug candidate, AOC 1044, can induce exon skipping specifically for exon 44, and clinical trials are planned for 2022. [207]

Table 6. RNA therapies for neuromuscular and neurological diseases

Neurological and	**Drug name/lab code**	**Type of RNA**	**Target**	**Company**	**Development stage**	**Clinical trial number**

Neuromuscular Disease						
Duchenne muscular dystrophy	Exondys 51	ASO	Exon 51 dystrophin	Sarepta Therapeutics	FDA approval in 2016 [200]	
Duchenne muscular dystrophy	Vyondys 53	ASO	Exon 53 dystrophin	Sarepta Therapeutics	FDA approval in 2019 [201]	
hATTR amyloidosis-polyneuropathy	Tegsedi	ASO	Transthyretin mRNA	Ionis Pharmaceuticals	FDA approval in 2018 [203]	
hATTR amyloidosis-polyneuropathy	Onpattro	siRNA	Transthyretin mRNA	Alnylam	FDA approval in 2018 [209]	
Spinal muscular atrophy	Spinraza	ASO	Survival of motor neuron 2 (SMN2) mRNA	Ionis Pharmaceuticals	FDA approval in 2016 [202]	
Amyotrophic lateral sclerosis	Tofersen	ASO	Superoxide dismutase 1 (SOD1)	Ionis Pharmaceuticals / Biogen	Phase III	NCT04856982 [210]
Duchenne muscular dystrophy (DMD)	SRP-5044	ASO	Exon 44 dystrophin	Sarepta Therapeutics	Preclinical [205]	
Duchenne muscular dystrophy (DMD)	SRP-5050	ASO	Exon 50 dystrophin	Sarepta Therapeutics	Preclinical [205]	
Myotonic dystrophy type 1 (DM1)	AOC 1001	Antibody oligo-nucleotide conjugates (AOC)	Myotonic dystrophy protein kinase (DMPK)	Avidity Biosciences	Phase I/II	NCT05027269 [211]
Duchenne muscular	AOC 1044	Antibody oligo-nucleotide	Exon 44 dystrophin	Avidity Biosciences	Preclinical [207]	

dystrophy (DMD)		conjugates (AOC)				

Eye diseases including visual impairment affect 2.2 billion people globally. [212] Macular degeneration, which causes loss in the center of the field of vision and is irreversible, affects 196 million people. [213] Macugen, developed by Gilead Sciences, was the first FDA-approved (2014) RNA aptamer treatment for neovascular age-related macular degeneration. [214] Macugen targets the VEGF protein in the eye, reducing the growth of blood vessels to control the leakage and swelling that cause vision loss. [215] As age-related macular degeneration progresses, geographic atrophy (GA), a chronic progressive degeneration of the macula, can develop. [216] Zimura by Iver Bio is another RNA aptamer therapeutic and is currently in a phase III clinical trial. Zimura inhibits complement C5 [217], which is involved in the development and progression of AMD. In clinical trials, Zimura slowed the progression of GA over 12 months in individuals with age-related macular degeneration. [216] QR-504a from ProQR Therapeutics is an investigational RNA ASO therapeutic that is in ongoing phase I/II clinical trials. [218] QR-504a is designed to slow vision loss in individuals with Fuchs endothelial corneal dystrophy (FECD) which results from trinucleotide repeat expansion mutations in the *TCF4* gene. [219]

Table 7. RNA therapies for eye diseases

Eye Disease	**Drug name/lab code**	**Type of RNA**	**Target**	**Company**	**Development stage**	**Clinical trial number**
Macular degeneration	Macugen	Aptamer (RNA)	Vascular endothelial growth factor (VEGF) protein	Gilead Sciences	FDA approval in 2014 [214]	
Geographic atrophy related to age-related macular degeneration	Zimura	Aptamer (RNA)	Complement component 5 (C5)	IVERIC Bio	Phase III	NCT04435366 [217]
Fuchs endothelial corneal dystrophy	QR-504a	ASO	Transcription factor 4 (TCF4)	ProQR Therapeutics	Phase I/II	NCT05052554 [218]

Kidney disease affects over 850 million people worldwide. [220] Primary hyperoxaluria type 1 (PH1) is a genetic kidney disease characterized by the overproduction of oxalate, which leads to kidney

stones, kidney failure, and systemic oxalosis. [213, 220] The siRNA therapeutic Oxlumo was developed by Alynlam and approved by the FDA in 2020. [221] Oxlumo targets the hydroxy acid oxidase 1 gene (*HAO1*), which encodes glycolate oxidase. [221] Oxlumo reduced urinary oxalate excretion in patients with progressive kidney failure in PH1. [221, 222] The majority of patients had normal or near-normal levels of oxalate after 6 months of treatment. Autosomal dominant polycystic kidney disease (ADPKD) is caused by mutations in the *PKD1* or *PKD2* gene. [223] It is characterized by cysts within the kidneys, often leading to kidney failure. To treat ADPKD, Regulus designed RGLS4326, a second-generation oligonucleotide that inhibits miR-17, which produces kidney cysts. RGLS4326 binds to miR-17 microRNAs, inhibits miR-17 activity, and reduces disease progression. [224] IgA nephropathy is a chronic kidney disease that is caused by deposits of protein immunoglobulin A (IgA) inside the glomeruli of the kidneys. [224, 225] Overproduction of complement factor B (FB) is associated with increased IgA nephropathy. Ionis has partnered with Roche to develop a ligand-conjugated ASO therapeutic, IONIS-FB-LRx, which targets FB to reduce the production of IgA and alleviate the symptoms of IgA nephropathy. [154]

Table 8. RNA therapies for kidney diseases

Kidney Disease	**Drug name/lab code**	**Type of RNA**	**Target**	**Company**	**Development stage**	**Clinical trial number**
Primary hyperoxaluria type 1	Oxlumo	siRNA	Hydroxy acid oxidase 1 (HAO1)	Alnylam	FDA approval 2020	NCT04152200 [226]
Autosomal dominant polycystic kidney disease (ADPKD)	RGLS4326	Oligonucleotide	miR-17	Regulus	Phase I	NCT04536688 [227]
IGA nephropathy	IONIS-FB-LRx	ASO	Complement factor B	Ionis/Roche	Phase II	NCT04014335 [228]

Respiratory diseases affect more than 1 billion people worldwide. [229] COVID-19 brought acute respiratory distress syndrome (ARDS) to the forefront of medical news during the pandemic. ARDS is a severe inflammatory lung disease with a mortality rate of over 40%. [230] Inflammation leads to lung tissue injury and leakage of blood and plasma into air spaces, resulting in low oxygen levels and often requiring mechanical ventilation. [230] Descartes-30 is an engineered mRNA cell therapy product by Cartesian Therapeutics. It comprises human mesenchymal stem cells that secret two human DNases for degrading ARDS-causing neutrophil extracellular traps (NETS). [178]. Descartes-30 is currently in phase I/II clinical trials. [231]

Cystic fibrosis is caused by a dysfunctional cystic fibrosis transmembrane conductance regulator (CFTR) protein, resulting from mutations in the *CFTR* gene. [232] Without CFTR, mucus in various organs including the lungs is extremely thick and sticky. MRT5005, developed by Translate Bio, is an mRNA therapeutic that bypasses this mutation by delivering mRNA encoding a fully functional CFTR protein to the cells in the lungs through nebulization. [233] Although initial interim results from clinical studies

showed promise, [234] results from the second interim phase I/II clinical trial showed no increase in the lung function of individuals receiving MRT5005. [235] The siRNA therapeutic ARO-ENaC is designed by Arrowhead Pharmaceuticals to reduce epithelial sodium channel alpha subunit (αENaC) in the lungs and airways. Increased ENaC contributes to airway dehydration and increased mucus. [236] However, Arrowhead paused a phase I/II study of ARO-ENaC in July 2021 after safety studies showed local lung inflammation in rats. [237]

Table 9. RNA therapies for respiratory diseases

Respiratory Disease	**Drug name/lab code**	**Type of RNA**	**Target**	**Company**	**Development stage**	**Clinical trial number**
Acute respiratory distress syndrome	Descartes-30	mRNA	Neutrophil extracellular traps (NETs)	Cartesian Therapeutics	Phase I/II	NCT04524962 [231]
Cystic fibrosis	MRT5005	mRNA	Cystic fibrosis transmembrane conductance regulator (CFTR)	Translate Bio	Phase I/II	NCT03375047 [238]
Cystic fibrosis	ARO-ENaC	siRNA	Epithelial sodium channel α subunit	Arrowhead Pharmaceuticals	Phase I/IIa	NCT04375514 [239]

Blood diseases affect one or more components of blood. Sickle cell disease is a type of blood disease that causes red blood cells to become misshapen and break down. [240] Beta-thalassemia syndromes are a group of blood diseases that result in reduced levels of hemoglobin in red blood cells. [241] BEAM-101, Beam Therapeutics leading *ex vivo* base editor, is a patient-specific, autologous hematopoietic investigational cell therapy. BEAM-101 introduces base edits that mimic the single nucleotide polymorphisms found in individuals with hereditary persistence of fetal hemoglobin (HPFH), which could alleviate the effects of mutations causing sickle cell disease or beta-thalassemia since the fetal hemoglobin doesn't become misshapen. [242] Beam plans to initiate a phase I/II clinical trial to assess the safety and efficacy of BEAM-101 for the treatment of sickle cell disease. [242] BEAM-101 uses an electroporation delivery system. [158] Silence Therapeutics has RNA therapeutics for blood diseases such as thalassemia, myelodysplastic syndrome, and rare iron-loading anemias. [243] Their siRNA therapeutic SLN124 targets *TMPRSS6*, a gene that prevents the liver from producing hepcidin. [243] Phase I clinical trial data showed that SLN124 improved red blood cell production and reduced anemia by increasing the levels of hepcidin. [243, 244]

Table 10. RNA therapies for blood disease

Blood Disease	Drug name/lab code	Type of RNA	Target	Company	Development stage	Clinical trial number
Sickle cell disease/beta thalassemia	BEAM-101	CRISPR	Fetal hemoglobin activation	Beam Therapeutics	Preclinical [158]	
Thalassemia/low-risk myelodysplastic syndrome	SLN124	siRNA	Transmembrane serine protease 6 (TMPRSS6)	Silence Therapeutics	Phase I	NCT04718844 [245]

Keloid scarring is a type of pathological condition that forms abnormally thick scarring after a skin injury. STP705, from Sirnaomics, is a siRNA therapeutic in phase II clinical trial for the treatment of keloid scars. [246] STP705 targets both TGF-β1 and COX-2 gene expression with polypeptide nanoparticle-enhanced delivery. [247] The synergistic effect of simultaneous silencing TGF-β1 and COX-2 may reverse skin fibrotic scarring by decreasing inflammation and activating fibroblast apoptosis. This mechanism of action of STP705 can be widely applied for the treatment of other fibrotic conditions. [247]

Autoimmune diseases are characterized by immune activation in response to normal antigens. [248] mRNA-6231 from Moderna is an mRNA encoding IL-2 mutein that activates and expands the regulatory T cell population and dampens self-reactive lymphocytes. [249]

Alcohol use disorder (AUD) is the inability to control alcohol use despite adverse social, occupational, or health consequences. According to the World Health Organization, AUD affects over 283 million people globally. [250] Alcohol is metabolized in the liver to acetaldehyde via alcohol dehydrogenase and then to acetic acid by aldehyde dehydrogenase 2 (ALDH2). [251] Inhibiting ALDH2 results in unpleasant symptoms due to the increased acetaldehyde when alcohol is not fully metabolized. [252] The siRNA therapeutic DCR-AUD by Dicerna knocks down ALDH2 protein expression in the liver, which results in increased acetaldehyde levels that discourage continued alcohol use in AUD patients. [252]

Table 11. RNA therapies for other diseases

Other Disease	Drug name/lab code	Type of RNA	Target	Company	Development stage	Clinical trial number
Keloid scarless healing	STP705	siRNA	Transforming growth factor beta 1(TGF-β1)/ cyclooxygenase-2 (COX-2)	Sirnaomics	Phase II	NCT04844840 [246]
Autoimmune diseases	mRNA-6231	mRNA	Interleukin-2 (IL-2)	Moderna	Phase I	NCT04916431 [253]

Alcohol use disorder	DCR-AUD	siRNA	Aldehyde dehydrogenase 2 (ALDH2)	Dicerna Pharmaceuticals	Phase I	NCT05021640 [254]

Chemical modifications for improving RNA stability and target specificity

RNA is composed of nucleosides that consist of a nucleic acid base attached to a D-ribose through a β-N-glycosyl bond between the ribose and the pyrimidine bases (uracil and cytosine) or the purine bases (adenosine and guanosine). The nucleosides are connected by a phosphodiester bond using the phosphate between the 3' and 5' carbons on adjacent ribose molecules (Figure 17). [255] RNAs can be modified on the nucleic acid base and on the phosphate and the ribose of the sugar-phosphate backbone, as shown illustrated Figure 17 with color circles.

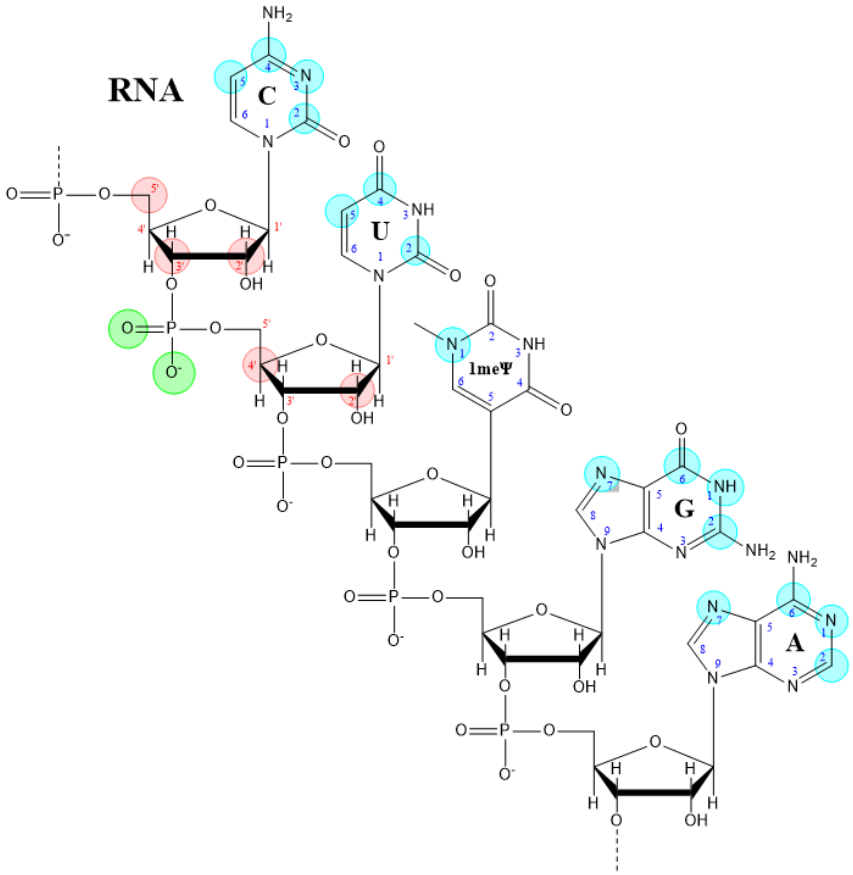


Figure 17. An example of RNA structure and modification sites. Green circles, modification sites on the phosphate; red circles, attachment sites for modifications on the ribose; blue circles, attachment sites for modifications on the nucleic acid base: 1-methylpseudouridine (1meΨ), cytosine (C), uridine (U), guanosine (G), and adenosine (A).

The nucleic acid side chains extending from the sugar-phosphate backbone form hydrogen bonds between the nucleic acid bases of complementary RNA chains; U bonds with A, and G bonds with C. Double-stranded RNA structures form as a result of intramolecular and intermolecular base pairing. Base pairing between loops of double-stranded stem-loops can yield 3D structures and triple helixes can form within single strands or between multiple strands.

Chemical modifications on RNA can improve the stability and reduce the immunogenicity of therapeutic RNAs. RNA is very susceptible to nucleases and hydrolysis by basic compounds. Chemical modification of RNA protects the vulnerable sugar-phosphate backbone from nuclease degradation and lowers the risk of off-target effects. For RNAs that form a duplex with a target sequence, mutations that lower the melting temperature of the duplex destabilize the complex and improve target specificity by decreasing base pairing with non-target RNA. RNA modifications can also improve the delivery of the RNA into the cell through the plasma membrane and enhance the activity of the RNA. [105]

Nucleic acid base modifications

Figure 18 shows chemical structures of commonly seen base modifications and rare bases. Cytidines or uridines can be methylated at the N-5 position to be 5-methylcytidines or 5-methyluridines. Cytidines can have the oxygen replaced with a sulfur at the N-2 position to be 2-thiocytidines. When uridines have the oxygen replaced with a sulfur at either the N-2 or the N-4 position, they become either 2-thiouridines or 4-thiouridines. Uridines can also be reduced on the base ring and become 5,6-dihydrouridines. Another commonly seen base modification is methylation at the N-7 position of the guanosine, which often occurs for the mRNA cap modifications. The guanosines can also be modified at the N-7 position by converting the nitrogen to a carbon and become 7-deazaguanosine. Similar modifications can happen to adenosines resulting in 7-methyladenosines and 7-deazaadenosines. Pseudouridines and inosines are also regularly seen as rare bases in modified RNA sequences. Additional modified or rare bases are shown in Supplement Figure S7. According to the data in the CAS Content Collection [83], the use of RNA modifications has been increasing since the beginning of 1995 (Supplemental Figure S6).

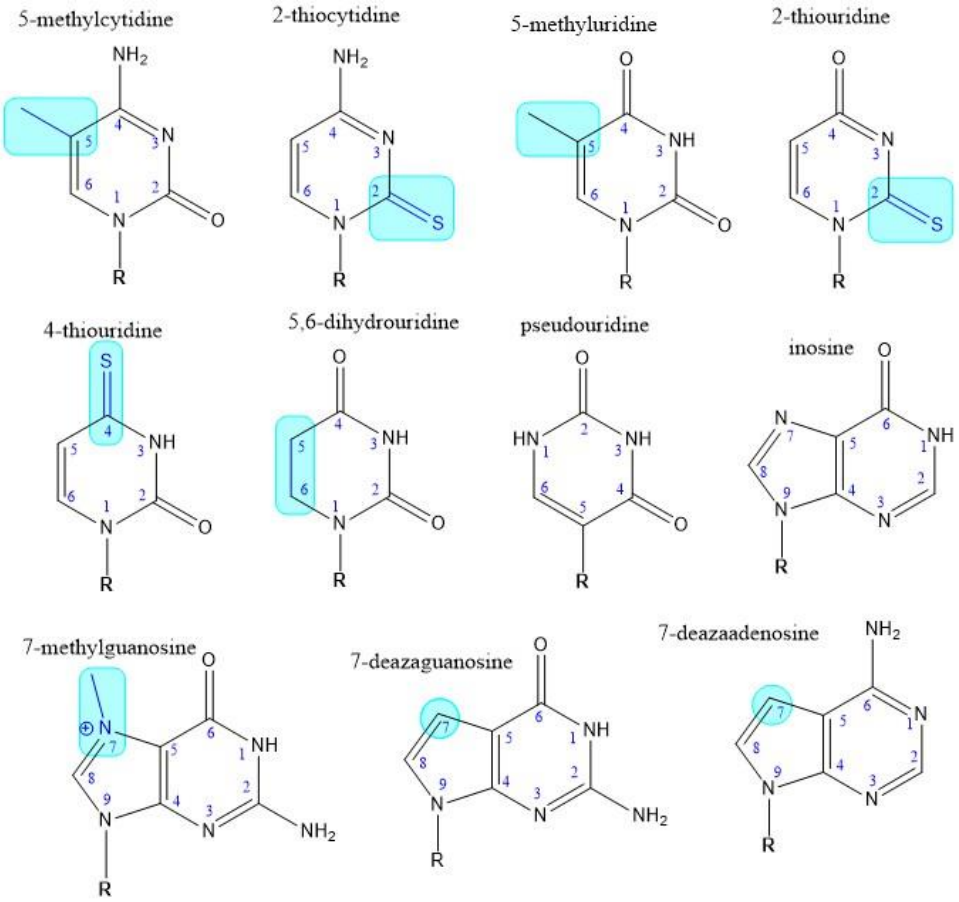


Figure 18. Examples of modified and rare bases. R = D-ribose; locations of modifications are shown in blue.

Base modifications that interfere with the formation of hydrogen bonds can thermally destabilize duplex formation with the target and thus improve target specificity by limiting off-target binding. [256] In addition, modifications improve the performance of therapeutic RNA. Replacing uridine with the modified base 1-methylpseudouridine (Figure 17) in therapeutic mRNAs, such as the COVID-19 vaccines (Pfizer's Comirnaty and Moderna's Spikevac), improves translation and lowers cytotoxic side effects and immune responses to the mRNA. [257] Both Pfizer's Comirnaty and Moderna's Spikevac mRNA vaccines also use a 7-methylguanosine cap linked by a 5' triphosphate to the 5' end of the mRNA, replicating the normal mRNA caps that prevent degradation of the 5' end of the mRNA. [258]

Modifications on ribose

The hydroxyl group on the C-2' position of the ribose destabilizes RNA compared to DNA. Modification of this hydroxyl protects against nuclease digestion and can lower the thermal stability of duplexes formed with a target RNA. Figure 19 shows the chemical structures of these modifications on ribose. The most common modifications at the C-2' position include 2'-O-methyl, 2'-fluoro, 2'-MOE, and 2'-amine. Another modification at the C-2' position is the LNA in which a 2'-O, 4'-C-methylene bridge connects the 2' position to the 4' position on the ribose. [259]

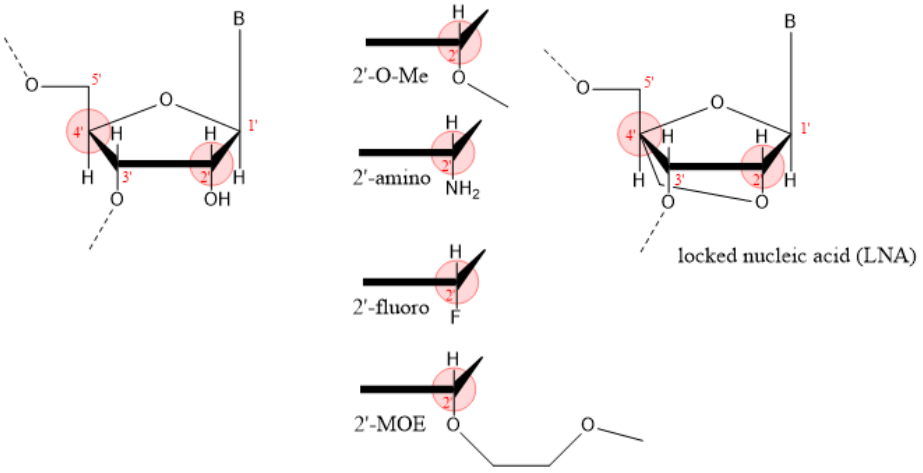


Figure 19. Common modifications on the 2'-hydroxyl group of D-ribose. B = nucleic acid base.

