RNA-DERIVED MEDICINES: A REVIEW OF THE RESEARCH TRENDS AND DEVELOPMENTS

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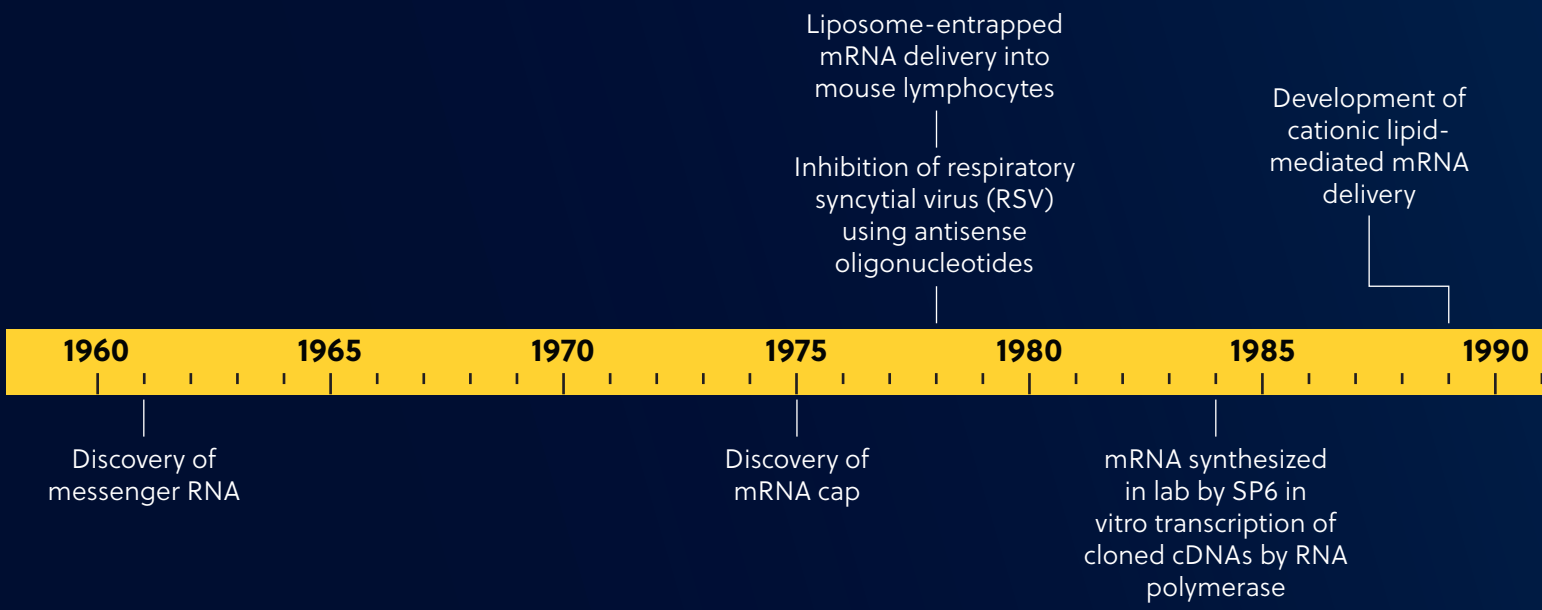
Introduction

In the last decade, there has been an encouraging shift in research, clinical development, and commercial activity to exploit the many biological roles of ribonucleic acid (RNA) for use in medicine. RNA technology provides an innovative approach to developing drugs against difficult or challenging therapeutic targets, holding potential across many diseases ranging from the largest global health challenges to extremely rare diseases.

However, RNA medicine has faced many challenges: RNA molecules are relatively unstable and transient, the limited translation into cellular protein expression can hinder efficacy, and foreign RNA molecules often trigger immunogenicity. Furthermore, the delivery of RNA molecules can be a challenge due to their relatively large size and high electric charge. Some of these practical problems can be mitigated by chemically modifying the RNA, providing the opportunity to develop therapeutics that are more stable, effective, and tolerable for patients.

The recent success of mRNA vaccines against COVID-19 and the approval of new RNA-based drugs has provided new momentum to the field, building on the key milestones and achievements of the last 60 years (**Figure 1**). Now, advances in our understanding of RNA structure and function, combined with a robust production pipeline, have substantially increased the capacity to develop clinically effective RNA-related applications.[1-12]

In this white paper, we used data from the CAS Content Collection™[13]—the largest human-curated collection of published scientific knowledge—to review the application of RNA in medicine and the use of chemical modifications and nanotechnology to improve the delivery and efficacy of RNA pharmaceuticals. We focus on chemical modifications to the nucleic acid base, backbone, and sugar molecules to increase RNA stability, along with the new delivery systems that are critical to the success of RNA medicine.