In addition, the 5' and 3' ends of RNA can be modified using cleavable linkers attached to GalNAc groups or lipophilic moieties to target the therapeutic RNA to the desired tissue. The linkers are cleaved by acid pH, redox potential, or degradative enzymes in cells but not in serum or blood. Cleavable linkers include acid-cleavable groups, ester-based cleavable groups, and peptide-based cleavable groups. [256]

Backbone modifications

Modifications to the phosphate group in the sugar-phosphate backbone can improve the resistance of therapeutic RNAs to extracellular and intracellular nucleases. In addition, the negative charge on the phosphate group also interferes with the delivery of the RNA into the cell through the lipid bilayer membrane, which is impermeable to polar molecules. Thus, replacing the oxygens on the phosphates with neutral groups or complexing the phosphate groups to cations like sodium can improve RNA delivery. [260]

A widely used backbone modification, phosphorothioate as shown in Figure 20, which replaces an oxygen in the phosphate group with sulfur, reduces the activity of extracellular and intracellular nucleases. [261] RNA molecules with phosphorothioate linkages at the ends resist exonucleases, whereas RNA molecules with phosphorothioate linkages within the RNA resist endonucleases. However, the sulfur on the phosphate group creates stereogenic α-phosphorus atoms resulting in diastereomers with different functional properties that can affect duplex formation. Careful spacing of the phosphorothioate linkages within the RNA can ameliorate this problem. [259, 260]

Other backbone modifications replace the D-ribose with either an L-ribose or a non-ribose moiety (Figure 20). Phosphorodiamidate morpholino oligonucleotides (PMO) contain morpholino groups linked by phosphorodiamidate groups rather than ribose linked by phosphates. Glycol nucleic acids

(GNA) have a backbone of repeating glycerol units linked by phosphodiester bonds. [262] In PNAs the sugar-phosphate backbone is replaced with a flexible N-(2-aminoethyl)glycine polymer with the nucleobases attached via a methylene carbonyl linkage. [263] PMO, GNA, and PNA all resist nuclease degradation. PNA forms duplexes with complementary DNA or RNA with higher affinity and specificity than unmodified DNA-DNA or DNA-RNA duplexes. However, duplexes containing PNA, PMO, and LNA resist RNase H degradation, inhibiting gene knockdown through targeted mRNA degradation. [259]

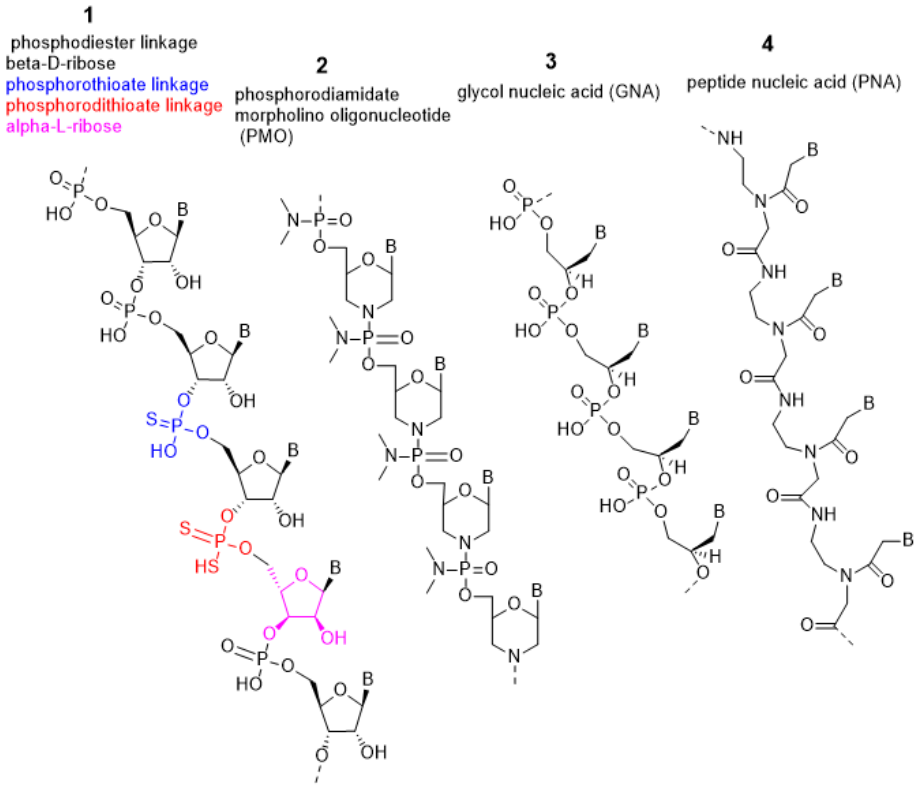


Figure 20. Examples of modified RNA backbones. Backbone 1 shows phosphate-ribose backbone linkages, which include the classic phosphodiester (black), phosphorothioate (blue), and phosphorodithioate (red). The purple ribose, α-L-ribose, has an alternative stereochemistry compared to the normal β-**D**-ribose moieties shown in black. Backbone 2 is a phosphorodiamidate morpholino (PMO) backbone, backbone 3 is (R)-glycol nucleic acid ((R)-GNA), and backbone 4 is peptide nucleic acid (PNA). B = nucleic acid base.

Trends of RNA chemical modifications

Sequence data for RNAs and RNA modifications are annotated and collected with CAS Registry Sequence Guidelines [264] from published documents and stored in the CAS Content Collection [83]. To better understand the chemical modification trends on RNA molecules, we extracted ~170,000 modified RNA sequences from the CAS Content Collection. Figure 21 shows the number of the modified RNA sequences distribution of these modified sequences along the sequence length. The predominance of

modified nucleotide RNAs for lengths of 18-27 bases reflects the fact that this sequence length is commonly used in siRNAs and ASOs; processed, naturally occurring double-stranded siRNAs are typically 21 or 23 bp long. The double-stranded nature of siRNAs accounts for the large number of modifications for nucleotides with a length of 42 and 44; two 21-nucleotide RNAs produce 42 nucleotides of RNA, while a 21-nucleotide and a 23-nucleotide RNA produce 44 nucleotides. The percentage of modified RNA sequences in the total RNA sequences was also shown along the sequence length, suggesting that sequences less than 100 base pairs are more frequently being modified than the longer sequences.

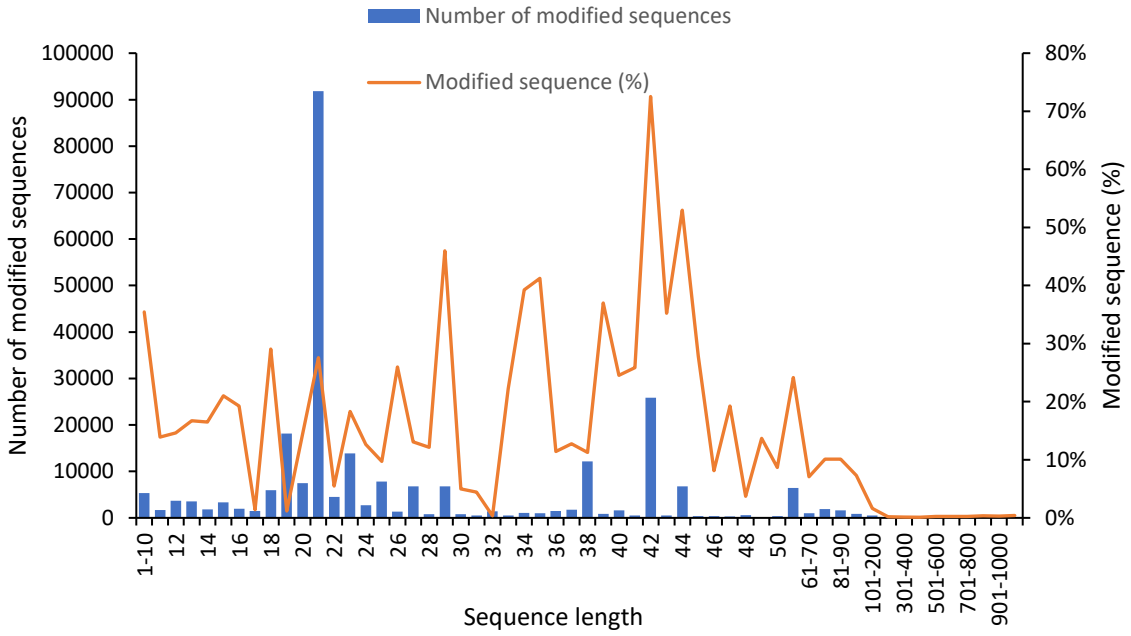


Figure 21. RNA sequences containing modifications and their distribution with respect to sequence lengths (from the CAS Content Collection). Blue bars: the absolute number of modified RNA sequences; orange line: the percentage of modified RNA sequences in the total RNA sequences with same sequence length.

Chemical modifications were further analyzed using this data set with specific types of modifications and sequence lengths. A heatmap (Figure 22) was constructed based on the relative frequencies of specific types of modification in the total modification event in that sequence length. The heat map of modification type versus sequence length shows a sharp change in the types of modifications in sequences <100 nucleotides vs. >100 nucleotides. Sequences >100 nucleotides are modified much less than shorter sequences, which reflects the use of chemical synthesis methods like solid-support synthesis to produce small RNAs and the use of *in vitro* transcription to produce larger RNAs. Longer sequences are distinct with more triphosphates and 7-methylguanosines, suggesting that they are mRNAs with 5' end caps consisting of 7-methylguanosine linked to the 5' end of the mRNA with a triphosphate group. Since therapeutic mRNAs are translated by the ribosomes to produce an active protein, excessive modification might provide steric hindrance that inhibits translation.

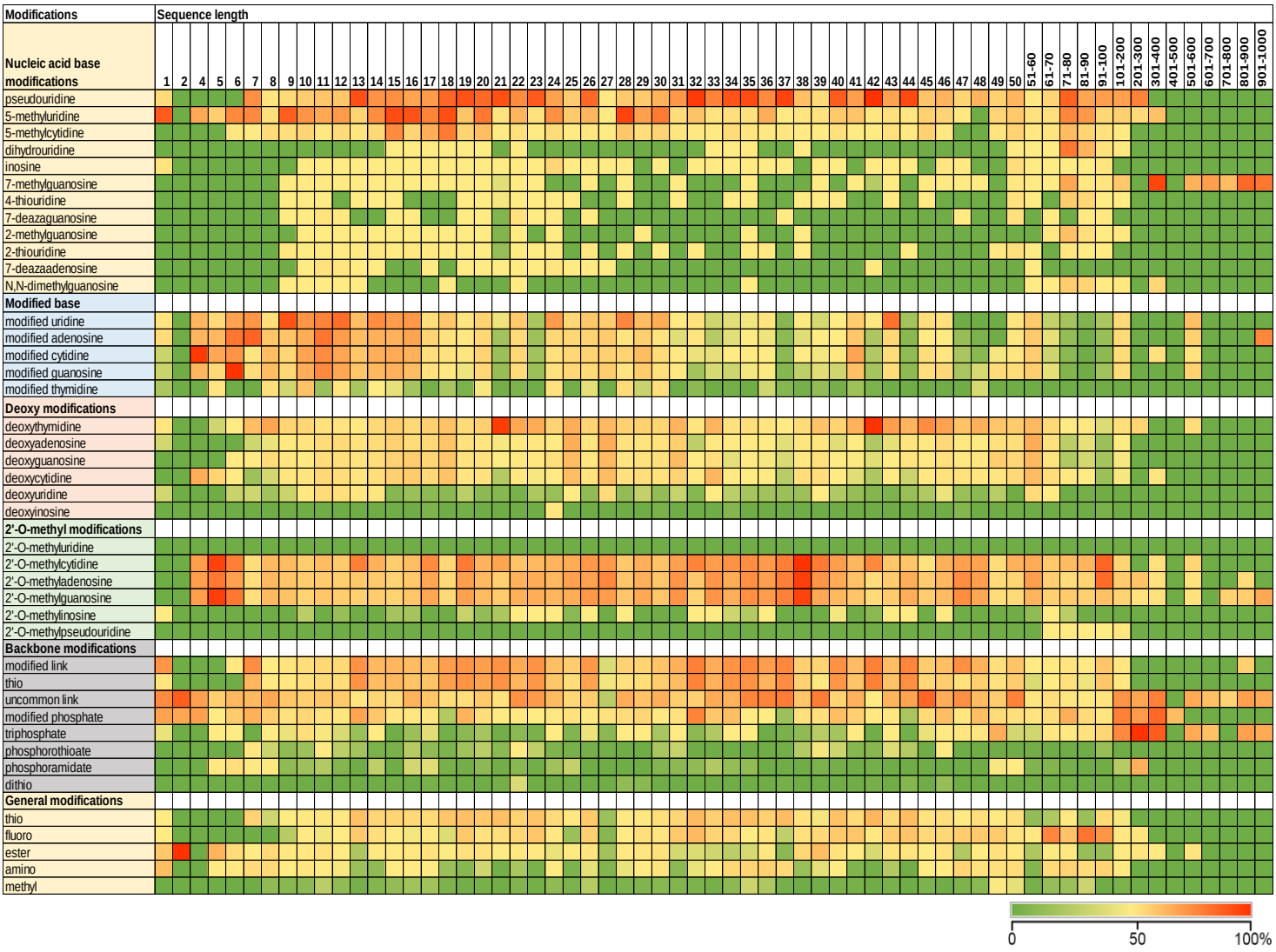


Figure 22. Frequencies of modifications of RNA and their distributions based on sequence lengths (CAS Content Collection).

The dominant nucleobase modifications for RNAs <100 nucleotides long are 5-methyluridine (m^5U) and 5-methylcytosine (m^5C). The rare base pseudouridine appeared frequently in the database, followed by the rare bases dihydrouracil and inosine. Bases in which the N-7 position is converted to a carbon (7-deazaguanine and 7-deazaadenine) appeared with some frequency as did the replacement by sulfur of the oxygen double-bonded to the N-4 or N-2 position of uracil or the N-2 position of cytosine. Less common modifications included benzoyl groups attached to N-3 in cytosine and the nitrogens in adenosine.

Modifications at the C-2’ ribose position are also common, with 2’-O-methyl and 2’-deoxy modifications as the most highly represented and 2’-fluoro and 2’-MOE somewhat less. The prevalence of thymidine (deoxythymidine) at sequence lengths of 21 and 42 is because thymidine at the 3’ end of artificial siRNAs protects them from exonucleases. Artificial siRNAs are predominantly 21-mers, and since they are double-stranded, they are highly represented at both 21 and 42 nucleotides.

Phosphorothioate is the most common phosphate modification for the sugar-phosphate backbone, although phosphorodithioate also occurs. Altered ribose stereochemistry, such as L-ribose in place of D-ribose, is a relatively rare modification, as are replacement backbones, such as PMO, GNA, and PNA.

Heat maps for modifications versus disease targets show little correlation between the disease and the modifications on the therapeutic RNA (Supplemental Figure S3 and S4). The strongest associations between modifications and disease targets are for age-related macular degeneration and pancreatic cancer, and these associations appear to be related to the types of RNA that target these diseases. Age-related macular degeneration (Table 7) and pancreatic cancer (Table 4) are targeted by aptamers, which are extensively modified. However, overall, there is little correlation between the type of RNA and the targeted diseases (Supplemental Figure S1), suggesting any RNA can be explored to target any disease.

Approved RNA medicines show a correlation between the type of RNA and specific modifications (Table 12). The mRNA vaccines BNT162/Comirnaty and mRNA-1273/Spikevax have 7-methylguanosine caps connected to the 5’ end of the mRNA by a triphosphate group and have all their uridines replaced with 1-methylpseudouridine, which improves mRNA stability and translation.

Table 12. The number of RNA modifications in approved RNA therapeutics. The color intensity reflects the number of RNA modifications.

		RNA modifications																	
Approved RNA drug	CAS Registry Number	RNA type	sequence length	deoxythymidine	2'-fluoro, 2'-deoxy	2'-O-methyl	3'-methyl	5'-ester	3'-ester	3'-glycosylated	2'-O-(2-methoxyethyl)	1me-pseudouridine	5-methylcytidine	7-methylguanosine	5-methyluridine	5'-5'-triphosphate	P-thio	modified base	uncommon link	RNA-containing
Kynamro	629167-92-6	ASO	20								10		9		1		19			10
Waylivra	1573402-50-2	ASO	20								10		5		6		19			10
Vitravene	160369-77-7	ASO	21														20			
Exondys 51	1173755-55-9	ASO	30					1							7			30	29	
Vyondys 53	1422959-91-8	ASO	25															25	24	
Tegsedi	1432726-13-0	ASO	20								10		5		4		19			10
Spinraza	1258984-36-9	ASO	18								18		4		7		17			
Leqvio	1639324-58-5	siRNA	44,23,21	1	12	30			1	1							6			
Givlaari	1639325-43-1	siRNA	44,23,21		16	28			1	1							6			
Onpattro	1386913-72-9	siRNA	42,21,21	4		11														
Oxlumo	1834612-06-4	siRNA	44, 23,21		10	34			1	1							6			
Macugen	222716-86-1	Aptamer	28	1	13	12		1											1	
BNT162	2417899-77-3	mRNA	4284			1	1					801		1		1				
mRNA-1273	2430046-03-8	mRNA	4101			1						626		1		1				

Of the approved RNA therapeutics, only ASOs have m^5C and m^5U. The ASOs have either a PMO backbone, a phosphorothioate backbone where all the nucleotides contain 2'-MOE groups, or a phosphorothioate backbone with blocks of nucleotides at the 5' and 3' ends containing 2'-MOE groups and internal 2'-deoxyribonucleotide residues. While the 2'-MOE groups protect the ASO from degradation, they also prevent RNase H digestion of the target mRNA. Thus, an ASO with an internal gap between the protected ends permits RNase H recognition of the target, which is the case for three of the four ASOs with 2'-MOE groups (Table 12). [259] ASOs with PMO backbones (see Figure 21) regulate their targets through steric hindrance, not nuclease digestion. [265] For the seven ASOs in Table 12, five have phosphorothioate backbones and two, Exondys 51 and Vyondys 53, have PMO backbones together with a triethylene glycol extension at their 5' end (Figure 23).

Aptamers have ester linkages to protective groups and can have extensive sugar-phosphate backbone modifications, such as PEGylation (the covalent attachment of polyethylene glycol) on their 5' ends. Because aptamers bind to their targets as a result of their tertiary structure and do not rely on nucleic acid hybridization for function, they have fewer constraints on modifications compared to other therapeutic RNAs. [105] The single FDA-approved aptamer, Macugen, has extensive 2'-O-methyl and 2'-

fluoro modifications, a 3'-to-3' link to thymidine at its 3' end, as well as double PEGylation at its 5' end (Figure 23).

siRNAs have a moderate number of phosphorothioate linkages, with 2'-fluoro and 2'-O-methyl modification of the ribose and are often modified on their 3' ends. The three approved therapeutic siRNAs, Leqvio, Givlaari/Girosiran, and Oxlumo, are 3'-glycosylated with the trivalent GalNAc conjugate [266] shown in Figure 23. This trivalent branched linker containing GalNAc residues targets the siRNAs to hepatocytes to treat liver diseases. [267] The other approved siRNA, Onpattro, has two 3'-terminal thymidine (dT) residues on both RNA strands to protect the siRNA from exonucleases.

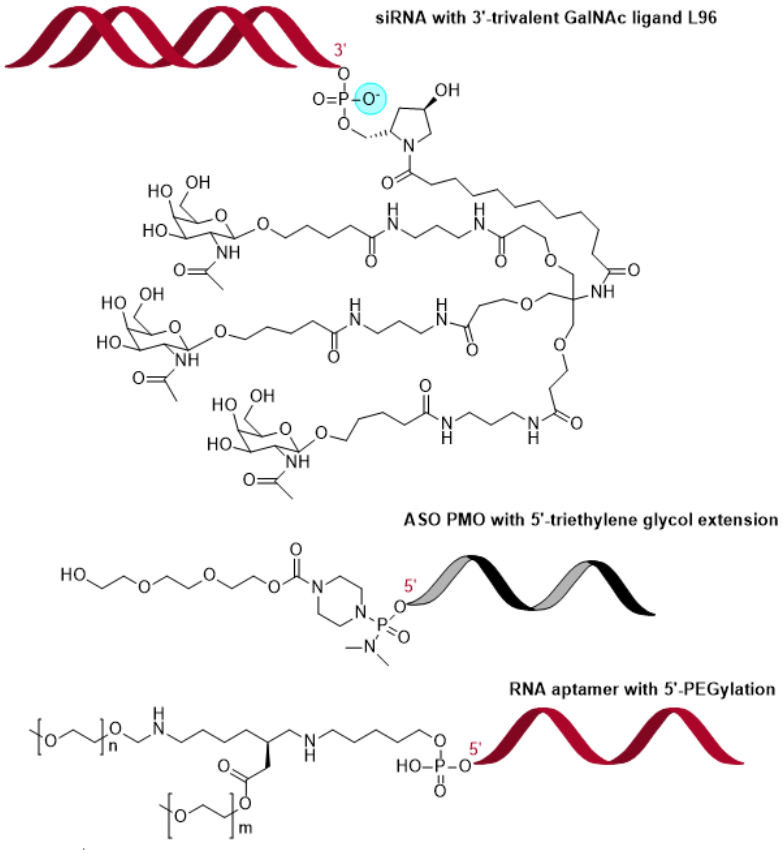


Figure 23. Examples of terminal modifications for therapeutic RNAs. siRNA with 3' tripartite GalNAc ligand L96 (O in the blue circle can be replaced with S); ASO with a PMO backbone (black) and triethylene glycol; RNA aptamer with double 5'-PEGylation. [268-270]

In summary, chemical modifications protect therapeutic RNAs from exonucleases, endonucleases, and the cellular environment, and they enhance pharmaceutical activity. The choice of the backbone determines whether an ASO blocks cellular processes such as translation, transcription, or splicing or targets an RNA for nuclease digestion. The 2'-ribose modifications on siRNAs mitigate off-target effects by lowering the thermal stability, thereby enhancing target-specific binding. 1-methylpseudouridine improves stability and translation of therapeutic mRNAs. Since therapeutic RNAs are extensively modified, they frequently are not designated as RNA vs. DNA but have names such as ASO (antisense oligonucleotide) and siNA (short interfering nucleic acid) for siRNAs. [271]

RNA delivery systems

RNA therapeutics, which are hydrophilic and negatively charged, cannot diffuse across cell membranes; thus, they require delivery vectors and/or chemical modification to reach their targets. When administered systemically, RNA delivery systems need to protect the RNA against serum nucleases, bypass the immune system, avoid non-specific interactions with serum proteins, and block renal clearance. [11] While biological barriers such as immunogenicity and nucleases are usually addressed by modifying the RNA chemically, encapsulation of RNA into nanocarriers can both protect and deliver RNA to cells. [272] Nanomaterials of low toxicity, biodegradability, and biocompatibility are used as RNA carriers. These include lipids, chitosan, cyclodextrin, polyethyleneimine (PEI), poly(lactic-co-glycolic acid), dendrimers, magnetic nanoparticles, carbon nanotubes, gold nanoparticles, silica nanoparticles, and others (Figure 24).

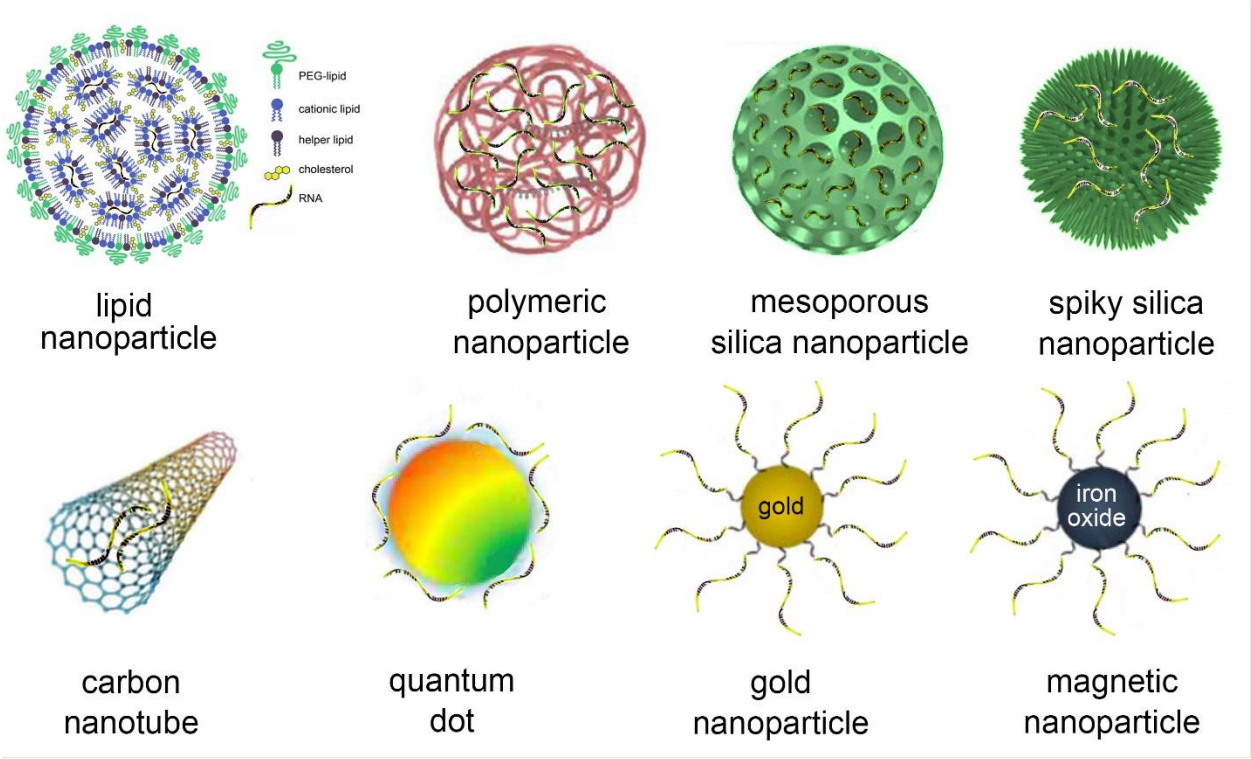


Figure 24. Examples of RNA nanocarriers.

Research publications on RNA delivery systems

Nearly 7,000 scientific publications on RNA delivery systems, including patents and non-patents (journal articles, books, dissertations, meeting abstracts, etc.) are in the CAS Content Collection [83, 273] Publications of studies involving RNA carriers are dominated by lipid nanoparticles, followed closely by polymeric nanocarriers (Figure 25).

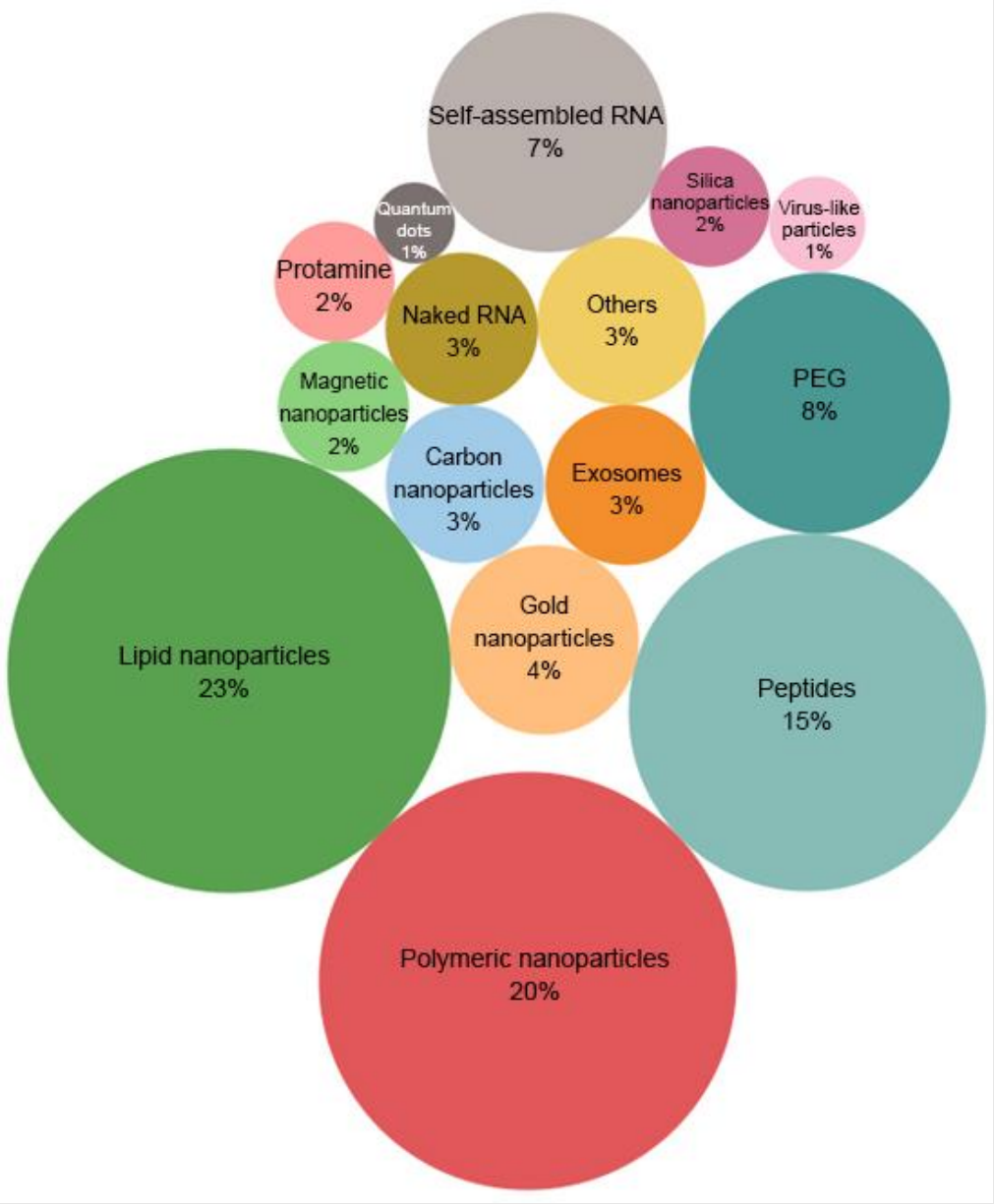


Figure 25. Percentage distribution of RNA nanocarrier-related documents in the CAS Content Collection [83].

Lipid nanoparticles

Lipid nanoparticles, comprising stable complexes between synthetic cationic lipids and anionic nucleic acids, are currently the most widely used non-viral delivery system for nucleic acid drugs and vaccines. [20, 274-276] The advantages of lipid systems include ease of production, biodegradability, protection of entrapped nucleic acids from nuclease degradation and renal clearance, promotion of cellular uptake, and endosomal escape. [277]

Since the first cationic lipids successfully delivered plasmids into cells in 1987, many more have been synthesized and tested as nucleic acid carriers. [20] Cationic lipids differ from natural lipids by having an ionizable (cationic) head group in place of the zwitterionic or anionic head group of the natural lipids. They include two hydrophobic alkyl chains or a cholesterol moiety, a positively charged polar head group, and a linker joining them. Ionizable lipids are positively charged inside the cell but are neutral in the bloodstream due to pH differences, and they are less toxic than nonionizable cationic lipids. [278] Examples of cationic lipid structures used as vectors for gene delivery are provided in Figure 26, and a comprehensive list, including the chemical structures of the~50 most frequently used cationic lipids in drug delivery [83], is available. [276]

The head groups of cationic lipids are typically amine derivatives such as primary, secondary, and tertiary amines (e.g., DOGS, DC-Chol), quaternary ammonium (e.g., DOTMA, DOTAP, DORIE, DMRIE), combinations of amines (e.g., DOSPA, GAP-DLRIE), and amidinium salts (e.g., diC14-amidine) (Figure 26). Guanidine and imidazole groups [279] as well as pyridinium, piperazine, and amino acid headgroups such as lysine, arginine, ornithine, and tryptophan [280, 281] have also been used. Headgroups with multiple cationic charges such as DOSPA, DOGS [282] may be more efficient than single-charged lipids [283] since the multiple charges of the headgroups condense nucleic acids. However multivalent cationic lipids bind strongly to the nucleic acid preventing intracellular release, and they tend to form toxic micelles. [284] Combinations of quaternary amines and polyamines enhance transfection efficiency. The first cationic lipid to combine these two functionalities, Lipofectamine, is the long-standing gold standard in nucleic acid delivery efficiency. It comprises DOSPA formulated with the helper lipid dioleoyl phosphatidylethanolamine (DOPE).[285]

Complexation with cationic lipids stabilizes nucleic acids, protects them from nuclease degradation, and facilitates delivery to the target cells. Lipid nanoparticles carrying nucleic acids adsorb to the cell membrane, are endocytosed, and release the nucleic acids into the cell. Cell membranes have negative charges that attract the cationic lipid nanoparticles and drive the adsorption and fusion of the lipid nanoparticle with the cell membrane. The anionic lipids of the cells are thought to neutralize the charge of the cationic lipid carriers, thereby eliminating the electrostatic interactions between the lipid carriers and the nucleic acids and facilitating the release of the nucleic acids. Neutralization of the cationic lipids also destroys the nanoparticles by promoting the formation of nonlamellar structures [286], which are associated with the efficacy of cationic lipid carriers in delivering nucleic acids into cells.[287] Despite their benefits, lipid nanoparticles show cell toxicity, stimulate the release of systemic inflammatory cytokines, and lipid aggregates may accumulate in the liver and spleen causing hepatotoxicity. [276, 288]

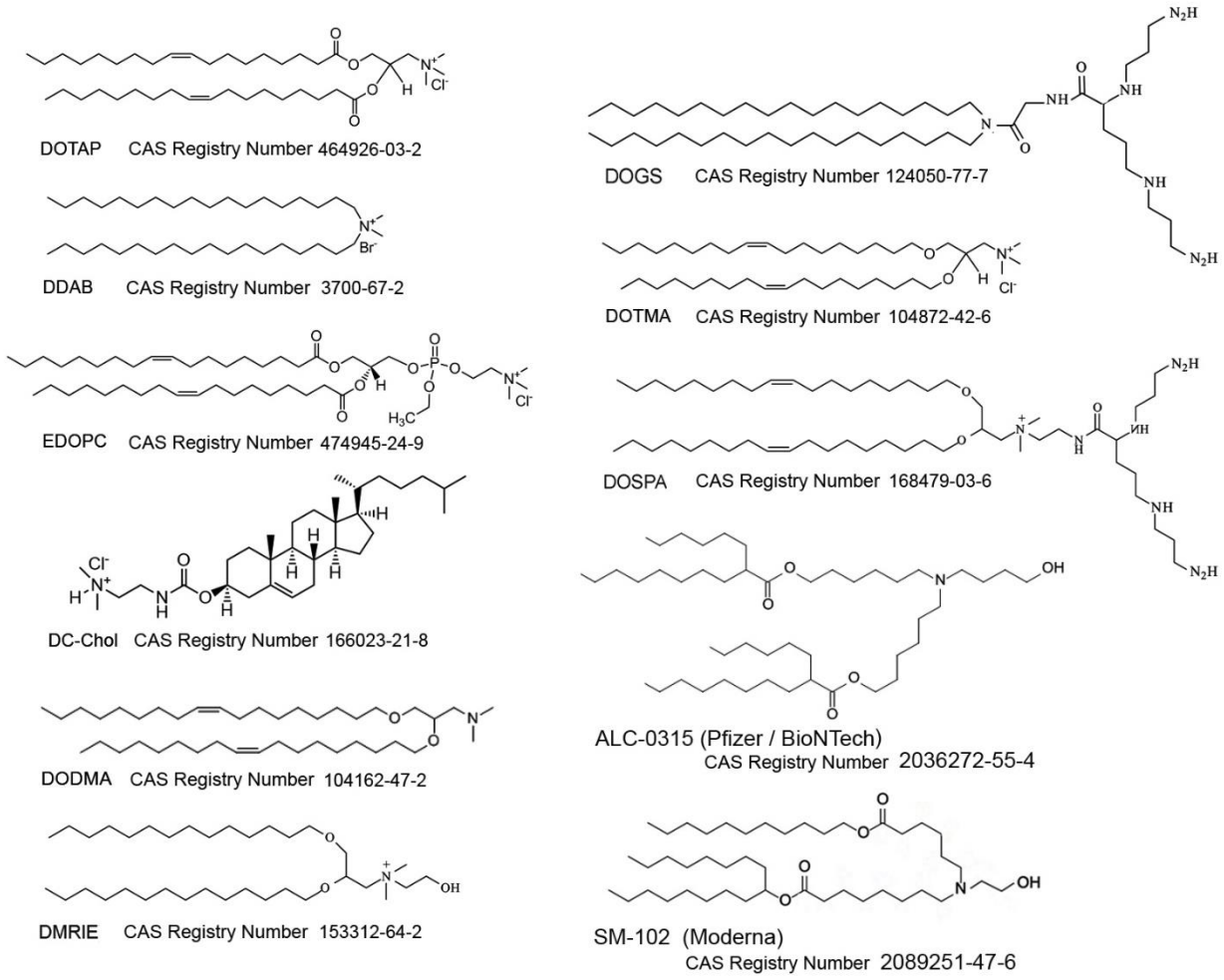


Figure 26. Examples of cationic lipids used as nucleic acid carriers. (An extensive list with the structures of the most frequently used cationic lipids in lipid nanoparticle pharmaceutical formulations according to the CAS Content Collection is available)[276]

Nearly 20 years after scientists first prepared liposomes (the first-generation lipid nanoparticles), they found similar~100 nm extracellular lipid vesicles in most eukaryotic cells. They are exosomes that form naturally in the endosomes and are released from cells normally or as result of some pathologies. They are formed by invaginations of the endosomal membrane and are subsequently released in the extracellular space via fusion with the plasma membrane. [289, 290] Although their functions are largely unknown, they are believed to be associated with important physiological processes such as the regulation of the intercellular communications and signaling, and of transmission of macromolecules between cells. [291] Exosomes, which are secreted by most cells, may be important in intercellular communication and signaling, and in the transport of proteins, lipids, and nucleic acids between cells. Since they are rich in mRNAs and small RNAs and can transport their contents to recipient cells, they are considered good candidates for RNA delivery. Exosome-mediated delivery of β-secretase-1 siRNA (targeting the enzyme β-secretase, which is important in Alzheimer's disease) to the brain resulted in highly efficient β-secretase-1 gene knockdown

in the mouse brain cortex. [292] Electroporation has been applied to load exosomes with exogenous siRNA. [293, 294]

Exosomes exhibit certain advantages over conventional delivery systems. They are natural transporters [295] and hence less likely to be toxic or to cause immune responses. Moreover, exosomes can cross biological barriers such as the blood-brain barrier. The unique membrane composition of exosomes is key to their ability to enter target cells. The membranes of exosomes are enriched in cholesterol and phosphatidylserine. [296] However, vesicles comprising only the lipids from the exosomal membrane are unable to fuse with cells, indicating that exosomal membrane proteins are also important for their activity. [296] A notable advantage of the exosomes as compared to other nanoparticle delivery vehicles is that they do not lead to a harmful accumulation of therapeutic RNAs in the liver. [297, 298]

Polymeric nanoparticles

Polymers comprise the second largest group of nucleic acid delivery vehicles after lipids. Cationic polymers form stable complexes (polyplexes) with anionic nucleic acids and offer a versatile, scalable, and easily adjustable platform for efficient nucleic acid delivery while minimizing immune responses and cellular toxicity. [299]

Linear cationic polymers are the most widely studied polymeric nucleic acid carriers. [300] PEI, poly(L-lysine) (PLL), poly(2-(dimethylamino)ethyl methacrylate) (PDMAEMA), poly 2-aminoethyl methacrylamide (PAEMA), poly(amidoamines) (PAAs), and poly(β-amino esters) (PBAEs) have been studied for drug delivery (Figure 27). These linear polymers differ in their cation types (primary amines such as PLL, secondary amines such as PEI, or tertiary amines such as PDMAEMA), and their cationic charge density.

Block linear copolymers comprising polycationic homopolymers and nonionic hydrophilic blocks compact nucleic acids into polyplex micelles. PEG, hydrophilic acrylamide, acrylate, and methacrylate polymers can arrange the nucleic acid in a micelle core and create a hydrophilic protective shell. For example, the PEG-*b*-PLL diblock copolymer assembles into monodisperse micelles with ASOs. [301]

Branched polycations include a type that has randomly distributed secondary chains branching from the primary polymer backbone. There are also dendrimers—polymers with fractal branching from a core. Nucleic acid carriers include branched PEI, branched PBAEs, as well as PLL, PAMAM, and poly(propyleneimine) (PPI) dendrimers (Figure 27). Branched PEI, one of the most widely studied polycationic nucleic acid carriers, comprises primary, secondary, and tertiary amines, with different pKa values. Branched PEI provides efficient nucleic acid binding and extensive buffering capacity, which likely contribute to its outstanding performance. [302]

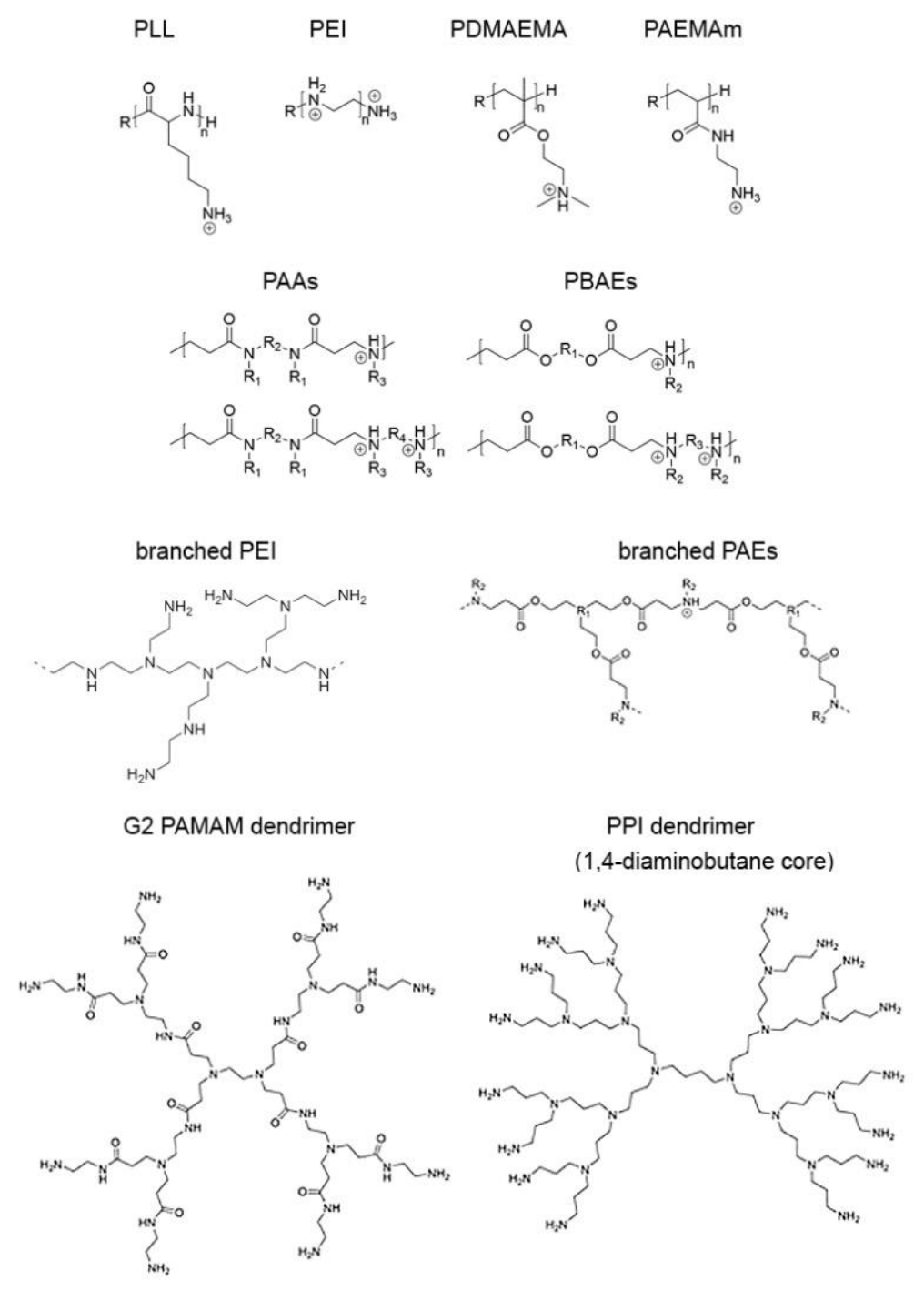


Figure 27. Examples of structures of polymeric nucleic acid carriers.