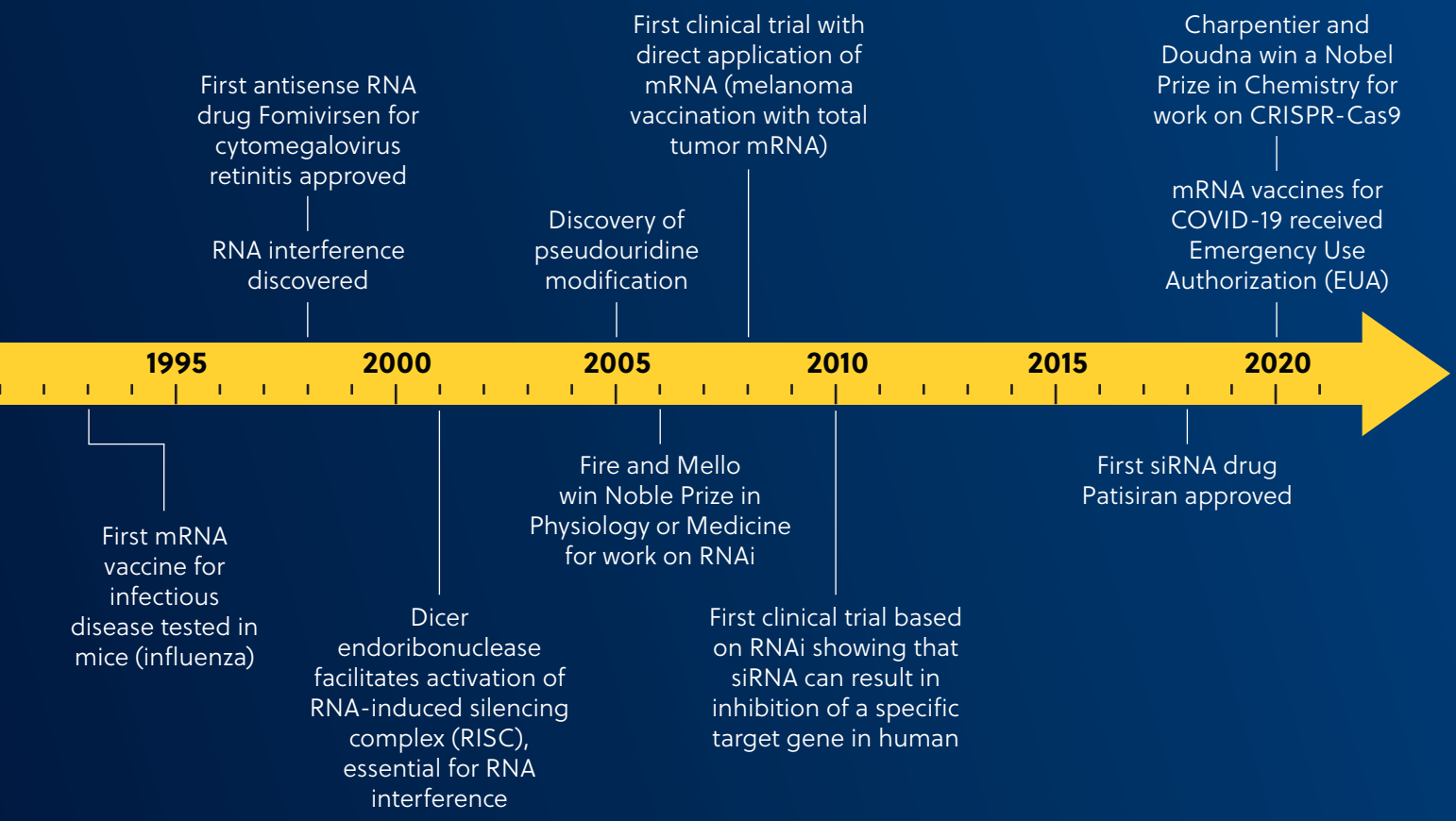


Figure 1. Timeline of major RNA research and development milestones. A more detailed timeline table complete with references can be found at https://www.cas.org/rna

Publication trends in RNA research: insights from the CAS Content Collection

Publication trends by type of RNA

Led by our team of scientists and AI experts, the CAS Content Collection[13] was analyzed to determine the number of publications discussing different types of RNA over the past 25 years (**Figure 2**). In addition to the steady increase in the number of journal publications and patents relating to RNA, the research has gradually become more diversified as new types of RNA are being discovered. This can be seen particularly in the areas of siRNA, miRNA, lncRNA, and CRISPR-related research. Notably, CRISPR technology has had a rapid increase recently in the volume of patent publications, accounting for 20% of the overall RNA-related patent publications in the year 2020.