PAMAM dendrimers, the most widely used dendrimer for nucleic acid delivery, comprise a hydrogen-bonding amide and tertiary amine groups in their cores and primary amine end groups in their coronas. Structural modifications have been attempted to lessen potential toxicity, enhance circulation time, and/or enhance targeting. [303-306]

Natural polymers including proteins and polysaccharides, particularly cationic collagen derivatives and chitosan, have been studied as nucleic acid delivery vehicles. [307] Chitosan, a linear cationic polysaccharide, has been formulated into polyplexes to deliver miRNA to osteopathic tumors, for multiple myeloma, and for bone regeneration. [308-310] An advantage of chitosan is that nucleic acids easily dissociate from the polymer upon cellular internalization. [311] Cationic collagen derivatives have been used for nucleic acid delivery to articular cartilage, for bone regeneration, and for treating tumor metastases. [312-314]

Cyclodextrins (CDs) are natural carbohydrate polymers for the delivery of therapeutic nucleic acids [315] that have shown clinical success. [316]CDs are biocompatible cyclic oligosaccharides with a hydrophilic exterior surface and a hydrophobic interior cavity. Cationic CD derivatives assemble with siRNA via electrostatic interactions into stable gene delivery nanostructures of 50–200 nm. [317]

Similar to cationic lipid nanoparticles, polymeric nucleic acid carriers bind non-specifically to the negatively charged cell membrane via electrostatic interactions [318] and are internalized by endocytosis. [319] Following endocytosis into early endosomes, polyplexes move through the progressively more acidic endosomes (pH 6.0−4.8). Escaping endosomal confinement is critical for nucleic acid delivery; therefore, scientists are developing and modifying polymer-based systems to promote endosomal escape. [318, 320-322]

Peptides

Peptides are structurally and functionally versatile, biocompatible, and can target cells; thus, they are attractive RNA carriers. Peptides that penetrate the cellular membrane and transfer to the cytoplasm, CPPs, are used most frequently for RNA delivery (Table 13). CPPs have variable sequences, lengths, and polarities, and they can enter cells via multiple pathways, such as by forming a hole in the membrane or by endocytosis. CPPs deliver nucleic acids into cells via chemical conjugation or noncovalent complex formation with nucleic acids. Electrostatic and hydrophobic interactions between CPPs and nucleic acids result in self-assembly into peptide-based nanoparticles. [323, 324] Amphipathic CPPs have been developed using hydrophilic and hydrophobic domains to provide both nucleic acid complexation and membrane interactions. [325] CPPs are promising, non-viral alternatives to lipid- or polymer-based carriers of therapeutic nucleic acids. [12]

Table 13. Examples of CPPs used in RNA delivery [326]

CPP	Amino acid sequence
Natural CPPs	
HIV Tat	YGRKKRRQRRR
HIV Rev	TRQARRNRRRRWRERQR
FHV coat	RRRRNRTRRNRRRVR
Penetratin	RQIKIWFQNRRMKWKK
MPG	GALFLGFLGAAGSTMGAWSQPKKKRKV
Polyarginines	
PR9	FFLIPKGRRRRRRRRR
SR9	RRRRRRRRR

IR9	GLFEAIEGFIENGWEGMIDGWYGRRRRRRRRR
HR9	CHHHHHRRRRRRRRRHHHHHC

Artificial/Engineered CPPs

Transportan	CLIKKALAALAKLNIKLLYGASNLTWG
CADY	GLWRALWRLLRSLWRLLWRA
C6	RLLRLLLRLWRRLLRLLR
PF20	LLKLLKKLLKLLKKLLKLL
NAP	KALKLKLALALLAKLKLA
POD	GGG[ARKKAAKA]4

The Tat peptide originated from the Tat protein of the human immunodeficiency virus (HIV). [327-329] The positively charged oligoarginine peptides promote internalization by forming hydrogen bonds with the membrane sulfates and the nucleic acid phosphate groups. [330, 331] The histidine-rich peptide is efficient in siRNA delivery. [332] The skin permeating and cell entering (SPACE) peptides facilitate the penetration of conjugated cargoes into the epidermis and dermis. [330, 331, 333] Recently, fusion peptides were developed comprising SPACE and cationic oligoarginine linked by a GCG sequence. [334] Nanocomplexes of fusion peptides and siRNA exhibited enhanced cellular uptake, gene silencing, and retention, possibly due to the synergistic effect of the oligoarginine and SPACE peptides. Oligoarginine electrostatically attracts siRNAs to form nanocomplexes, and the SPACE peptide interacts with the cellular membrane via hydrogen bonding.

Noncovalent complexation of cationic peptides that comprise hydrophilic and hydrophobic domains with RNA provide efficient gene silencing. [335, 336] The hydrophilic domain of positively charged amino acids, such as arginine, lysine, and histidine, provides a net positive charge of at least +8 that condenses the RNA and promotes hydrogen bonding with the anionic cellular membrane to stimulate cellular uptake. [337] The hydrophobic peptide domain of tryptophan and phenylalanine enhances interactions with the lipid bilayer. Terminal modification of the cationic peptides with hydrophobic molecules such as cholesterol, stearic acid, or cholic acid increases the hydrophobicity. [338, 339] The other terminus of the peptide can be modified with hydrophilic molecules such as PEG. These peptides form a micelle-like structure with siRNA and deliver them efficiently to target cells. [338, 339]

Protamines are naturally occurring cationic peptides involved in the condensation of chromatin during spermatogenesis. Comprising >65% positively charged arginines, protamines form noncovalent electrostatic complexes with nucleic acids and protect them from enzymatic degradation. Although protamines are intrinsically disordered peptides, in the presence of nucleic acids they switch from a random coil to a structure with one or more α-helices. [340] Protamine-RNA complexes show limited efficacy, possibly due to the tight interaction between the protamine and RNA. [341]

Other carriers

Gold nanoparticles (AuNP) carriers are biocompatible, protect RNA from degradation, and have tunable shape, size, and optical properties. [342, 343] Two approaches for the design of AuNP nucleic acid

carriers are covalent attachment and supramolecular assembly. To covalently conjugate RNA with the AuNP core, a thiol group is introduced onto the RNA [344] to create RNA monolayers by linking RNAs directly to the AuNP surface via Au–thiol bonds. The dense shell of oligonucleotides on the surface of the AuNPs inhibits nuclease degradation. Since AuNP-RNA conjugates can be delivered transdermally, they may be useful for gene therapy to treat cutaneous tumors, skin inflammation, and other skin disorders. To electrostatically attract the negatively charged nucleic acids, AuNPs have been functionalized by structurally modifying them adding positively charged molecules such as polymers and amino acids. [345]

Spherical nucleic acids are three-dimensional nucleic acid nanostructures that are functionalized, packed densely, and oriented spherically around a nanoparticle core. [346] Since they are resistant to nuclease degradation and are taken up efficiently by cells they show promise as therapeutic agents. [347] Despite their high negative charge (zeta potential less than −30 mV), they rapidly and efficiently enter cells by caveolin-mediated endocytosis and regulate gene expression. Immunomodulatory spherical nucleic acids that stimulate or regulate immunity by engaging toll-like receptors are a promising immunotherapy for cancers including triple-negative breast cancer, prostate cancer, and melanoma. [348]

Magnetic nanoparticles deliver nucleic acid by magnetofection, which is the application of a magnetic field. [349, 350] The nanoparticles need functionalization (i.e., surface modifications) to enhance their performance as a magnetic gene delivery vector. The earliest magnetic core-shell nanoparticles for gene delivery were iron oxide nanoparticles stabilized with high molecular weight PEI, with a hydrodynamic diameter of ~200 nm and positive zeta potential. [351] Nucleic acids with magnetic nanoparticles increased transfection efficiency by about 1000-fold compared to non-magnetic vectors. A variety of formulations of PEI-coated superparamagnetic iron oxide nanoparticles complexed with nucleic acids have been reported. [352] Other polymers and lipids have also been used to functionalize iron oxide nanoparticles for nucleic acid delivery. [353-357] These include biodegradable polylactide magnetic nanoparticles containing oleate-coated magnetite and surface modified with PEI-oleate for nucleic acid binding [358] and mesoporous silica nanoparticles decorated with magnetite nanocrystals to yield highly efficient transfection agents. [359]

Mesoporous silica nanoparticles (MSN) are hollow particles that can encapsulate a wide range of drugs after surface modification. Silica nanoparticles allow slow release of the cargo as they degrade slowly in the body to nontoxic byproducts. The advantages of MSNs as drug delivery vehicles include a large surface area, tunable pore sizes, simple surface modifications, and efficient encapsulation of cargo molecules. [360] Mesoporous and solid silica nanoparticles are synthesized using the sol-gel method in which a silica precursor is hydrolyzed and then condensed to generate spherical particles. Tetraethyl orthosilicate, the usual silica precursor, provides good control by modification of the synthesis conditions. Silica nanoparticles with a spiky nanotopography can enlarge the surface area for binding RNA molecules. To bind the negatively charged nucleic acids and enhance cellular uptake, silica particles are modified with positively charged compounds such as PLL or PEI, or with branched PEI to enhance the binding of the nucleic acids. [83, 361]. After cell entry by endocytosis, the acidic pH in the endosomes changes the charge of PEI resulting in the dissociation and release of the RNA.

Calcium phosphate nanoparticles are promising non-viral vectors for gene therapy, an emerging strategy for effective bone repair and regeneration. The calcium phosphate naturally present in bones

makes calcium phosphate nanoparticles a good choice for RNA delivery for bone repair. [362] There is particular interest in the use of calcium phosphate nanoparticles with RNAi to regulate gene expression in bone tissue engineering because of their osteoinductivity, osteoconductivity, and affinity for nucleic acids. [363] Calcium phosphate nanoparticles are endocytosed by cells and dissolve in the acidic endosomes and lysosomes resulting in the release of the nucleic acid cargo.[364] Calcium phosphate nanocarriers are biocompatible, biodegradable, non-toxic and non-immunogenic, inexpensive, and easily synthesized. However, they become unstable over time because of the growth of crystals [362], and they provide only limited nuclease protection. Surface functionalization of calcium phosphate particles can facilitate their endocytosis and subsequent endosomal escape. [362, 365]

Although many vehicles or ligands have been used for the delivery of nucleic acids, they may exhibit low immunocompatibility, toxicity, poor stability, inefficient drug release, or restricted tissue accessibility. [366] However, RNA itself can self-assemble into complex programmable structures. [367, 368] Ligand-conjugated RNA nanoparticles based on bacteriophage phi29 packaging RNA provide a targeted ribozyme delivery system. [369] RNA nanoparticles can also carry CpG DNA to macrophages. [368] Origami-like RNA nanostructures are stable and efficient as drug carriers for controlled drug release. [370, 371]

Virus-like particles (VLPs) are organized protein complexes resembling a native virus capsid. VLPs are either naturally occurring empty virus shells or are synthesized by the expression of viral structural proteins that self-assemble into a virus-like structure. VLPs are attractive drug delivery platforms due to their biocompatibility, biodegradability, and targeting ability. [372] VLPs produced *in vivo* are used for various medical applications such as drug and vaccine delivery [373], including the COVID-19 vaccine Novavax (NVX-CoV2373) currently in phase III trials [374]. *In vivo* and *in vitro* cargo loading has been demonstrated for VLPs. [375]

Quantum dots (QDs) are semiconductor crystalline nanoparticles with unique tunable optical properties including nearly 100-fold greater brightness and 1000-fold better stability against photobleaching versus organic dyes and luminescent proteins. Their fluorescence emission can be adjusted by the particle size, known as the quantum size effect. [376] QDs emit narrow wavelength bands under a wide excitation range, can be appropriately functionalized, and are desirable vectors for imaging-guided therapy. Since they can efficiently deliver RNAs into target cells and can be used to track the RNA distribution in cells[377], they may serve as an effective theranostic RNA delivery agent.[378, 379] The application of theranostic agents with appropriate targeted, controlled delivery and imaging capabilities has the potential to significantly advance gene therapy.

Carbon nanotubes (CNTs) – tubes made of carbon with nanosized diameters – have attracted increasing attention in biomedicine because of their unique structures and properties, being the strongest and stiffest materials yet found in terms of tensile strength and elastic modulus. With functionalization, CNTs have been used as nanocarriers for nucleic acids including siRNA, oligonucleotides, and RNA/DNA aptamers. [380] Amino-functionalized multiwalled CNTs deliver proprietary toxic siRNA to human lung carcinoma cells.[381] Appropriately designed amino-functional segments on the CNTs promote internalization in the cell and gene silencing. [382] Cationic single-walled CNTs deliver siRNA to Lewis lung carcinoma cells. [383]

Despite the widespread use of carriers for RNA delivery, naked RNA has also been delivered *in vivo* [384], including delivery of mRNA by intramuscular, subcutaneous, or intradermal injections; the latter

can promote wound healing by *in situ* expression of specific proteins in the skin. [385-389] Local injections circumvent complications associated with systemic administration such as clearance from the bloodstream.[389] Naked mRNA administered subcutaneously produces a more efficient translation of the protein than mRNA-loaded nanoparticles. [390, 391]

PEGylation allows protein drugs to avoid the immune response [392], but it also improves the surface properties of biomolecules and drug delivery systems. It blocks surface access by steric hindrance increasing the circulation time in blood. [393] Thus, it may improve pharmacokinetic properties and enhance the efficacy of drugs, including RNA therapeutics.[394] PEGylation of the RNA nanocarriers reduces nonspecific interactions with serum proteins and prevents recognition by the immune system, increasing the blood circulation time. PEGylation stabilizes RNAs with longer PEGs stabilizing oligonucleotide better than shorter ones. [395] The aptamer drug Macugen comprises an oligonucleotide modified with branched 40 kDa PEG at the 5' terminus, thus enhancing the nuclease resistance of the PEG-aptamer conjugate. PEGylation markedly improves the pharmacokinetics and boosts the neutralizing activity of anti-IL-17A aptamers, an important advance in the development of therapeutic aptamers. [396]

Conclusions

Our understanding of the types and functions of RNA has increased dramatically over the last 50 years. Originally identified simply as coding or non-coding, RNA was recognized early as central to the process of transcribing and translating a gene into a protein. Over the last few decades, many new types of RNA have been discovered with specific functions, including snRNA, snoRNA, shRNA, miRNA, tmRNA, siRNA, saRNA, piRNA, and circRNA. These RNA molecules participate in a complex network that regulates how RNA is used in the cell and modifies gene expression. The multiple functions of RNA provide multiple pathways for exploiting RNA as a therapeutic molecule.

RNA can be engineered for its intended therapeutic functions by *in vitro* transcription or solid-support synthesis. Therapeutic mRNAs, which are translated *in vivo* to deliver a therapeutic or vaccine protein, require extensive replacement of uridine with 1-methylpseudouridine to decrease the immune response to the mRNA and improve the translation. ASOs and therapeutic siRNAs have phosphorothioate and 2'-ribose modifications to protect the RNA from degradation and to improve target specificity. Aptamers, whose 3-D structure rather than primary sequence determines their function, are heavily modified. For all RNAs, the degree of modification can be adjusted to enhance the effectiveness of the therapeutic RNA.

There are now approved RNA medicines for cardiovascular, metabolic, liver, infectious, neurological, and neuromuscular, kidney, and eye diseases with many more in the research phase. Although there are many types of therapeutic RNAs, ASOs dominate the approved drugs followed by siRNAs. CRISPR and AOC therapeutics currently have high research interests still in preclinical stage. The top RNA medicine companies such as Moderna, Ionis, BioNTech, Alnylam, and Sirnaomics all specialize in one type of RNA but research and treat as many as nine different diseases. RNA therapeutics have the potential to treat a wide range of diseases from the most common to the extremely rare.

RNA carriers are important in overcoming the physiological barriers of systemic administration of RNA-based drugs. However, they provide a challenge in the translation of RNA medicines to the clinical setting. After the recent success of the lipid nanoparticle-based mRNA vaccines against COVID-19, lipid carriers have become the primary RNA delivery vehicle. However, since lipid nanoparticles can be toxic to cells and stimulate the release of systemic inflammatory cytokines, interest in natural transporters such as exosomes is gaining momentum. Other nontoxic and nonimmunogenic carriers such as structurally and functionally versatile peptides are also attracting attention. Novel technologies including carbon nanotubes and other inorganic biocompatible polymers, carbon nanodots, and functionalized or hybrid systems are also exciting avenues of exploration and evaluation.

Our growing understanding of the many types and functions of RNA has been combined with the ability to synthesize modified RNAs with improved stability and pharmaceutical activity. Nanotechnology-based systems to deliver those RNAs to the cell have resulted in an explosion of new therapeutic options for diseases ranging from viral infections to cancer. This arsenal of multiple specifically targeted RNA therapies has the potential to revolutionize the treatment of human disease.

Acknowledgments

We sincerely appreciate Zach Baum for help with RNA modification data handling, Laura Czuba for project coordination, and Peter Jap and Cristina Tomeo for insightful discussion. We are also grateful to Manuel Guzman, Gilles Georges, Michael Dennis, Carmin Gade, Dawn George, Cynthia Casebolt, and Hong Xie for executive sponsorship.

Supplemental Information:

1. Figure S1. Number of documents for various RNA types applied in medical studies in the years of 1995-2020, categorized by the type of diseases
2. Figure S2. Top companies with RNA therapeutics and vaccines in clinical trials
3. Figure S3. Frequencies of various types of modifications on RNA sequences and their distributions with respect to disease types obtained from the CAS Content Collection
4. Figure S4. Frequencies of various types of modifications on RNA sequences and their distributions with respect to specific diseases acquired from the CAS Content Collection
5. Figure S5. The co-occurrence of RNA modifications on the same sequences
6. Figure S6. Document numbers per year related to modified RNAs. Data were obtained from a SciFinder^{n} search
7. Figure S7. Modified and rare nucleic acid bases
8. Supplemental Table 1: Timeline and milestones of RNA research and development
9. Supplemental Table 2: RNA therapies and vaccines for various diseases in the development stages

References

1. Titze-de-Almeida, S. S., Brandão, P. R. d. P., Faber, I.,Titze-de-Almeida, R., Leading RNA Interference Therapeutics Part 1: Silencing Hereditary Transthyretin Amyloidosis, with a Focus on Patisiran. *Molecular Diagnosis & Therapy* **2020,** *24*, 49-59.
2. Dolgin, E., INJECTION OF HOPE. *Nature* **2019,** *574*, S10-S12.
3. Ozata, D. M., Gainetdinov, I., Zoch, A., O'Carroll, D.,Zamore, P. D., PIWI-interacting RNAs: small RNAs with big functions. *Nat Rev Genet* **2019,** *20*, 89-108.
4. Ramat, A.,Simonelig, M., Functions of PIWI Proteins in Gene Regulation: New Arrows Added to the piRNA Quiver. *Trends Genet* **2021,** *37*, 188-200.
5. Yoon, S.,Rossi, J. J., Therapeutic Potential of Small Activating RNAs (saRNAs) in Human Cancers. *Curr Pharm Biotechnol* **2018,** *19*, 604-610.
6. Jiang, F.,Doudna, J. A., CRISPR–Cas9 Structures and Mechanisms. *Annual Review of Biophysics* **2017,** *46*, 505-529.
7. Yao, R. W., Wang, Y.,Chen, L. L., Cellular functions of long noncoding RNAs. *Nat Cell Biol* **2019,** *21*, 542-551.
8. Li, X., Yang, L.,Chen, L. L., The Biogenesis, Functions, and Challenges of Circular RNAs. *Mol Cell* **2018,** *71*, 428-442.
9. Wong, E.,Goldberg, T., Mipomersen (kynamro): a novel antisense oligonucleotide inhibitor for the management of homozygous familial hypercholesterolemia. *P & T : a peer-reviewed journal for formulary management* **2014,** *39*, 119-122.
10. Davis, M. E., Zuckerman, J. E., Choi, C. H. J., Seligson, D., Tolcher, A., Alabi, C. A., Yen, Y., Heidel, J. D.,Ribas, A., Evidence of RNAi in humans from systemically administered siRNA via targeted nanoparticles. *Nature* **2010,** *464*, 1067-1070.
11. Whitehead, K. A., Langer, R.,Anderson, D. G., Knocking down barriers: advances in siRNA delivery. *Nat Rev Drug Discov* **2009,** *8*, 129-38.
12. Roberts, T. C., Langer, R.,Wood, M. J. A., Advances in oligonucleotide drug delivery. *Nature Reviews Drug Discovery* **2020,** *19*, 673-694.
13. Brenner, S., Meselson, M.,Jacob, F., An unstable intermediate carrying information from genes to ribosomes for protein synthesis. *Nature* **1961,** *190*, 576-+.
14. Muthukrishnan, S., Both, G. W., Furuichi, Y.,Shatkin, A. J., 5'-Terminal 7-methylguanosine in eukaryotic messenger-RNA is required for translation. *Nature* **1975,** *255*, 33-37.
15. Furuichi, Y.,Miura, K. I., Blocked structure at 5' terminus of messenger-RNA from cytoplasmic polyhedrosis-virus. *Nature* **1975,** *253*, 374-375.
16. Papahadjopoulos, D., Vail, W. J., Jacobson, K.,Poste, G., Cochleate lipid cylinders: formation by fusion of unilamellar lipid vesicles. *Biochim Biophys Acta* **1975,** *394*, 483-91.
17. Dimitriadis, G. J., Translation of rabbit globin mRNA introduced by liposomes into mouse lymphocytes. *Nature* **1978,** *274*, 923-924.
18. Zamecnik, P. C.,Stephenson, M. L., Inhibition of Rous sarcoma virus replication and cell transformation by a specific oligodeoxynucleotide. *Proceedings of the National Academy of Sciences of the United States of America* **1978,** *75*, 280-284.
19. Krieg, P. A.,Melton, D. A., Functional messenger-RNAs are produced by SP6 invitro transcription of cloned cDNAs. *Nucleic Acids Research* **1984,** *12*, 7057-7070.
20. Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M.,Danielsen, M., Lipofection - A Highly Efficient, Lipid-Mediated DNA-Transfection Procedure. *Proceedings of the National Academy of Sciences of the United States of America* **1987,** *84*, 7413-7417.
21. Malone, R. W., Felgner, P. L.,Verma, I. M., Cationic liposome-mediated RNA transfection. *Proceedings of the National Academy of Sciences of the United States of America* **1989,** *86*, 6077-6081.