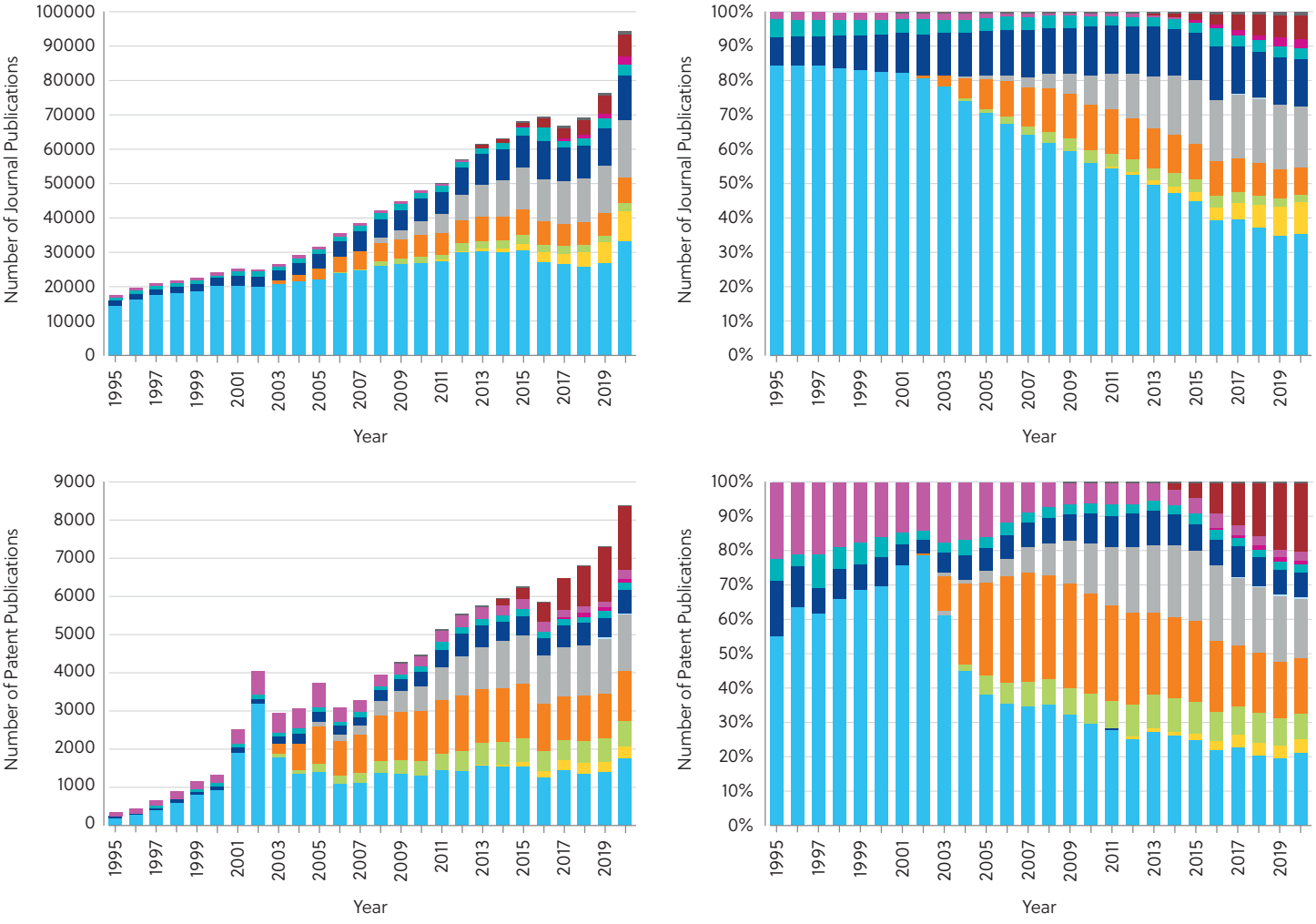


Figure 2. Document publication trends for different types of RNA from 1995-2020. Top two panels: journal publications in absolute numbers and given year percentages. Bottom two panels: patent publications (counted once per patent family) in absolute numbers and given year percentages

We also explored the publication volume trends of different types of RNA (**Figure 3**) and although the cumulative publication numbers for circRNA, exosome RNA, lncRNA, and CRISPR are relatively small compared with others (**Figure 2**), they are increasing at much faster rates.