22. Fire, A., Xu, S. Q., Montgomery, M. K., Kostas, S. A., Driver, S. E.,Mello, C. C., Potent and specific genetic interference by double-stranded RNA in Caenorhabditis elegans. *Nature* **1998,** *391*, 806-811.
23. Piascik, P., Fomiversen sodium approved to treat CMV retinitis. *J Am Pharm Assoc (Wash)* **1999,** *39*, 84-5.
24. Bernstein, E., Caudy, A. A., Hammond, S. M.,Hannon, G. J., Role for a bidentate ribonuclease in the initiation step of RNA interference. *Nature* **2001,** *409*, 363-366.
25. Kariko, K., Buckstein, M., Ni, H. P.,Weissman, D., Suppression of RNA recognition by Toll-like receptors: The impact of nucleoside modification and the evolutionary origin of RNA. *Immunity* **2005,** *23*, 165-175.
26. Weide, B., Pascolo, S., Scheel, B., Derhovanessian, E., Pflugfelder, A., Eigentler, T. K., Pawelec, G., Hoerr, I., Rammensee, H.-G.,Garbe, C., Direct Injection of Protamine-protected mRNA: Results of a Phase 1/2 Vaccination Trial in Metastatic Melanoma Patients. *Journal of Immunotherapy* **2009,** *32*.
27. Adams, D., Gonzalez-Duarte, A., O'Riordan, W. D., Yang, C. C., Ueda, M., Kristen, A. V., Tournev, I., Schmidt, H. H., Coelho, T., Berk, J. L., Lin, K. P., Vita, G., Attarian, S., Planté-Bordeneuve, V., Mezei, M. M., Campistol, J. M., Buades, J., Brannagan, T. H., 3rd, Kim, B. J., Oh, J., Parman, Y., Sekijima, Y., Hawkins, P. N., Solomon, S. D., Polydefkis, M., Dyck, P. J., Gandhi, P. J., Goyal, S., Chen, J., Strahs, A. L., Nochur, S. V., Sweetser, M. T., Garg, P. P., Vaishnaw, A. K., Gollob, J. A.,Suhr, O. B., Patisiran, an RNAi Therapeutic, for Hereditary Transthyretin Amyloidosis. *N Engl J Med* **2018,** *379*, 11-21.
28. Vaccines and Related Biological Products Advisory Committee Meeting. Moderna COVID-19 Vaccine. FDA Briefing Document. https://www.fda.gov/media/144434/download (accessed December 22, 2020).
29. Emergency use authorization (EUA) of the Pfizer-BioNTech COVID-19 vaccine to prevent coronavirus disease 2019 (COVID-19) in individuals 16 years of age and older. https://www.fda.gov/media/144414/download (accessed December 22, 2020).
30. FDA Approves First COVID-19 Vaccine. https://www.fda.gov/news-events/press-announcements/fda-approves-first-covid-19-vaccine (accessed Dec 27, 2021).
31. Castanotto, D., Zhang, X., Rüger, J., Alluin, J., Sharma, R., Pirrotte, P., Joenson, L., Ioannou, S., Nelson, M. S., Vikeså, J., Hansen, B. R., Koch, T., Jensen, M. A., Rossi, J. J.,Stein, C. A., A Multifunctional LNA Oligonucleotide-Based Strategy Blocks AR Expression and Transactivation Activity in PCa Cells. *Molecular Therapy - Nucleic Acids* **2021,** *23*, 63-75.
32. Cooper, G. M., RNA Synthesis and Processing. In *The Cell: A Molecular Approach.*, Sinauer Associates: Sunderland, MA, 2000.
33. Alberts, B., Johnson, A., Lewis, J.,al., e., How Cells Read the Genome: From DNA to Protein. In *Molecular Biology of the Cell*, 4th Edition ed.; Garland Science: New York, 2002.
34. Valadkhan, S.,Gunawardane, L. S., Role of small nuclear RNAs in eukaryotic gene expression. *Essays Biochem* **2013,** *54*, 79-90.
35. Matera, A. G., Terns, R. M.,Terns, M. P., Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs. *Nat Rev Mol Cell Biol* **2007,** *8*, 209-20.
36. Morais, P., Adachi, H.,Yu, Y. T., Spliceosomal snRNA Epitranscriptomics. *Front Genet* **2021,** *12*, 652129.
37. Wajahat, M., Bracken, C. P.,Orang, A., Emerging Functions for snoRNAs and snoRNA-Derived Fragments. *Int J Mol Sci* **2021,** *22*.
38. van der Werf, J., Chin, C. V.,Fleming, N. I., SnoRNA in Cancer Progression, Metastasis and Immunotherapy Response. *Biology (Basel)* **2021,** *10*.
39. Liang, J., Wen, J., Huang, Z., Chen, X. P., Zhang, B. X.,Chu, L., Small Nucleolar RNAs: Insight Into Their Function in Cancer. *Front Oncol* **2019,** *9*, 587.
40. Roy, K.,Chanfreau, G. F., The Diverse Functions of Fungal RNase III Enzymes in RNA Metabolism. *Enzymes* **2012,** *31*, 213-35.

41. Williams, G. T.,Farzaneh, F., Are snoRNAs and snoRNA host genes new players in cancer? *Nat Rev Cancer* **2012,** *12*, 84-8.
42. Holley, C. L.,Topkara, V. K., An introduction to small non-coding RNAs: miRNA and snoRNA. *Cardiovasc Drugs Ther* **2011,** *25*, 151-9.
43. Bachellerie, J. P., Cavaillé, J.,Hüttenhofer, A., The expanding snoRNA world. *Biochimie* **2002,** *84*, 775-90.
44. Ro-Choi, T. S., Nucleolar snoRNA and ribosome production. *Mol Cells* **1997,** *7*, 451-67.
45. Bridges, M. C., Daulagala, A. C.,Kourtidis, A., LNCcation: lncRNA localization and function. *J Cell Biol* **2021,** *220*.
46. Ferrè, F., Colantoni, A.,Helmer-Citterich, M., Revealing protein-lncRNA interaction. *Brief Bioinform* **2016,** *17*, 106-16.
47. Jathar, S., Kumar, V., Srivastava, J.,Tripathi, V., Technological Developments in lncRNA Biology. *Adv Exp Med Biol* **2017,** *1008*, 283-323.
48. Li, J., Meng, H., Bai, Y.,Wang, K., Regulation of lncRNA and Its Role in Cancer Metastasis. *Oncol Res* **2016,** *23*, 205-17.
49. Peng, W. X., Koirala, P.,Mo, Y. Y., LncRNA-mediated regulation of cell signaling in cancer. *Oncogene* **2017,** *36*, 5661-5667.
50. Statello, L., Guo, C. J., Chen, L. L.,Huarte, M., Gene regulation by long non-coding RNAs and its biological functions. *Nat Rev Mol Cell Biol* **2021,** *22*, 96-118.
51. Yang, Z., Jiang, S., Shang, J., Jiang, Y., Dai, Y., Xu, B., Yu, Y., Liang, Z.,Yang, Y., LncRNA: Shedding light on mechanisms and opportunities in fibrosis and aging. *Ageing Res Rev* **2019,** *52*, 17-31.
52. Backes, C., Meese, E.,Keller, A., Specific miRNA Disease Biomarkers in Blood, Serum and Plasma: Challenges and Prospects. *Mol Diagn Ther* **2016,** *20*, 509-518.
53. Bernardo, B. C., Ooi, J. Y., Lin, R. C.,McMullen, J. R., miRNA therapeutics: a new class of drugs with potential therapeutic applications in the heart. *Future Med Chem* **2015,** *7*, 1771-92.
54. Cai, Y., Yu, X., Hu, S.,Yu, J., A brief review on the mechanisms of miRNA regulation. *Genomics Proteomics Bioinformatics* **2009,** *7*, 147-54.
55. Cao, T.,Zhen, X. C., Dysregulation of miRNA and its potential therapeutic application in schizophrenia. *CNS Neurosci Ther* **2018,** *24*, 586-597.
56. Lee, Y. S.,Dutta, A., MicroRNAs in cancer. *Annu Rev Pathol* **2009,** *4*, 199-227.
57. Lu, T. X.,Rothenberg, M. E., MicroRNA. *J Allergy Clin Immunol* **2018,** *141*, 1202-1207.
58. Tiwari, A., Mukherjee, B.,Dixit, M., MicroRNA Key to Angiogenesis Regulation: MiRNA Biology and Therapy. *Curr Cancer Drug Targets* **2018,** *18*, 266-277.
59. Piatek, M. J.,Werner, A., Endogenous siRNAs: regulators of internal affairs. *Biochem Soc Trans* **2014,** *42*, 1174-9.
60. Crooke, S. T., Witztum, J. L., Bennett, C. F.,Baker, B. F., RNA-Targeted Therapeutics. *Cell Metab* **2018,** *27*, 714-739.
61. Schütze, N., siRNA technology. *Mol Cell Endocrinol* **2004,** *213*, 115-9.
62. Rossi, J. J.,Rossi, D. J., siRNA Drugs: Here to Stay. *Mol Ther* **2021,** *29*, 431-432.
63. Pushparaj, P. N., Aarthi, J. J., Manikandan, J.,Kumar, S. D., siRNA, miRNA, and shRNA: in vivo applications. *J Dent Res* **2008,** *87*, 992-1003.
64. Qureshi, A., Tantray, V. G., Kirmani, A. R.,Ahangar, A. G., A review on current status of antiviral siRNA. *Rev Med Virol* **2018,** *28*, e1976.
65. Hu, B., Weng, Y., Xia, X. H., Liang, X. J.,Huang, Y., Clinical advances of siRNA therapeutics. *J Gene Med* **2019,** *21*, e3097.
66. Gavrilov, K.,Saltzman, W. M., Therapeutic siRNA: principles, challenges, and strategies. *Yale J Biol Med* **2012,** *85*, 187-200.

67. Hu, B., Zhong, L., Weng, Y., Peng, L., Huang, Y., Zhao, Y.,Liang, X. J., Therapeutic siRNA: state of the art. *Signal Transduct Target Ther* **2020,** *5*, 101.
68. Singh, A., Trivedi, P.,Jain, N. K., Advances in siRNA delivery in cancer therapy. *Artif Cells Nanomed Biotechnol* **2018,** *46*, 274-283.
69. Nikam, R. R.,Gore, K. R., Journey of siRNA: Clinical Developments and Targeted Delivery. *Nucleic Acid Ther* **2018,** *28*, 209-224.
70. Saw, P. E.,Song, E. W., siRNA therapeutics: a clinical reality. *Sci China Life Sci* **2020,** *63*, 485-500.
71. Czech, B., Munafò, M., Ciabrelli, F., Eastwood, E. L., Fabry, M. H., Kneuss, E.,Hannon, G. J., piRNA-Guided Genome Defense: From Biogenesis to Silencing. *Annu Rev Genet* **2018,** *52*, 131-157.
72. Ishizu, H., Nagao, A.,Siomi, H., Gatekeepers for Piwi-piRNA complexes to enter the nucleus. *Curr Opin Genet Dev* **2011,** *21*, 484-90.
73. Iwasaki, Y. W., Siomi, M. C.,Siomi, H., PIWI-Interacting RNA: Its Biogenesis and Functions. *Annu Rev Biochem* **2015,** *84*, 405-33.
74. Lenart, P., Novak, J.,Bienertova-Vasku, J., PIWI-piRNA pathway: Setting the pace of aging by reducing DNA damage. *Mech Ageing Dev* **2018,** *173*, 29-38.
75. Liu, Y., Dou, M., Song, X., Dong, Y., Liu, S., Liu, H., Tao, J., Li, W., Yin, X.,Xu, W., The emerging role of the piRNA/piwi complex in cancer. *Mol Cancer* **2019,** *18*, 123.
76. Mani, S. R.,Juliano, C. E., Untangling the web: the diverse functions of the PIWI/piRNA pathway. *Mol Reprod Dev* **2013,** *80*, 632-64.
77. Yuan, Z. H.,Zhao, Y. M., The regulatory functions of piRNA/PIWI in spermatogenesis. *Yi Chuan* **2017,** *39*, 683-691.
78. Chang, W.,Wang, J., Exosomes and Their Noncoding RNA Cargo Are Emerging as New Modulators for Diabetes Mellitus. *Cells* **2019,** *8*.
79. Chu, Y. L., Li, H., Ng, P. L. A., Kong, S. T., Zhang, H., Lin, Y., Tai, W. C. S., Yu, A. C. S., Yim, A. K. Y., Tsang, H. F., Cho, W. C. S.,Wong, S. C. C., The potential of circulating exosomal RNA biomarkers in cancer. *Expert Rev Mol Diagn* **2020,** *20*, 665-678.
80. Mathivanan, S., Ji, H.,Simpson, R. J., Exosomes: extracellular organelles important in intercellular communication. *J Proteomics* **2010,** *73*, 1907-20.
81. Xie, Y., Dang, W., Zhang, S., Yue, W., Yang, L., Zhai, X., Yan, Q.,Lu, J., The role of exosomal noncoding RNAs in cancer. *Mol Cancer* **2019,** *18*, 37.
82. Zhang, J., Li, S., Li, L., Li, M., Guo, C., Yao, J.,Mi, S., Exosome and exosomal microRNA: trafficking, sorting, and function. *Genomics Proteomics Bioinformatics* **2015,** *13*, 17-24.
83. CAS Content Collection. https://www.cas.org/about/cas-content (accessed November 25, 2021).
84. Crick, F. H., On protein synthesis. *Symp Soc Exp Biol* **1958,** *12*, 138-63.
85. Palade , G. E., A SMALL PARTICULATE COMPONENT OF THE CYTOPLASM. *The Journal of Biophysical and Biochemical Cytology* **1955,** *1*, 59-68.
86. Holley, R. W., Apgar, J., Everett, G. A., Madison, J. T., Marquisee, M., Merrill, S. H., Penswick, J. R.,Zamir, A., Structure of a Ribonucleic Acid. *Science* **1965,** *147*, 1462-1465.
87. Esteller, M., Non-coding RNAs in human disease. *Nat Rev Genet* **2011,** *12*, 861-74.
88. Hombach, S.,Kretz, M., Non-coding RNAs: Classification, Biology and Functioning. *Adv Exp Med Biol* **2016,** *937*, 3-17.
89. Yang, J. X., Rastetter, R. H.,Wilhelm, D., Non-coding RNAs: An Introduction. *Adv Exp Med Biol* **2016,** *886*, 13-32.
90. Xu, J., Bai, J.,Xiao, J., Computationally Modeling ncRNA-ncRNA Crosstalk. *Adv Exp Med Biol* **2018,** *1094*, 77-86.
91. Liu, N., Wang, Z. Z., Zhao, M., Zhang, Y.,Chen, N. H., Role of non-coding RNA in the pathogenesis of depression. *Gene* **2020,** *735*, 144276.

92. Anastasiadou, E., Jacob, L. S.,Slack, F. J., Non-coding RNA networks in cancer. *Nat Rev Cancer* **2018,** *18*, 5-18.
93. Panni, S., Lovering, R. C., Porras, P.,Orchard, S., Non-coding RNA regulatory networks. *Biochim Biophys Acta Gene Regul Mech* **2020,** *1863*, 194417.
94. Mattick, J. S.,Makunin, I. V., Non-coding RNA. *Hum Mol Genet* **2006,** *15 Spec No 1*, R17-29.
95. Jiao, A. L.,Slack, F. J., RNA-mediated gene activation. *Epigenetics* **2014,** *9*, 27-36.
96. Kwok, A., Raulf, N.,Habib, N., Developing small activating RNA as a therapeutic: current challenges and promises. *Ther Deliv* **2019,** *10*, 151-164.
97. Laham-Karam, N., Laitinen, P., Turunen, T. A.,Ylä-Herttuala, S., Activating the Chromatin by Noncoding RNAs. *Antioxid Redox Signal* **2018,** *29*, 813-831.
98. Li, L. C., Small RNA-Guided Transcriptional Gene Activation (RNAa) in Mammalian Cells. *Adv Exp Med Biol* **2017,** *983*, 1-20.
99. Li, S. M.,Hu, J., Small Activating RNAs: Towards Development of New Therapeutic Agents and Clinical Treatments. *Curr Pharm Biotechnol* **2018,** *19*, 622-630.
100. Zheng, L., Wang, L., Gan, J.,Zhang, H., RNA activation: promise as a new weapon against cancer. *Cancer Lett* **2014,** *355*, 18-24.
101. Zhao, X., Voutila, J., Habib, N.,al., e., RNA Activation. In *Innovative Medicine: Basic Research and Development* Nakao, K.; Minato, N.; Uemoto, S., Eds. Springer: Tokyo, 2015.
102. Yu, C. Y.,Kuo, H. C., The emerging roles and functions of circular RNAs and their generation. *J Biomed Sci* **2019,** *26*, 29.
103. Heiser, A., Coleman, D., Dannull, J., Yancey, D., Maurice, M. A., Lallas, C. D., Dahm, P., Niedzwiecki, D., Gilboa, E.,Vieweg, J., Autologous dendritic cells transfected with prostate-specific antigen RNA stimulate CTL responses against metastatic prostate tumors. *J Clin Invest* **2002,** *109*, 409-17.
104. Sahin, U., Karikó, K.,Türeci, Ö., mRNA-based therapeutics — developing a new class of drugs. *Nature Reviews Drug Discovery* **2014,** *13*, 759-780.
105. Damase, T. R., Sukhovershin, R., Boada, C., Taraballi, F., Pettigrew, R. I.,Cooke, J. P., The Limitless Future of RNA Therapeutics. *Frontiers in Bioengineering and Biotechnology* **2021,** *9*.
106. Hanna, J., Hossain, G. S.,Kocerha, J., The Potential for microRNA Therapeutics and Clinical Research. *Frontiers in Genetics* **2019,** *10*.
107. Bobba, C. M., Fei, Q., Shukla, V., Lee, H., Patel, P., Putman, R. K., Spitzer, C., Tsai, M., Wewers, M. D., Lee, R. J., Christman, J. W., Ballinger, M. N., Ghadiali, S. N.,Englert, J. A., Nanoparticle delivery of microRNA-146a regulates mechanotransduction in lung macrophages and mitigates injury during mechanical ventilation. *Nature Communications* **2021,** *12*, 289.
108. Dovgan, I., Koniev, O., Kolodych, S.,Wagner, A., Antibody–Oligonucleotide Conjugates as Therapeutic, Imaging, and Detection Agents. *Bioconjugate Chemistry* **2019,** *30*, 2483-2501.
109. DeLong, R., Ushering in a new era of RNA-based therapies. *Communications Biology* **2021,** *4*, 577.
110. LIU, D., R., LEVY, J., Ma, YEH, W., Hsi, COMANDER, J., TACHIDA, Y., PIERCE, E., A., LIU, Q.,PENDSE, N. Targeted base editing of the USH2A gene. WO2021222318, 2021.
111. Darmostuk, M., Rimpelova, S., Gbelcova, H.,Ruml, T., Current approaches in SELEX: An update to aptamer selection technology. *Biotechnology Advances* **2015,** *33*, 1141-1161.
112. Lamberti, G.,Barba, A. A., Drug Delivery of siRNA Therapeutics. *Pharmaceutics* **2020,** *12*, 178.
113. Saeedi, S., Israel, S., Nagy, C.,Turecki, G., The emerging role of exosomes in mental disorders. *Translational Psychiatry* **2019,** *9*, 122.
114. Condrat, C. E., Thompson, D. C., Barbu, M. G., Bugnar, O. L., Boboc, A., Cretoiu, D., Suciu, N., Cretoiu, S. M.,Voinea, S. C., miRNAs as Biomarkers in Disease: Latest Findings Regarding Their Role in Diagnosis and Prognosis. *Cells* **2020,** *9*, 276.

115. Tribolet, L., Kerr, E., Cowled, C., Bean, A. G. D., Stewart, C. R., Dearnley, M.,Farr, R. J., MicroRNA Biomarkers for Infectious Diseases: From Basic Research to Biosensing. *Frontiers in Microbiology* **2020,** *11*.
116. Ravkin, H. D., Givton, O., Geffen, D. B.,Rubin, E., Direct comparison shows that mRNA-based diagnostics incorporate information which cannot be learned directly from genomic mutations. *BMC Bioinformatics* **2020,** *21*, 196.
117. Kaminski, M. M., Abudayyeh, O. O., Gootenberg, J. S., Zhang, F.,Collins, J. J., CRISPR-based diagnostics. *Nature Biomedical Engineering* **2021,** *5*, 643-656.
118. Mermade 192X Synthesizer. https://shop.biosearchtech.com/nucleic-acid-chemistry-reagents-and-instruments/dna-and-rna-synthesis-instruments-and-accessories/dna-and-rna-oligonucleotide-synthesizers/mermade-192x/mermade-192x-synthesizer/p/NACINS-007 (accessed 20 Nov 2021).
119. Tremblay, F., Bondurant, L. D., McIninch, J. D., Castoreno, A., Schlegel, M. K.,Kaittanis, C. New double stranded RNA agent capable of inhibiting expression of apolipoprotein C3 in cell, used for treating subject having disorder e.g. hypertriglyceridemia, non-alcoholic fatty liver disease, and obesity that would benefit from reduction in apolipoprotein C3 expression. WO2021167841-A1; US2021292756-A1; US11162103-B2.
120. The Basics: In Vitro Transcription. https://www.thermofisher.com/us/en/home/references/ambion-tech-support/probe-labeling-systems/general-articles/the-basics-in-vitro-transcription.html (accessed 25 Nov 2021).
121. Moderna. https://www.modernatx.com/about-us (accessed 20 Nov 2021).
122. BioNTech. https://biontech.de/ (accessed 20 Nov 2021).
123. CureVac. https://www.curevac.com/en/ (accessed 20 Nov 2021).
124. Stemirna Therapeutics. https://www.stemirna.com/en/about/index.aspx (accessed 20 Nov 2021).
125. Cartesian Therapeutics. https://www.cartesiantherapeutics.com/rna-cell-therapy/ (accessed 20 Nov 2021).
126. Alnylam https://www.alnylam.com/about-alnylam/ (accessed 20 Nov 2021).
127. Dicerna https://dicerna.com/ (accessed 20 Nov 2021).
128. Sirnaomics https://sirnaomics.com/overview/ (accessed 20 Nov 2021).
129. Arrowhead Pharmaceuticals. https://arrowheadpharma.com/about/ (accessed 20 Nov 2021).
130. Silence Therapeutics. https://silence-therapeutics.com/about-us/default.aspx (accessed 20 Nov 2021).
131. Ionis: A force for life. https://www.ionispharma.com/about/ (accessed 20 Niv 2021).
132. Sarepta Therapeutics. https://www.sarepta.com/about-us (accessed 20 Nov 2021).
133. NOXXON Pharma. https://www.noxxon.com/ (accessed 20 Nov 2021).
134. Beam Therapeutics. https://beamtx.com/ (accessed 20 Nov 2021).
135. AstraZeneca. https://www.astrazeneca.com/our-company.html (accessed 20 Nov 2021).
136. CureVac pipeline. https://www.curevac.com/en/pipeline/ (accessed 20 Nov 2021).
137. Cardiovascular diseases. https://www.who.int/health-topics/cardiovascular-diseases#tab=tab_1 (accessed 20 Nov 2021).
138. KYNAMRO- mipomersen sodium injection, solution. https://www.kynamro.com/media/pdfs/Kynamro_Prescribing_information.pdf (accessed 20 Nov 2021).
139. FDA approves Novartis Leqvio® (inclisiran), first-in-class siRNA to lower cholesterol and keep it low with two doses a year. https://www.novartis.com/news/media-releases/fda-approves-novartis-leqvio-inclisiran-first-class-sirna-lower-cholesterol-and-keep-it-low-two-doses-year (accessed Dec 27, 2021).
140. Delivery Platforms. https://www.alnylam.com/our-science/sirna-delivery-platforms/ (accessed 20 Nov 2021).

141. Study to Investigate Safety, Tolerability, PK and PD Response of SLN360 in Subjects With Elevated Lipoprotein(a). https://clinicaltrials.gov/ct2/show/NCT04606602?term=NCT04606602&draw=2&rank=1 (accessed 20 Nov 2021).
142. Alnylam pipeline. https://www.alnylam.com/alnylam-rnai-pipeline/ (accessed 20 Nov 2021).
143. Zilebesiran (ALN-AGT), in Development for the Treatment of Hypertension. https://www.alnylam.com/wp-content/uploads/2021/06/2021-RNAi-Roundtables-Zilebesiran_FINAL.pdf (accessed 20 Nov 2021).
144. Moderna's pipeline. https://www.modernatx.com/pipeline (accessed 20 Nov 2021).
145. Anttila, V., Saraste, A., Knuuti, J., Jaakkola, P., Hedman, M., Svedlund, S., Lagerström-Fermér, M., Kjaer, M., Jeppsson, A.,Gan, L.-M., Synthetic mRNA Encoding VEGF-A in Patients Undergoing Coronary Artery Bypass Grafting: Design of a Phase 2a Clinical Trial. *Molecular Therapy - Methods & Clinical Development* **2020,** *18*, 464-472.
146. Taimeh, Z., Loughran, J., Birks, E. J.,Bolli, R., Vascular endothelial growth factor in heart failure. *Nat Rev Cardiol* **2013,** *10*, 519-30.
147. Genzyme and Isis Announce FDA Approval of KYNAMRO™ (mipomersen sodium) Injection for the Treatment of Homozygous Familial Hypercholesterolemia. https://ir.ionispharma.com/news-releases/news-release-details/genzyme-and-isis-announce-fda-approval-kynamrotm-mipomersen (accessed 20 Nov 2021).
148. AZD8601 Study in CABG Patients. https://clinicaltrials.gov/ct2/show/NCT03370887?term=NCT03370887&draw=2&rank=1 (accessed 20 Nov 2021).
149. A Study to Evaluate ALN-AGT01 in Patients With Hypertension. https://clinicaltrials.gov/ct2/show/NCT03934307?term=NCT03934307&draw=2&rank=1 (accessed 20 Nov 2021).
150. Saklayen, M. G., The Global Epidemic of the Metabolic Syndrome. *Curr Hypertens Rep* **2018,** *20*, 12.
151. Diabetes. https://www.who.int/health-topics/diabetes#tab=tab_1 (accessed 20 Nov 2021).
152. Paik, J.,Duggan, S., Volanesorsen: First Global Approval. *Drugs* **2019,** *79*, 1349-1354.
153. Witztum, J. L., Gaudet, D., Freedman, S. D., Alexander, V. J., Digenio, A., Williams, K. R., Yang, Q., Hughes, S. G., Geary, R. S., Arca, M., Stroes, E. S. G., Bergeron, J., Soran, H., Civeira, F., Hemphill, L., Tsimikas, S., Blom, D. J., O'Dea, L.,Bruckert, E., Volanesorsen and Triglyceride Levels in Familial Chylomicronemia Syndrome. *New England Journal of Medicine* **2019,** *381*, 531-542.
154. The Ionis antisense pipeline. https://www.ionispharma.com/ionis-innovation/pipeline/ (accessed 20 Nov 2021).
155. Morgan, E. S., Tai, L. J., Pham, N. C., Overman, J. K., Watts, L. M., Smith, A., Jung, S. W., Gajdošík, M., Kršśák, M., Krebs, M., Geary, R. S., Baker, B. F.,Bhanot, S., Antisense Inhibition of Glucagon Receptor by IONIS-GCGR(Rx) Improves Type 2 Diabetes Without Increase in Hepatic Glycogen Content in Patients With Type 2 Diabetes on Stable Metformin Therapy. *Diabetes Care* **2019,** *42*, 585-593.
156. Strnad, P., McElvaney, N. G.,Lomas, D. A., Alpha(1)-Antitrypsin Deficiency. *N Engl J Med* **2020,** *382*, 1443-1455.
157. Wooddell, C. I., Blomenkamp, K., Peterson, R. M., Subbotin, V. M., Schwabe, C., Hamilton, J., Chu, Q., Christianson, D. R., Hegge, J. O., Kolbe, J., Hamilton, H. L., Branca-Afrazi, M. F., Given, B. D., Lewis, D. L., Gane, E., Kanner, S. B.,Teckman, J. H., Development of an RNAi therapeutic for alpha-1-antitrypsin liver disease. *JCI Insight* **2020,** *5*.
158. Precise, Versatile Editing Platform. https://beamtx.com/our-portfolio/ (accessed 20 Nov 2021).
159. Understanding the Human Genome. https://beamtx.com/our-science/ (accessed 20 Nov 2021).

160. Methylmalonic acidemia. https://medlineplus.gov/genetics/condition/methylmalonic-acidemia/ (accessed 20 Nov 2021).
161. Moderna Announces First Patient Dosed in Phase 1/2 Study of mRNA-3705 for Methylmalonic Acidemia. https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-first-patient-dosed-phase-12-study-mrna-3705 (accessed 20 Nov 2021).
162. Safety, Tolerability and Efficacy of ISIS-GCGRRx in Type 2 Diabetes. https://clinicaltrials.gov/ct2/show/NCT01885260?term=NCT01885260&draw=2&rank=1 (accessed 20 Nov 2021).
163. A Study to Assess Safety, Pharmacokinetics, and Pharmacodynamics of mRNA-3705 in Participants With Isolated Methylmalonic Acidemia. https://clinicaltrials.gov/ct2/show/NCT04899310?term=NCT04899310&draw=2&rank=1 (accessed 20 Nov 2021).
164. Study of ARO-AAT in Patients With Alpha-1 Antitrypsin Deficiency Associated Liver Disease (AATD). https://clinicaltrials.gov/ct2/show/NCT03946449?term=NCT03946449&draw=2&rank=1 (accessed 20 Nov 2021).
165. Moon, A. M., Singal, A. G.,Tapper, E. B., Contemporary Epidemiology of Chronic Liver Disease and Cirrhosis. *Clin Gastroenterol Hepatol* **2020,** *18*, 2650-2666.
166. Asrani, S. K., Devarbhavi, H., Eaton, J.,Kamath, P. S., Burden of liver diseases in the world. *J Hepatol* **2019,** *70*, 151-171.
167. Acute Hepatic Porphyria (AHP). https://liverfoundation.org/acute-hepatic-porphyria-ahp/#what-are-some-symptoms-of-ahp (accessed 20 Nov 2021).
168. Alnylam Announces Approval of GIVLAARI™ (givosiran) by the U.S. Food and Drug Administration (FDA). https://investors.alnylam.com/press-release?id=24281 (accessed 20 Nov 2021).
169. A Study to Assess Safety, Tolerability, PK and PD of AZD2693 in Non-alcoholic Steatohepatitis Patients. https://clinicaltrials.gov/ct2/show/NCT04483947?term=NCT04483947&draw=2&rank=1 (accessed 20 Nov 2021).
170. BasuRay, S., Wang, Y., Smagris, E., Cohen, J. C.,Hobbs, H. H., Accumulation of PNPLA3 on lipid droplets is the basis of associated hepatic steatosis. *Proc Natl Acad Sci U S A* **2019,** *116*, 9521-9526.
171. FDA approves givosiran for acute hepatic porphyria. https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-givosiran-acute-hepatic-porphyria (accessed 20 Nov 2021).
172. Sung, H., Ferlay, J., Siegel, R. L., Laversanne, M., Soerjomataram, I., Jemal, A.,Bray, F., Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians* **2021,** *71*, 209-249.
173. NOXXON 's pipeline. https://www.noxxon.com/index.php?option=com_content&view=article&id=15&Itemid=503 (accessed 20 Nov 2021).
174. NOX-A12 https://www.noxxon.com/index.php?option=com_content&view=article&id=21&Itemid=478 (accessed 20 Nov 2021).
175. Guo, F., Wang, Y., Liu, J., Mok, S. C., Xue, F.,Zhang, W., CXCL12/CXCR4: a symbiotic bridge linking cancer cells and their stromal neighbors in oncogenic communication networks. *Oncogene* **2016,** *35*, 816-26.
176. Sirnaomics pipeline. https://sirnaomics.com/pipeline/ (accessed 20 Nov 2021).
177. Sirnaomics Receives FDA Approval of IND for Phase 1 Clinical Trial of Systemic RNAi Therapeutic STP707 for Solid Tumor Treatment. https://sirnaomics.com/news/sirnaomics-receives-fda-approval-of-ind-for-phase-1-clinical-trial-of-systemic-rnai-therapeutic-stp707-for-solid-tumor-treatment/ (accessed 20 Nov 2021).

178. Cartesian's pipeline. https://www.cartesiantherapeutics.com/clinical-trials/ (accessed 20 Nov 2021).
179. What Is Multiple Myeloma? https://www.cancer.org/cancer/multiple-myeloma/about/what-is-multiple-myeloma.html (accessed 20 Nov 2021).
180. Glioblastoma Treatment With Irradiation and Olaptesed Pegol (NOX-A12) in MGMT Unmethylated Patients (GLORIA). https://clinicaltrials.gov/ct2/show/NCT04121455?term=NCT04121455&draw=2&rank=1 (accessed 20 Nov 2021).
181. Olaptesed With Pembrolizumab and Nanoliposomal Irinotecan or Gemcitabine/Nab-Paclitaxel in MSS Pancreatic Cancer (OPTIMUS). https://clinicaltrials.gov/ct2/show/NCT04901741?term=NCT04901741&draw=2&rank=1 (accessed 20 Nov 2021).
182. Ph. 1, Evaluation of Safety, Tolerability, PK, Anti-tumor Activity of STP707 IV in Subjects With Solid Tumors. https://clinicaltrials.gov/ct2/show/NCT05037149?term=NCT05037149&draw=2&rank=1 (accessed 20 Nov 2021).
183. Descartes-11 in Multiple Myeloma. https://clinicaltrials.gov/ct2/show/NCT03994705?term=NCT03994705&draw=2&rank=1 (accessed 20 Nov 2021).
184. First antisense drug is approved with fleeting success. https://www.nature.com/articles/d42859-019-00080-6 (accessed 20 Nov 2021).
185. Vitravene. https://www.rxlist.com/vitravene-drug.htm#dosage (accessed 20 Nov 2021).
186. WHO Coronavirus (COVID-19) Dashboard. https://covid19.who.int/ (accessed 28 Dec 2021).
187. Pfizer-BioNTech COVID-19 Vaccine COMIRNATY® Receives Full U.S. FDA Approval for Individuals 16 Years and Older. https://www.pfizer.com/news/press-release/press-release-detail/pfizer-biontech-covid-19-vaccine-comirnatyr-receives-full (accessed 20 Nov 2021).
188. Moderna COVID-19 Vaccine. https://www.fda.gov/emergency-preparedness-and-response/coronavirus-disease-2019-covid-19/moderna-covid-19-vaccine (accessed 20 Nov 2021).
189. Coronavirus (COVID-19) Vaccinations. https://ourworldindata.org/covid-vaccinations?country=OWID_WRL (accessed 28 Dec 2021).
190. AB-729 (GalNAc-RNAi). http://www.arbutusbio.com/portfolio/ab-729-galnac-rnai.php (accessed 20 Nov 2021).
191. Kapoor, R.,Kottilil, S., Strategies to eliminate HBV infection. *Future Virol* **2014,** *9*, 565-585.
192. Moderna Announces First Participant Dosed in Phase 1/2 Study of Its Quadrivalent Seasonal Flu mRNA Vaccine. https://investors.modernatx.com/news-releases/news-release-details/moderna-announces-first-participant-dosed-phase-12-study-its (accessed 20 Nov 2021).
193. Moderna Receives FDA Fast Track Designation for Respiratory Syncytial Virus (RSV) Vaccine (mRNA-1345). https://investors.modernatx.com/news-releases/news-release-details/moderna-receives-fda-fast-track-designation-respiratory (accessed 20 Nov 2021).
194. Xu, L., Ma, Z., Li, Y., Pang, Z.,Xiao, S., Antibody dependent enhancement: Unavoidable problems in vaccine development. *Adv Immunol* **2021,** *151*, 99-133.
195. Safety, Tolerability, and Pharmacokinetics of AB-836 in Healthy Subjects and Subjects With Chronic HBV Infection. https://clinicaltrials.gov/ct2/show/NCT04775797?term=NCT04775797&draw=2&rank=1 (accessed 20 Nov 2021).
196. A Study of mRNA-1010 Seasonal Influenza Vaccine in Healthy Adults. https://clinicaltrials.gov/ct2/show/NCT04956575?term=NCT04956575&draw=2&rank=1 (accessed 20 Nov 2021).

197. A Dose Escalation Study to Evaluate Safety, Reactogenicity, and Immunogenicity of mRNA-1345 in Healthy Adults and in Children Who Are Respiratory Syncytial Virus (RSV)-Seropositive. https://clinicaltrials.gov/ct2/show/NCT04528719?term=NCT04528719&draw=2&rank=1 (accessed 20 Nov 2021).
198. Neuromuscular Disorders. https://www.cedars-sinai.org/health-library/diseases-and-conditions/n/neuromuscular-disorders.html (accessed 20 Nov 2021).
199. Neurological Disorders. https://dphhs.mt.gov/schoolhealth/chronichealth/neurologicaldisorders (accessed 20 Nov 2021).
200. FDA grants accelerated approval to first drug for Duchenne muscular dystrophy. https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-first-drug-duchenne-muscular-dystrophy (accessed 20 Nov 2021).
201. FDA grants accelerated approval to first targeted treatment for rare Duchenne muscular dystrophy mutation. https://www.fda.gov/news-events/press-announcements/fda-grants-accelerated-approval-first-targeted-treatment-rare-duchenne-muscular-dystrophy-mutation (accessed 20 Nov 2021).
202. Ottesen, E. W., ISS-N1 makes the First FDA-approved Drug for Spinal Muscular Atrophy. *Transl Neurosci* **2017,** *8*, 1-6.
203. Gales, L., Tegsedi (Inotersen): An Antisense Oligonucleotide Approved for the Treatment of Adult Patients with Hereditary Transthyretin Amyloidosis. *Pharmaceuticals* **2019,** *12*, 78.
204. Gomez-Aguado, I., Rodriguez-Castejon, J., Vicente-Pascual, M., Rodriguez-Gascon, A., Solinis, M. A.,del Pozo-Rodriguez, A., Nanomedicines to Deliver mRNA: State of the Art and Future Perspectives. *Nanomaterials* **2020,** *10*.
205. Building an industry-leading genetic medicine pipeline. https://www.sarepta.com/products-pipeline/pipeline (accessed 20 Nov 2021).
206. What is ALS? https://www.als.org/understanding-als/what-is-als (accessed 20 Nov 2021).
207. Delivering on the RNA Revolution. https://www.aviditybiosciences.com/pipeline/pipeline-overview/ (accessed 20 Nov 2021).
208. Holt, I., Jacquemin, V., Fardaei, M., Sewry, C. A., Butler-Browne, G. S., Furling, D., Brook, J. D.,Morris, G. E., Muscleblind-like proteins: similarities and differences in normal and myotonic dystrophy muscle. *The American journal of pathology* **2009,** *174*, 216-227.
209. Akinc, A., Maier, M. A., Manoharan, M., Fitzgerald, K., Jayaraman, M., Barros, S., Ansell, S., Du, X. Y., Hope, M. J., Madden, T. D., Mui, B. L., Semple, S. C., Tam, Y. K., Ciufolini, M., Witzigmann, D., Kulkarni, J. A., van der Meel, R.,Cullis, P. R., The Onpattro story and the clinical translation of nanomedicines containing nucleic acid-based drugs. *Nature Nanotechnology* **2019,** *14*, 1084-1087.
210. A Study of BIIB067 When Initiated in Clinically Presymptomatic Adults With a Confirmed Superoxide Dismutase 1 Mutation (ATLAS). https://clinicaltrials.gov/ct2/show/NCT04856982?term=NCT04856982&draw (accessed 20 Nov 2021).
211. Study of AOC 1001 in Adult Myotonic Dystrophy Type 1 (DM1) Patients (MARINA). https://clinicaltrials.gov/ct2/show/NCT05027269?term=NCT05027269&draw=2&rank=1 (accessed 20 Nov 2021).
212. Blindness and vision impairment. https://www.who.int/news-room/fact-sheets/detail/blindness-and-visual-impairment (accessed 20 Nov 2021).
213. Wong, W. L., Su, X., Li, X., Cheung, C. M., Klein, R., Cheng, C. Y.,Wong, T. Y., Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *Lancet Glob Health* **2014,** *2*, e106-16.
214. Nagpal, M., Nagpal, K.,Nagpal, P. N., A comparative debate on the various anti-vascular endothelial growth factor drugs: pegaptanib sodium (Macugen), ranibizumab (Lucentis) and bevacizumab (Avastin). *Indian journal of ophthalmology* **2007,** *55*, 437-439.

215. Adamis, A. P., Altaweel, M., Bressler, N. M., Cunningham, E. T., Jr., Davis, M. D., Goldbaum, M., Gonzales, C., Guyer, D. R., Barrett, K.,Patel, M., Changes in retinal neovascularization after pegaptanib (Macugen) therapy in diabetic individuals. *Ophthalmology* **2006,** *113*, 23-8.
216. Jaffe, G. J., Westby, K., Csaky, K. G., Monés, J., Pearlman, J. A., Patel, S. S., Joondeph, B. C., Randolph, J., Masonson, H.,Rezaei, K. A., C5 Inhibitor Avacincaptad Pegol for Geographic Atrophy Due to Age-Related Macular Degeneration: A Randomized Pivotal Phase 2/3 Trial. *Ophthalmology* **2021,** *128*, 576-586.
217. A Phase 3 Safety and Efficacy Study of Intravitreal Administration of Zimura (Complement C5 Inhibitor). https://clinicaltrials.gov/ct2/show/NCT04435366?term=NCT04435366&draw=2&rank=1 (accessed 20 Nov 2021).
218. Study With QR-504a to Evaluate Safety, Tolerability & Corneal Endothelium Molecular Biomarker(s) in Subjects With FECD3 (Fuchs Focus). https://clinicaltrials.gov/ct2/show/NCT05052554 (accessed 20 Nov 2021).
219. QR-504a for Fuchs endothelial corneal dystrophy. https://www.proqr.com/qr-504a-for-fuchs-endothelial-corneal-dystrophy (accessed 20 Nov 2021).
220. 850 Million People Worldwide Have Kidney Disease. https://www.webmd.com/kidney-stones/news/20180705/850-million-people-worldwide-have-kidney-disease (accessed 20 Nov 2021).
221. Scott, L. J.,Keam, S. J., Lumasiran: First Approval. *Drugs* **2021,** *81*, 277-282.
222. Garrelfs, S. F., Frishberg, Y., Hulton, S. A., Koren, M. J., O'Riordan, W. D., Cochat, P., Deschênes, G., Shasha-Lavsky, H., Saland, J. M., Van't Hoff, W. G., Fuster, D. G., Magen, D., Moochhala, S. H., Schalk, G., Simkova, E., Groothoff, J. W., Sas, D. J., Meliambro, K. A., Lu, J., Sweetser, M. T., Garg, P. P., Vaishnaw, A. K., Gansner, J. M., McGregor, T. L.,Lieske, J. C., Lumasiran, an RNAi Therapeutic for Primary Hyperoxaluria Type 1. *N Engl J Med* **2021,** *384*, 1216-1226.
223. Lee, E. C., Valencia, T., Allerson, C., Schairer, A., Flaten, A., Yheskel, M., Kersjes, K., Li, J., Gatto, S., Takhar, M., Lockton, S., Pavlicek, A., Kim, M., Chu, T., Soriano, R., Davis, S., Androsavich, J. R., Sarwary, S., Owen, T., Kaplan, J., Liu, K., Jang, G., Neben, S., Bentley, P., Wright, T.,Patel, V., Discovery and preclinical evaluation of anti-miR-17 oligonucleotide RGLS4326 for the treatment of polycystic kidney disease. *Nature Communications* **2019,** *10*, 4148.
224. Schairer, A., Valencia, T., Lockton, S., Gatto, S., Kim, M., Wallace, D.,Lee, E. Efficacy of RGLS4326 in Human Primary 3D-Cyst Cultures derived from Autosomal Dominant Polycystic Kidney Disease (ADPKD) Donors http://regulusrx.com/wp-content/uploads/2018/11/ASN-2018-RGLS4326-Poster-3021589-FINAL.pdf (accessed 20 Nov 2021).
225. Ji, F. P., Wen, L., Zhang, Y. P., Liu, E. P.,Wen, J. G., Serum complement factor B is associated with disease activity and progression of idiopathic membranous nephropathy concomitant with IgA nephropathy. *Int Urol Nephrol* **2021**.
226. A Study to Evaluate Lumasiran in Patients With Advanced Primary Hyperoxaluria Type 1 (ILLUMINATE-C). https://clinicaltrials.gov/ct2/show/NCT04152200?term=NCT04152200&draw=2&rank=1 (accessed 20 Nov 2021).
227. A Study of RGLS4326 in Patients With Autosomal Dominant Polycystic Kidney Disease. https://clinicaltrials.gov/ct2/show/NCT04536688?term=NCT04536688&draw=2&rank=1 (accessed 20 Nov 2021).
228. A Study to Evaluate the Effectiveness and Safety of IONIS-FB-LRx, an Antisense Inhibitor of Complement Factor B, in Adult Participants With Primary IgA Nephropathy. https://clinicaltrials.gov/ct2/show/NCT04014335?term=NCT04014335&draw=2&rank=1 (accessed 20 Nov 2021).