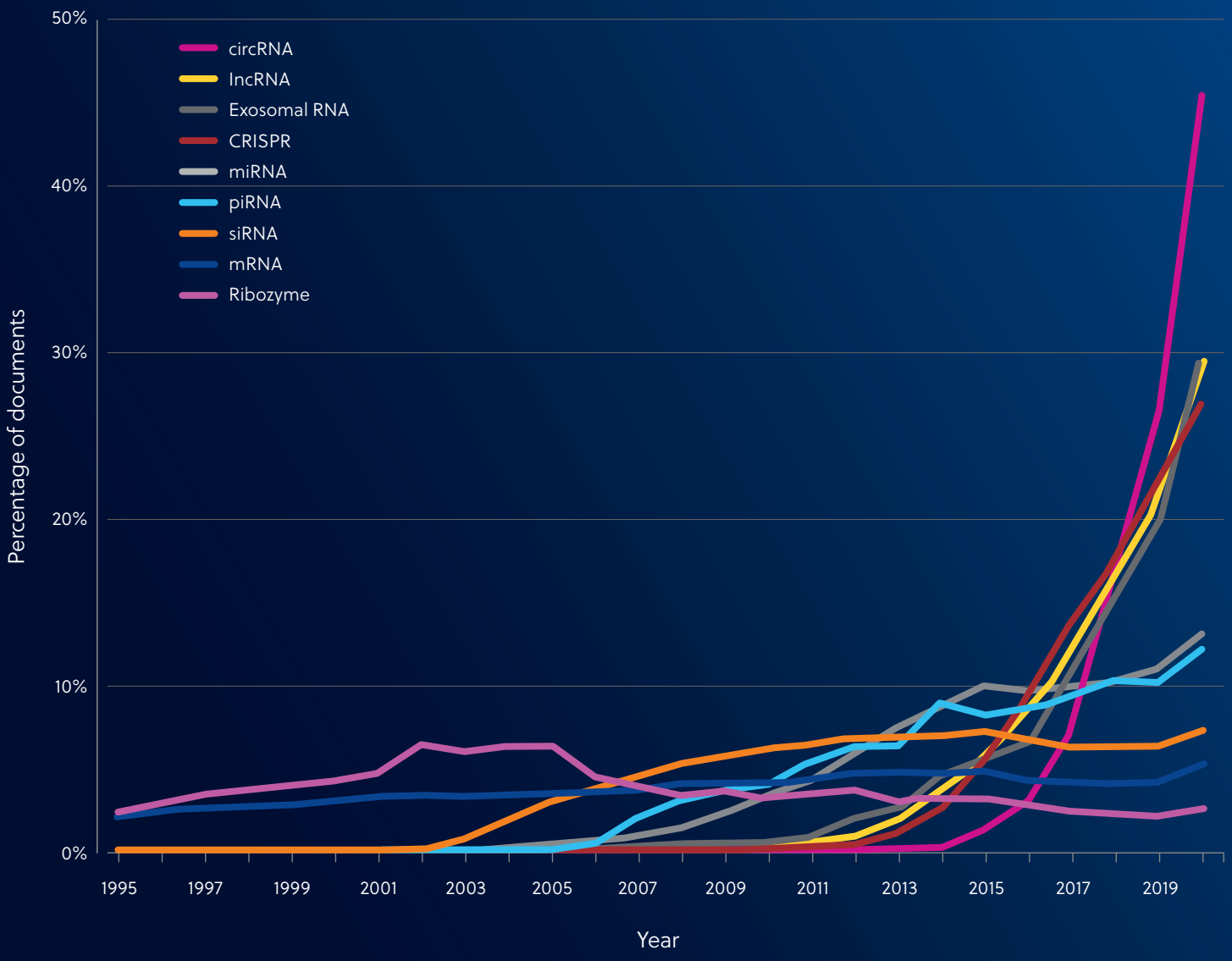


Figure 3. Trends in publication volume for different RNA types in the years 1995–2020. Percentages are calculated with yearly publication numbers normalized by total publications in the years 1995–2020 for each RNA type

Publication trends for RNAs used in medical applications

The broad application potential of RNA medicines—including as therapeutic RNAs, diagnostic biomarkers, and therapeutic targets—has generated notable attention in the scientific and medical community. The CAS Content Collection[13] shows that the number of journal publications and patents related to RNA applications in medicine has experienced a steady growth in the years 1995–2020 (**Figure 4**). The peak in patents around 2001–2002 is likely related to the first human clinical trial using the first dendritic cells transfected with mRNA encoding tumor antigens (a therapeutic mRNA cancer vaccine) in 2001,[14,15] while the spike in publication numbers in 2020 is likely a result of the interest in the COVID-19 mRNA vaccines.