229. The Global Impact of Respiratory Disease. https://www.who.int/gard/publications/The_Global_Impact_of_Respiratory_Disease.pdf (accessed 20 Nov 2021).
230. Cartesian Therapeutics Initiates Clinical Trial of First RNA-Engineered Cell Therapy for Acute Respiratory Distress Syndrome and COVID-19 https://www.prnewswire.com/news-releases/cartesian-therapeutics-initiates-clinical-trial-of-first-rna-engineered-cell-therapy-for-acute-respiratory-distress-syndrome-and-covid-19-301121921.html (accessed 20 Nov 2021).
231. Study of Descartes-30 in Acute Respiratory Distress Syndrome. https://clinicaltrials.gov/ct2/show/NCT04524962?term=NCT04524962&draw=2&rank=1 (accessed 20 Nov 2021).
232. Ratjen, F., Bell, S. C., Rowe, S. M., Goss, C. H., Quittner, A. L.,Bush, A., Cystic fibrosis. *Nature reviews. Disease primers* **2015,** *1*, 15010-15010.
233. Translate Bio pipeline - Present and future focus. https://translate.bio/pipeline/ (accessed 20 Nov 2021).
234. Chow, M. Y. T., Qiu, Y.,Lam, J. K. W., Inhaled RNA Therapy: From Promise to Reality. *Trends in pharmacological sciences* **2020,** *41*, 715-729.
235. Translate Bio Announces Results from Second Interim Data Analysis from Ongoing Phase 1/2 Clinical Trial of MRT5005 in Patients with Cystic Fibrosis (CF). https://investors.translate.bio/news-releases/news-release-details/translate-bio-announces-results-second-interim-data-analysis (accessed 20 Nov 2021).
236. Arrowhead's pipeline - Novel Drugs toTreat Intractable Diseases. https://arrowheadpharma.com/pipeline/ (accessed 20 Nov 2021).
237. Arrowhead Pauses ARO-ENaC Phase 1/2 Clinical Study. https://ir.arrowheadpharma.com/news-releases/news-release-details/arrowhead-pauses-aro-enac-phase-12-clinical-study (accessed 20 Nov 2021).
238. Study to Evaluate the Safety & Tolerability of MRT5005 Administered by Nebulization in Adults With Cystic Fibrosis (RESTORE-CF). https://clinicaltrials.gov/ct2/show/NCT03375047?term=NCT03375047&draw=2&rank=1 (accessed 20 Nov 2021).
239. Study of ARO-ENaC in Healthy Volunteers and in Patients With Cystic Fibrosis. https://clinicaltrials.gov/ct2/show/NCT04375514?term=NCT04375514&draw=2&rank=1 (accessed 20 Nov 2021).
240. Salinas Cisneros, G.,Thein, S. L., Recent Advances in the Treatment of Sickle Cell Disease. *Frontiers in Physiology* **2020,** *11*.
241. Galanello, R.,Origa, R., Beta-thalassemia. *Orphanet journal of rare diseases* **2010,** *5*, 11-11.
242. Beam Therapeutics Provides Business and Pipeline Updates and Reports Third Quarter 2021 Financial Results. https://investors.beamtx.com/node/7596 (accessed 20 Nov 2021).
243. Silence Therapeutics pipeline - Our mRNAi GOLD™ Pipeline Targets. https://silence-therapeutics.com/our-pipeline/default.aspx (accessed 20 Nov 2021).
244. SLN-124. https://thalassaemia.org.cy/clinical-trial-updates/sln-124/ (accessed 20 Nov 2021).
245. A Study Investigate the Safety, Tolerability, Pharmacokinetic, and Pharmacodynamic Response of SLN124 in Adults With Alpha/Beta-thalassaemia and Very Low- and Low-risk Myelodysplastic Syndrome. https://clinicaltrials.gov/ct2/show/NCT04718844?term=NCT04718844&draw=2&rank=1 (accessed 20 Nov 2021).
246. A Study for Safety and Efficacy Evaluation of Various Doses of STP705 in Reducing Keloid Recurrence. https://clinicaltrials.gov/ct2/show/NCT04844840?term=NCT04844840&draw=2&rank=1 (accessed 20 Nov 2021).

247. Sirnaomics Doses First Patient in Phase 2 Study of STP705 for Keloid Scar Prevention. https://sirnaomics.com/news/sirnaomics-doses-first-patient-in-phase-2-study-of-stp705-for-keloid-scar-prevention/ (accessed 20 Nov 2021).
248. Janeway CA Jr, Travers P, Walport M,al., e., *Immunobiology: The Immune System in Health and Disease. 5th edition. New York: Garland Science; 2001. Autoimmune responses are directed against self antigens*.
249. Moderna Builds on Clinical Validation of Systemic Delivery with Two Additional Development Candidates in New Autoimmune Therapeutic Area. https://investors.modernatx.com/news-releases/news-release-details/moderna-builds-clinical-validation-systemic-delivery-two (accessed 20 Nov 2021).
250. Global alcohol action plan 2022-2030 to strengthen implementation of the Global Strategy to Reduce the Harmful Use of Alcohol. https://cdn.who.int/media/docs/default-source/alcohol/action-plan-on-alcohol_first-draft-final_formatted.pdf?sfvrsn=b690edb0_1&download=true (accessed 20 Nov 2021).
251. Alcohol Use Disorder (AUD). https://dicerna.com/patients/alcohol-use-disorder-aud/ (accessed 20 Nov 2021).
252. A New and Unique Approach to Potentially Improving Treatment Outcomes for People With Alcohol Use Disorder (AUD). https://dicerna.com/pipeline/dcr-aud/ (accessed 20 Nov 2021).
253. A Study to Evaluate the Safety, Tolerability, Pharmacokinetics, and Pharmacodynamics of mRNA-6231 in Healthy Adults. https://clinicaltrials.gov/ct2/show/NCT04916431?term=NCT04916431&draw=2&rank=1 (accessed 20 Nov 2021).
254. Study of DCR-AUD in Healthy Volunteers. https://clinicaltrials.gov/ct2/show/NCT05021640?term=NCT05021640&draw=2&rank=1 (accessed 20 Nov 2021).
255. Lehninger, A. L., *Biochemistry: the molecular basis of cell structure and function*. 2nd ed.; Worth Publishers: New york, NY, 1975.
256. MCININCH, J. D., KEATING, M., SCHLEGEL, M. K., CASTORENO, A., JADHAV, V. R., KAITTANIS, C., CASTELLANOS-RIZALDOS, E.,PANDYA, B. A. Compositions and methods for silencing VEGF-A expression. WO/2021/163066, 2021.
257. Parr, C. J. C., Wada, S., Kotake, K., Kameda, S., Matsuura, S., Sakashita, S., Park, S., Sugiyama, H., Kuang, Y.,Saito, H., N1-Methylpseudouridine substitution enhances the performance of synthetic mRNA switches in cells. *Nucleic Acids Research* **2020,** *48*, e35-e35.
258. Shatkin, A. J., Capping of eucaryotic mRNAs. *Cell* **1976,** *9*, 645-653.
259. Chan, J. H., Lim, S.,Wong, W. F., ANTISENSE OLIGONUCLEOTIDES: FROM DESIGN TO THERAPEUTIC APPLICATION. *Clinical and Experimental Pharmacology and Physiology* **2006,** *33*, 533-540.
260. Phosphorothioate Oligonucleotides. https://www.sigmaaldrich.com/US/en/technical-documents/technical-article/genomics/gene-expression-and-silencing/phosphorothioates (accessed 20 Nov 2021).
261. Putney, S. D., Benkovic, S. J.,Schimmel, P. R., A DNA fragment with an alpha-phosphorothioate nucleotide at one end is asymmetrically blocked from digestion by exonuclease III and can be replicated in vivo. *Proceedings of the National Academy of Sciences* **1981,** *78*, 7350-7354.
262. CANTLEY, W., MCININCH, J., D., CASTORENO, A., KAITTANIS, C.,SCHLEGEL, M., K. COMPOSITIONS AND METHODS FOR SILENCING SCN9A EXPRESSION. WO/2021/207189, 2021.
263. Nielsen, P. E.,Egholm, M., An introduction to peptide nucleic acid. *Curr Issues Mol Biol* **1999,** *1*, 89-104.
264. Blocking Groups and Annotations for Registry Sequences. https://www.cas.org/sites/default/files/documents/blocking.pdf (accessed Jan 1, 2022).

265. Summerton, J., Morpholino antisense oligomers: the case for an RNase H-independent structural type. *Biochim Biophys Acta* **1999,** *1489*, 141-58.
266. FITZGERALD, K., TREMBLAY, F.,MCININCH, J. D. G PROTEIN-COUPLED RECEPTOR 146 (GPR146) IRNA COMPOSITIONS AND METHODS OF USE THEREOF. WO/2021/174056, 2021.
267. GalNAc-siRNA Conjugates: Leading the Way for Delivery of RNAi Therapeutics. *Nucleic Acid Therapeutics* **2018,** *28*, 109-118.
268. FITZGERALD, K., TREMBLAY, F.,MCININCH, J. D. G PROTEIN-COUPLED RECEPTOR 146 (GPR146) IRNA COMPOSITIONS AND METHODS OF USE THEREOF. WO/2021/174056, 2021.
269. PubChem Substance Record for SID 135267507, AIW6036FAS. https://pubchem.ncbi.nlm.nih.gov/substance/135267507 (accessed Dec 9, 2021).
270. Pegaptanib. https://en.wikipedia.org/wiki/Pegaptanib#/media/File:Pegaptanib_sodium_skeletal.svg (accessed Dec 8, 2021).
271. VIEIRA ARAUJO SOARES DA SILVA, P. M. siRNA molecules, methods of production and uses thereof. US20210332364, 2021.
272. Kanasty, R., Dorkin, J. R., Vegas, A.,Anderson, D., Delivery materials for siRNA therapeutics. *Nature Materials* **2013,** *12*, 967-977.
273. CAS Data. https://www.cas.org/cas-data (accessed November 25, 2021).
274. Yang, N., Nonviral gene delivery system. *International journal of pharmaceutical investigation* **2012,** *2*, 97-98.
275. Koynova, R.,Tenchov, B., Cationic Lipids: Molecular Structure/Transfection Activity Relationships and Interactions with Biomembranes. In *Nucleic Acid Transfection*, Bielke, W.; Erbacher, C., Eds. Springer-Verlag: Berlin, Heidelberg, 2010; Vol. 296, pp 51-93.
276. Tenchov, R., Bird, R., Curtze, A. E.,Zhou, Q., Lipid Nanoparticles—From Liposomes to mRNA Vaccine Delivery, a Landscape of Research Diversity and Advancement. *ACS Nano* **2021**.
277. Mitchell, M. J., Billingsley, M. M., Haley, R. M., Wechsler, M. E., Peppas, N. A.,Langer, R., Engineering precision nanoparticles for drug delivery. *Nature Reviews Drug Discovery* **2021,** *20*, 101-124.
278. Hajj, K. A., Ball, R. L., Deluty, S. B., Singh, S. R., Strelkova, D., Knapp, C. M.,Whitehead, K. A., Branched-Tail Lipid Nanoparticles Potently Deliver mRNA *In Vivo* due to Enhanced Ionization at Endosomal pH. *Small* **2019,** *15*.
279. Heyes, J. A., Niculescu-Duvaz, D., Cooper, R. G.,Springer, C. J., Synthesis of novel cationic lipids: Effect of structural modification on the efficiency of gene transfer. *Journal of Medicinal Chemistry* **2002,** *45*, 99-114.
280. Karmali, P. P.,Chaudhuri, A., Cationic Liposomes as non-viral carriers of gene medicines: Resolved issues, open questions, and future promises. *Medicinal Research Reviews* **2007,** *27*, 696-722.
281. Karmali, P. P., Kumar, V. V.,Chaudhuri, A., Design, syntheses and in vitro gene delivery efficacies of novel mono-, di- and trilysinated cationic lipids: A structure-activity investigation. *Journal of Medicinal Chemistry* **2004,** *47*, 2123-2132.
282. Behr, J. P., Demeneix, B., Loeffler, J. P.,Mutul, J. P., Efficient Gene-Transfer Into Mammalian Primary Endocrine-Cells with Lipopolyamine-Coated DNA. *Proceedings of the National Academy of Sciences of the United States of America* **1989,** *86*, 6982-6986.
283. Ferrari, M. E., Nguyen, C. M., Zelphati, O., Tsai, Y. L.,Felgner, P. L., Analytical methods for the characterization of cationic lipid nucleic acid complexes. *Human Gene Therapy* **1998,** *9*, 341-351.
284. de Lima, M. C. P., Neves, S., Filipe, A., Duzgunes, N.,Simoes, S., Cationic liposomes for gene delivery: From biophysics to biological applications. *Current Medicinal Chemistry* **2003,** *10*, 1221-1231.
285. Wheeler, C. J., Felgner, P. L., Tsai, Y. J., Marshall, J., Sukhu, L., Doh, S. G., Hartikka, J., Nietupski, J., Manthorpe, M., Nichols, M., Plewe, M., Liang, X. W., Norman, J., Smith, A.,Cheng, S. H., A novel

cationic lipid greatly enhances plasmid DNA delivery and expression in mouse lung. *Proceedings of the National Academy of Sciences of the United States of America* **1996,** *93*, 11454-11459.
286. Tarahovsky, Y. S., Arsenault, A. L., MacDonald, R. C., McIntosh, T. J.,Epand, R. M., Electrostatic Control of Phospholipid Polymorphism. *Biophysical Journal* **2000,** *79*, 3193-3200.
287. Koynova, R., Wang, L.,MacDonald, R. C., An Intracellular Lamellar - Nonlamellar Phase Transition Rationalizes the Superior Performance of Some Cationic Lipid Transfection Agents. *Proceedings of the National Academy of Sciences of the United States of America* **2006,** *103*, 14373-14378.
288. Kedmi, R., Ben-Arie, N.,Peer, D., The systemic toxicity of positively charged lipid nanoparticles and the role of Toll-like receptor 4 in immune activation. *Biomaterials* **2010,** *31*, 6867-75.
289. Kalluri, R.,LeBleu, V. S., The biology, function, and biomedical applications of exosomes. *Science* **2020,** *367*, eaau6977.
290. Edgar, J. R., Q&A: What are exosomes, exactly? *BMC Biology* **2016,** *14*, 46.
291. Zhang, Y., Liu, Y., Liu, H.,Tang, W. H., Exosomes: biogenesis, biologic function and clinical potential. *Cell & Bioscience* **2019,** *9*, 19.
292. Alvarez-Erviti, L., Seow, Y., Yin, H., Betts, C., Lakhal, S.,Wood, M. J. A., Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nature Biotechnology* **2011,** *29*, 341-345.
293. Shtam, T. A., Kovalev, R. A., Varfolomeeva, E. Y., Makarov, E. M., Kil, Y. V.,Filatov, M. V., Exosomes are natural carriers of exogenous siRNA to human cells in vitro. *Cell Commun Signal* **2013,** *11*, 88-88.
294. Lamichhane, T. N., Raiker, R. S.,Jay, S. M., Exogenous DNA Loading into Extracellular Vesicles via Electroporation is Size-Dependent and Enables Limited Gene Delivery. *Mol Pharm* **2015,** *12*, 3650-3657.
295. Zhou, X., Brown, B. A., Siegel, A. P., El Masry, M. S., Zeng, X., Song, W., Das, A., Khandelwal, P., Clark, A., Singh, K., Guda, P. R., Gorain, M., Timsina, L., Xuan, Y., Jacobson, S. C., Novotny, M. V., Roy, S., Agarwal, M., Lee, R. J., Sen, C. K., Clemmer, D. E.,Ghatak, S., Exosome-Mediated Crosstalk between Keratinocytes and Macrophages in Cutaneous Wound Healing. *ACS Nano* **2020,** *14*, 12732-12748.
296. Subra, C., Laulagnier, K., Perret, B.,Record, M., Exosome lipidomics unravels lipid sorting at the level of multivesicular bodies. *Biochimie* **2007,** *89*, 205-12.
297. Ha, D., Yang, N.,Nadithe, V., Exosomes as therapeutic drug carriers and delivery vehicles across biological membranes: current perspectives and future challenges. *Acta Pharm Sin B* **2016,** *6*, 287-296.
298. Butreddy, A., Kommineni, N.,Dudhipala, N., Exosomes as Naturally Occurring Vehicles for Delivery of Biopharmaceuticals: Insights from Drug Delivery to Clinical Perspectives. *Nanomaterials (Basel)* **2021,** *11*, 1481.
299. Kumar, R., Santa Chalarca, C. F., Bockman, M. R., Bruggen, C. V., Grimme, C. J., Dalal, R. J., Hanson, M. G., Hexum, J. K.,Reineke, T. M., Polymeric Delivery of Therapeutic Nucleic Acids. *Chemical Reviews* **2021**.
300. Zhu, L.,Mahato, R. I., Lipid and polymeric carrier-mediated nucleic acid delivery. *Expert Opin Drug Deliv* **2010,** *7*, 1209-1226.
301. Kataoka, K., Togawa, H., Harada, A., Yasugi, K., Matsumoto, T.,Katayose, S., Spontaneous Formation of Polyion Complex Micelles with Narrow Distribution from Antisense Oligonucleotide and Cationic Block Copolymer in Physiological Saline. *Macromolecules* **1996,** *29*, 8556-8557.
302. Neu, M., Fischer, D.,Kissel, T., Recent advances in rational gene transfer vector design based on poly(ethylene imine) and its derivatives. *The Journal of Gene Medicine* **2005,** *7*, 992-1009.
303. Vandamme, T. F.,Brobeck, L., Poly(amidoamine) dendrimers as ophthalmic vehicles for ocular delivery of pilocarpine nitrate and tropicamide. *Journal of Controlled Release* **2005,** *102*, 23-38.
304. Urbiola, K., Blanco-Fernández, L., Ogris, M., Rödl, W., Wagner, E.,Tros de Ilarduya, C., Novel PAMAM-PEG-Peptide Conjugates for siRNA Delivery Targeted to the Transferrin and Epidermal Growth Factor Receptors. *Journal of Personalized Medicine* **2018,** *8*, 4.

305. Singh, P., Gupta, U., Asthana, A.,Jain, N. K., Folate and Folate−PEG−PAMAM Dendrimers: Synthesis, Characterization, and Targeted Anticancer Drug Delivery Potential in Tumor Bearing Mice. *Bioconjugate Chemistry* **2008,** *19*, 2239-2252.
306. Tang, Y., Li, Y.-B., Wang, B., Lin, R.-Y., van Dongen, M., Zurcher, D. M., Gu, X.-Y., Banaszak Holl, M. M., Liu, G.,Qi, R., Efficient in Vitro siRNA Delivery and Intramuscular Gene Silencing Using PEG-Modified PAMAM Dendrimers. *Mol Pharm* **2012,** *9*, 1812-1821.
307. Piotrowski-Daspit, A. S., Kauffman, A. C., Bracaglia, L. G.,Saltzman, W. M., Polymeric vehicles for nucleic acid delivery. *Adv Drug Deliv Rev* **2020,** *156*, 119-132.
308. Gaur, S., Wen, Y., Song, J. H., Parikh, N. U., Mangala, L. S., Blessing, A. M., Ivan, C., Wu, S. Y., Varkaris, A., Shi, Y., Lopez-Berestein, G., Frigo, D. E., Sood, A. K.,Gallick, G. E., Chitosan nanoparticle-mediated delivery of miRNA-34a decreases prostate tumor growth in the bone and its expression induces non-canonical autophagy. *Oncotarget* **2015,** *6*.
309. Cosco, D., Cilurzo, F., Maiuolo, J., Federico, C., Di Martino, M. T., Cristiano, M. C., Tassone, P., Fresta, M.,Paolino, D., Delivery of miR-34a by chitosan/PLGA nanoplexes for the anticancer treatment of multiple myeloma. *Sci Rep* **2015,** *5*, 17579.
310. Chen, X., Gu, S., Chen, B.-F., Shen, W.-L., Yin, Z., Xu, G.-W., Hu, J.-J., Zhu, T., Li, G., Wan, C., Ouyang, H.-W., Lee, T.-L.,Chan, W.-Y., Nanoparticle delivery of stable miR-199a-5p agomir improves the osteogenesis of human mesenchymal stem cells via the HIF1a pathway. *Biomaterials* **2015,** *53*, 239-250.
311. Köping-Höggård, M., Tubulekas, I., Guan, H., Edwards, K., Nilsson, M., Vårum, K. M.,Artursson, P., Chitosan as a nonviral gene delivery system. Structure–property relationships and characteristics compared with polyethylenimine in vitro and after lung administration in vivo. *Gene Therapy* **2001,** *8*, 1108-1121.
312. Curtin, C. M., Tierney, E. G., McSorley, K., Cryan, S.-A., Duffy, G. P.,O'Brien, F. J., Combinatorial Gene Therapy Accelerates Bone Regeneration: Non-Viral Dual Delivery of VEGF and BMP2 in a Collagen-Nanohydroxyapatite Scaffold. *Advanced Healthcare Materials* **2015,** *4*, 223-227.
313. Capito, R. M.,Spector, M., Collagen scaffolds for nonviral IGF-1 gene delivery in articular cartilage tissue engineering. *Gene Therapy* **2007,** *14*, 721-732.
314. Cohen-Sacks, H., Elazar, V., Gao, J., Golomb, A., Adwan, H., Korchov, N., Levy, R. J., Berger, M. R.,Golomb, G., Delivery and expression of pDNA embedded in collagen matrices. *Journal of Controlled Release* **2004,** *95*, 309-320.
315. Aoife, M. O., apos, Mahony, Martin, J. O., apos, Neill, Bruno, M. D. C. G., Raphael, D., John, F. C., Caitriona, M. O., apos,Driscoll, Cyclodextrins for Non-Viral Gene and siRNA Delivery. *Pharmaceutical Nanotechnology* **2013,** *1*, 6-14.
316. Chaturvedi, K., Ganguly, K., Kulkarni, A. R., Kulkarni, V. H., Nadagouda, M. N., Rudzinski, W. E.,Aminabhavi, T. M., Cyclodextrin-based siRNA delivery nanocarriers: a state-of-the-art review. *Expert Opin Drug Deliv* **2011,** *8*, 1455-1468.
317. Singh, R. P., Hidalgo, T., Cazade, P.-A., Darcy, R., Cronin, M. F., Dorin, I., O'Driscoll, C. M.,Thompson, D., Self-Assembled Cationic β-Cyclodextrin Nanostructures for siRNA Delivery. *Mol Pharm* **2019,** *16*, 1358-1366.
318. Mintzer, M. A.,Simanek, E. E., Nonviral Vectors for Gene Delivery. *Chemical Reviews* **2009,** *109*, 259-302.
319. El-Sayed, A.,Harashima, H., Endocytosis of Gene Delivery Vectors: From Clathrin-dependent to Lipid Raft-mediated Endocytosis. *Molecular Therapy* **2013,** *21*, 1118-1130.
320. Bus, T., Traeger, A.,Schubert, U. S., The great escape: how cationic polyplexes overcome the endosomal barrier. *Journal of Materials Chemistry B* **2018,** *6*, 6904-6918.
321. Degors, I. M. S., Wang, C., Rehman, Z. U.,Zuhorn, I. S., Carriers Break Barriers in Drug Delivery: Endocytosis and Endosomal Escape of Gene Delivery Vectors. *Accounts of Chemical Research* **2019,** *52*, 1750-1760.

322. Liang, K., Such, G. K., Zhu, Z., Dodds, S. J., Johnston, A. P. R., Cui, J., Ejima, H.,Caruso, F., Engineering Cellular Degradation of Multilayered Capsules through Controlled Cross-Linking. *ACS Nano* **2012,** *6*, 10186-10194.
323. Boisguérin, P., Deshayes, S., Gait, M. J., O'Donovan, L., Godfrey, C., Betts, C. A., Wood, M. J. A.,Lebleu, B., Delivery of therapeutic oligonucleotides with cell penetrating peptides. *Advanced Drug Delivery Reviews* **2015,** *87*, 52-67.
324. Kurrikoff, K.,Langel, Ü., Recent CPP-based applications in medicine. *Expert Opin Drug Deliv* **2019,** *16*, 1183-1191.
325. Boisguérin, P., Konate, K., Josse, E., Vivès, E.,Deshayes, S., Peptide-Based Nanoparticles for Therapeutic Nucleic Acid Delivery. *Biomedicines* **2021,** *9*, 583.
326. Huang, Y.-W., Lee, H.-J., Tolliver, L. M.,Aronstam, R. S., Delivery of Nucleic Acids and Nanomaterials by Cell-Penetrating Peptides: Opportunities and Challenges. *BioMed Research International* **2015,** *2015*, 834079.
327. Vivès, E., Brodin, P.,Lebleu, B., A truncated HIV-1 Tat protein basic domain rapidly translocates through the plasma membrane and accumulates in the cell nucleus. *J Biol Chem* **1997,** *272*, 16010-7.
328. Yi, A., Sim, D., Lee, Y.-J., Sarangthem, V.,Park, R.-W., Development of elastin-like polypeptide for targeted specific gene delivery in vivo. *Journal of Nanobiotechnology* **2020,** *18*, 15.
329. Kasai, H., Inoue, K., Imamura, K., Yuvienco, C., Montclare, J. K.,Yamano, S., Efficient siRNA delivery and gene silencing using a lipopolypeptide hybrid vector mediated by a caveolae-mediated and temperature-dependent endocytic pathway. *J Nanobiotechnology* **2019,** *17*, 11.
330. Guo, F., Fu, Q., Zhou, K., Jin, C., Wu, W., Ji, X., Yan, Q., Yang, Q., Wu, D., Li, A.,Yang, G., Matrix metalloprotein-triggered, cell penetrating peptide-modified star-shaped nanoparticles for tumor targeting and cancer therapy. *Journal of Nanobiotechnology* **2020,** *18*, 48.
331. Koren, E.,Torchilin, V. P., Cell-penetrating peptides: breaking through to the other side. *Trends Mol Med* **2012,** *18*, 385-93.
332. Langlet-Bertin, B., Leborgne, C., Scherman, D., Bechinger, B., Mason, A. J.,Kichler, A., Design and evaluation of histidine-rich amphipathic peptides for siRNA delivery. *Pharm Res* **2010,** *27*, 1426-36.
333. Hsu, T.,Mitragotri, S., Delivery of siRNA and other macromolecules into skin and cells using a peptide enhancer. *Proceedings of the National Academy of Sciences of the United States of America* **2011,** *108*, 15816-15821.
334. Ryu, Y. C., Kim, K. A., Kim, B. C., Wang, H.-M. D.,Hwang, B. H., Novel fusion peptide‐mediated siRNA delivery using self‐assembled nanocomplex. *Journal of Nanobiotechnology* **2021,** *19*, 44.
335. Shukla, R. S., Qin, B.,Cheng, K., Peptides Used in the Delivery of Small Noncoding RNA. *Mol Pharm* **2014,** *11*, 3395-3408.
336. Simeoni, F., Morris, M. C., Heitz, F.,Divita, G., Insight into the mechanism of the peptide-based gene delivery system MPG: implications for delivery of siRNA into mammalian cells. *Nucleic acids research* **2003,** *31*, 2717-2724.
337. Fang, B., Guo, H. Y., Zhang, M., Jiang, L.,Ren, F. Z., The six amino acid antimicrobial peptide bLFcin6 penetrates cells and delivers siRNA. *Febs j* **2013,** *280*, 1007-17.
338. Qin, B., Chen, Z., Jin, W.,Cheng, K., Development of cholesteryl peptide micelles for siRNA delivery. *J Control Release* **2013,** *172*, 159-168.
339. Guo, J., Cheng, W. P., Gu, J., Ding, C., Qu, X., Yang, Z.,O'Driscoll, C., Systemic delivery of therapeutic small interfering RNA using a pH-triggered amphiphilic poly-L-lysine nanocarrier to suppress prostate cancer growth in mice. *Eur J Pharm Sci* **2012,** *45*, 521-32.
340. Warrant, R. W.,Kim, S.-H., α-Helix–double helix interaction shown in the structure of a protamine-transfer RNA complex and a nucleoprotamine model. *Nature* **1978,** *271*, 130-135.
341. Schlake, T., Thess, A., Fotin-Mleczek, M.,Kallen, K. J., Developing mRNA-vaccine technologies. *RNA Biol* **2012,** *9*, 1319-30.

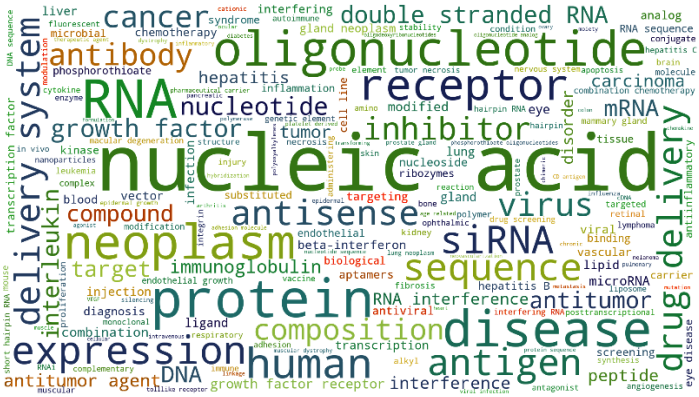
342. Rosi, N. L., Giljohann, D. A., Thaxton, C. S., Lytton-Jean, A. K., Han, M. S.,Mirkin, C. A., Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. *Science* **2006,** *312*, 1027-30.
343. Artiga, A., Serrano-Sevilla, I., De Matteis, L., Mitchell, S. G.,de la Fuente, J. M., Current status and future perspectives of gold nanoparticle vectors for siRNA delivery. *Journal of Materials Chemistry B* **2019,** *7*, 876-896.
344. Giljohann, D. A., Seferos, D. S., Prigodich, A. E., Patel, P. C.,Mirkin, C. A., Gene Regulation with Polyvalent siRNA−Nanoparticle Conjugates. *Journal of the American Chemical Society* **2009,** *131*, 2072-2073.
345. Ding, Y., Jiang, Z., Saha, K., Kim, C. S., Kim, S. T., Landis, R. F.,Rotello, V. M., Gold nanoparticles for nucleic acid delivery. *Molecular therapy : the journal of the American Society of Gene Therapy* **2014,** *22*, 1075-1083.
346. Cutler, J. I., Auyeung, E.,Mirkin, C. A., Spherical Nucleic Acids. *Journal of the American Chemical Society* **2012,** *134*, 1376-1391.
347. Seferos, D. S., Giljohann, D. A., Hill, H. D., Prigodich, A. E.,Mirkin, C. A., Nano-flares: probes for transfection and mRNA detection in living cells. *Journal of the American Chemical Society* **2007,** *129*, 15477-15479.
348. Wang, S., Qin, L., Yamankurt, G., Skakuj, K., Huang, Z., Chen, P.-C., Dominguez, D., Lee, A., Zhang, B.,Mirkin, C. A., Rational vaccinology with spherical nucleic acids. *Proceedings of the National Academy of Sciences* **2019,** *116*, 10473-10481.
349. Plank, C., Zelphati, O.,Mykhaylyk, O., Magnetically enhanced nucleic acid delivery. Ten years of magnetofection—Progress and prospects. *Advanced Drug Delivery Reviews* **2011,** *63*, 1300-1331.
350. Mykhaylyk, O., Sanchez-Antequera, Y., Vlaskou, D., Cerda, M. B., Bokharaei, M., Hammerschmid, E., Anton, M.,Plank, C., Magnetic nanoparticle and magnetic field assisted siRNA delivery in vitro. *Methods Mol Biol* **2015,** *1218*, 53-106.
351. Scherer, F., Anton, M., Schillinger, U., Henke, J., Bergemann, C., Krüger, A., Gänsbacher, B.,Plank, C., Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo. *Gene Therapy* **2002,** *9*, 102-109.
352. Steitz, B., Hofmann, H., Kamau, S. W., Hassa, P. O., Hottiger, M. O., von Rechenberg, B., Hofmann-Amtenbrink, M.,Petri-Fink, A., Characterization of PEI-coated superparamagnetic iron oxide nanoparticles for transfection: Size distribution, colloidal properties and DNA interaction. *Journal of Magnetism and Magnetic Materials* **2007,** *311*, 300-305.
353. Tai, M. F., Chi, K. M., Lau, K. H. W., Baylink, D. J.,Chen, S. T., Generation of magnetic retroviral vectors with magnetic nanoparticles. *Reviews on Advanced Materials Science* **2003,** *5*, 319-323.
354. Ito, A., Takahashi, T., Kameyama, Y., Kawabe, Y.,Kamihira, M., Magnetic Concentration of a Retroviral Vector Using Magnetite Cationic Liposomes. *Tissue Engineering Part C: Methods* **2009,** *15*, 57-64.
355. Namiki, Y., Namiki, T., Yoshida, H., Ishii, Y., Tsubota, A., Koido, S., Nariai, K., Mitsunaga, M., Yanagisawa, S., Kashiwagi, H., Mabashi, Y., Yumoto, Y., Hoshina, S., Fujise, K.,Tada, N., A novel magnetic crystal–lipid nanostructure for magnetically guided in vivo gene delivery. *Nature Nanotechnology* **2009,** *4*, 598-606.
356. Pan, B., Cui, D., Sheng, Y., Ozkan, C., Gao, F., He, R., Li, Q., Xu, P.,Huang, T., Dendrimer-modified magnetic nanoparticles enhance efficiency of gene delivery system. *Cancer Res* **2007,** *67*, 8156-63.
357. Zheng, X., Lu, J., Deng, L., Xiong, Y.,Chen, J., Preparation and characterization of magnetic cationic liposome in gene delivery. *International Journal of Pharmaceutics* **2009,** *366*, 211-217.
358. Chorny, M., Fishbein, I., Alferiev, I.,Levy, R. J., Magnetically responsive biodegradable nanoparticles enhance adenoviral gene transfer in cultured smooth muscle and endothelial cells. *Mol Pharm* **2009,** *6*, 1380-7.

359. Yiu, H. H., McBain, S. C., Lethbridge, Z. A., Lees, M. R.,Dobson, J., Preparation and characterization of polyethylenimine-coated Fe3O4-MCM-48 nanocomposite particles as a novel agent for magnet-assisted transfection. *J Biomed Mater Res A* **2010,** *92*, 386-92.
360. Hom, C., Lu, J., Liong, M., Luo, H., Li, Z., Zink, J. I.,Tamanoi, F., Mesoporous Silica Nanoparticles Facilitate Delivery of siRNA to Shutdown Signaling Pathways in Mammalian Cells. *Small* **2010,** *6*, 1185-1190.
361. Song, H., Yu, M., Lu, Y., Gu, Z., Yang, Y., Zhang, M., Fu, J.,Yu, C., Plasmid DNA Delivery: Nanotopography Matters. *Journal of the American Chemical Society* **2017,** *139*, 18247-18254.
362. Bose, S.,Tarafder, S., Calcium phosphate ceramic systems in growth factor and drug delivery for bone tissue engineering: A review. *Acta Biomaterialia* **2012,** *8*, 1401-1421.
363. Levingstone, T. J., Herbaj, S., Redmond, J., McCarthy, H. O.,Dunne, N. J., Calcium Phosphate Nanoparticles-Based Systems for RNAi Delivery: Applications in Bone Tissue Regeneration. *Nanomaterials (Basel)* **2020,** *10*.
364. Feray, B. Gene Delivery by Hydroxyapatite and Calcium Phosphate Nanoparticles: A Review of Novel and Recent Applications. https://doi.org/10.5772/intechopen.71062 (accessed Aug 6, 2021).
365. Wagner, D. E.,Bhaduri, S. B., Progress and Outlook of Inorganic Nanoparticles for Delivery of Nucleic Acid Sequences Related to Orthopedic Pathologies: A Review. *Tissue Engineering Part B: Reviews* **2012,** *18*, 1-14.
366. van den Boorn, J. G., Schlee, M., Coch, C.,Hartmann, G., SiRNA delivery with exosome nanoparticles. *Nature Biotechnology* **2011,** *29*, 325-326.
367. Lu, H., Wang, J., Wang, T., Zhong, J., Bao, Y.,Hao, H., Recent Progress on Nanostructures for Drug Delivery Applications. *Journal of Nanomaterials* **2016,** *2016*, 5762431.
368. Khisamutdinov, E. F., Li, H., Jasinski, D. L., Chen, J., Fu, J.,Guo, P., Enhancing immunomodulation on innate immunity by shape transition among RNA triangle, square and pentagon nanovehicles. *Nucleic Acids Research* **2014,** *42*, 9996-10004.
369. Hoeprich, S., Zhou, Q., Guo, S., Shu, D., Qi, G., Wang, Y.,Guo, P., Bacterial virus phi29 pRNA as a hammerhead ribozyme escort to destroy hepatitis B virus. *Gene Therapy* **2003,** *10*, 1258-1267.
370. Geary, C., Rothemund, P. W. K.,Andersen, E. S., A single-stranded architecture for cotranscriptional folding of RNA nanostructures. *Science* **2014,** *345*, 799-804.
371. Li, H., Lee, T., Dziubla, T., Pi, F., Guo, S., Xu, J., Li, C., Haque, F., Liang, X.-J.,Guo, P., RNA as a stable polymer to build controllable and defined nanostructures for material and biomedical applications. *Nano Today* **2015,** *10*, 631-655.
372. Rohovie, M. J., Nagasawa, M.,Swartz, J. R., Virus-like particles: Next-generation nanoparticles for targeted therapeutic delivery. *Bioengineering & Translational Medicine* **2017,** *2*, 43-57.
373. Qian, C., Liu, X., Xu, Q., Wang, Z., Chen, J., Li, T., Zheng, Q., Yu, H., Gu, Y., Li, S.,Xia, N., Recent Progress on the Versatility of Virus-Like Particles. *Vaccines* **2020,** *8*, 139.
374. Keech, C., Albert, G., Cho, I., Robertson, A., Reed, P., Neal, S., Plested, J. S., Zhu, M., Cloney-Clark, S., Zhou, H., Smith, G., Patel, N., Frieman, M. B., Haupt, R. E., Logue, J., McGrath, M., Weston, S., Piedra, P. A., Desai, C., Callahan, K., Lewis, M., Price-Abbott, P., Formica, N., Shinde, V., Fries, L., Lickliter, J. D., Griffin, P., Wilkinson, B.,Glenn, G. M., Phase 1–2 Trial of a SARS-CoV-2 Recombinant Spike Protein Nanoparticle Vaccine. *New England Journal of Medicine* **2020,** *383*, 2320-2332.
375. Bajaj, S.,Banerjee, M., Engineering Virus Capsids Into Biomedical Delivery Vehicles: Structural Engineering Problems in Nanoscale. *Journal of Biomedical Nanotechnology* **2015,** *11*, 53-69.
376. Resch-Genger, U., Grabolle, M., Cavaliere-Jaricot, S., Nitschke, R.,Nann, T., Quantum dots versus organic dyes as fluorescent labels. *Nature Methods* **2008,** *5*, 763-775.
377. Wegner, K. D.,Hildebrandt, N., Quantum dots: bright and versatile in vitro and in vivo fluorescence imaging biosensors. *Chemical Society Reviews* **2015,** *44*, 4792-4834.

378. Knipe, J. M., Peters, J. T.,Peppas, N. A., Theranostic agents for intracellular gene delivery with spatiotemporal imaging. *Nano Today* **2013,** *8*, 21-38.
379. Wang, Z., Liu, G., Zheng, H.,Chen, X., Rigid nanoparticle-based delivery of anti-cancer siRNA: Challenges and opportunities. *Biotechnology Advances* **2014,** *32*, 831-843.
380. Kim, Y. D., Park, T. E., Singh, B., Maharjan, S., Choi, Y. J., Choung, P. H., Arote, R. B.,Cho, C. S., Nanoparticle-mediated delivery of siRNA for effective lung cancer therapy. *Nanomedicine (Lond)* **2015,** *10*, 1165-88.
381. Podesta, J. E., Al-Jamal, K. T., Herrero, M. A., Tian, B., Ali-Boucetta, H., Hegde, V., Bianco, A., Prato, M.,Kostarelos, K., Antitumor activity and prolonged survival by carbon-nanotube-mediated therapeutic siRNA silencing in a human lung xenograft model. *Small* **2009,** *5*, 1176-85.
382. Al-Jamal, K. T., Toma, F. M., Yilmazer, A., Ali-Boucetta, H., Nunes, A., Herrero, M. A., Tian, B., Eddaoudi, A., Al-Jamal, W. T., Bianco, A., Prato, M.,Kostarelo, K., Enhanced cellular internalization and gene silencing with a series of cationic dendron-multiwalled carbon nanotube:siRNA complexes. *Faseb j* **2010,** *24*, 4354-65.
383. Zhang, Z., Yang, X., Zhang, Y., Zeng, B., Wang, S., Zhu, T., Roden, R. B., Chen, Y.,Yang, R., Delivery of telomerase reverse transcriptase small interfering RNA in complex with positively charged single-walled carbon nanotubes suppresses tumor growth. *Clin Cancer Res* **2006,** *12*, 4933-9.
384. Wadhwa, A., Aljabbari, A., Lokras, A., Foged, C.,Thakur, A., Opportunities and Challenges in the Delivery of mRNA-based Vaccines. *Pharmaceutics* **2020,** *12*, 102.
385. Pardi, N., Tuyishime, S., Muramatsu, H., Kariko, K., Mui, B. L., Tam, Y. K., Madden, T. D., Hope, M. J.,Weissman, D., Expression kinetics of nucleoside-modified mRNA delivered in lipid nanoparticles to mice by various routes. *Journal of Controlled Release* **2015,** *217*, 345-351.
386. Zhang, C., Maruggi, G., Shan, H.,Li, J., Advances in mRNA Vaccines for Infectious Diseases. *Frontiers in Immunology* **2019,** *10*.
387. Pardi, N., Hogan, M. J., Pelc, R. S., Muramatsu, H., Andersen, H., DeMaso, C. R., Dowd, K. A., Sutherland, L. L., Scearce, R. M., Parks, R., Wagner, W., Granados, A., Greenhouse, J., Walker, M., Willis, E., Yu, J.-S., McGee, C. E., Sempowski, G. D., Mui, B. L., Tam, Y. K., Huang, Y.-J., Vanlandingham, D., Holmes, V. M., Balachandran, H., Sahu, S., Lifton, M., Higgs, S., Hensley, S. E., Madden, T. D., Hope, M. J., Karikó, K., Santra, S., Graham, B. S., Lewis, M. G., Pierson, T. C., Haynes, B. F.,Weissman, D., Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. *Nature* **2017,** *543*, 248-251.
388. Zakrewsky, M., Kumar, S.,Mitragotri, S., Nucleic acid delivery into skin for the treatment of skin disease: Proofs-of-concept, potential impact, and remaining challenges. *Journal of Controlled Release* **2015,** *219*, 445-456.
389. Golombek, S., Pilz, M., Steinle, H., Kochba, E., Levin, Y., Lunter, D., Schlensak, C., Wendel, H. P.,Avci-Adali, M., Intradermal Delivery of Synthetic mRNA Using Hollow Microneedles for Efficient and Rapid Production of Exogenous Proteins in Skin. *Molecular Therapy - Nucleic Acids* **2018,** *11*, 382-392.
390. Van Lint, S., Goyvaerts, C., Maenhout, S., Goethals, L., Disy, A., Benteyn, D., Pen, J., Bonehill, A., Heirman, C., Breckpot, K.,Thielemans, K., Preclinical Evaluation of TriMix and Antigen mRNA-Based Antitumor Therapy. *Cancer Research* **2012,** *72*, 1661-1671.
391. Sultana, N., Magadum, A., Hadas, Y., Kondrat, J., Singh, N., Youssef, E., Calderon, D., Chepurko, E., Dubois, N., Hajjar, R. J.,Zangi, L., Optimizing Cardiac Delivery of Modified mRNA. *Molecular Therapy* **2017,** *25*, 1306-1315.
392. Davis, F. F., The origin of pegnology. *Advanced Drug Delivery Reviews* **2002,** *54*, 457-458.
393. Zalipsky, S., Chemistry of polyethylene-glycol conjugates with biologically-active molecules. *Advanced Drug Delivery Reviews* **1995,** *16*, 157-182.
394. Ikeda, Y.,Nagasaki, Y., Impacts of PEGylation on the gene and oligonucleotide delivery system. *Journal of Applied Polymer Science* **2014,** *131*.

395. Bouchard, P. R., Hutabarat, R. M.,Thompson, K. M., Discovery and Development of Therapeutic Aptamers. *Annual Review of Pharmacology and Toxicology* **2010,** *50*, 237-257.
396. Haruta, K., Otaki, N., Nagamine, M., Kayo, T., Sasaki, A., Hiramoto, S., Takahashi, M., Hota, K., Sato, H.,Yamazaki, H., A Novel PEGylation Method for Improving the Pharmacokinetic Properties of Anti-Interleukin-17A RNA Aptamers. *Nucleic Acid Ther* **2017,** *27*, 36-44.

Table of Contents Graphic:



TOC graphic description: A word cloud image of key terms from the titles and abstracts of publications related to RNA medical applications