There is also a swell in publications between 2011–2016 as a result of a growing interest in siRNA and miRNA—an interest that decreased temporarily with the discovery of their off-target effects. Interest in mRNA also increased between 2011–2016 before tapering off, only to be recovered once mRNA vaccines took center stage in the fight against COVID-19.

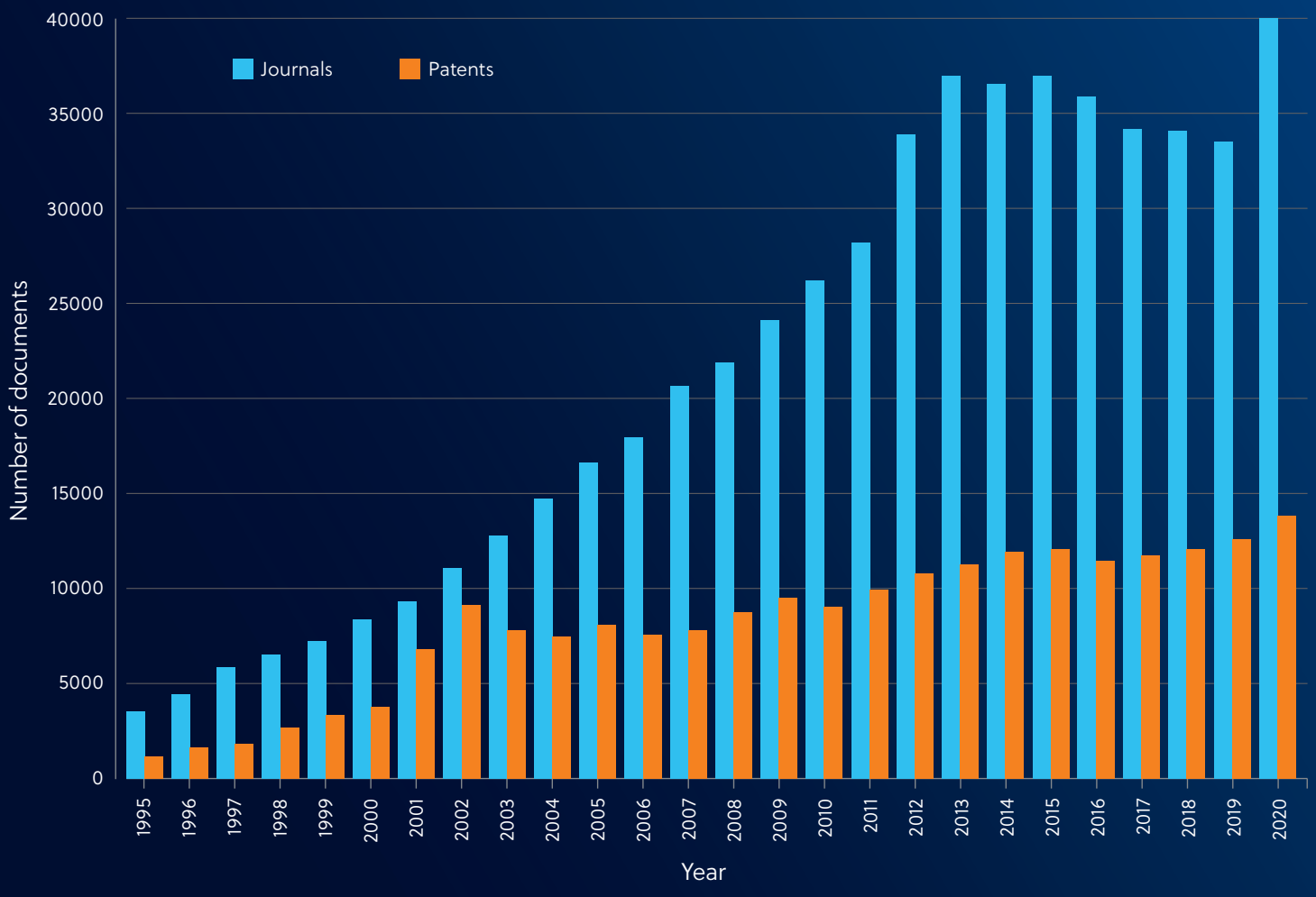


Figure 4. Numbers of journal documents and patents related to RNAs for medical use by year

The distribution of diseases associated with RNA medicine in journal and patent publications

Since the first approved antisense oligonucleotide (ASO) RNA therapeutics in 1998, the research and development of RNA as therapeutics, vaccines, and diagnostic agents has expanded across diverse disease areas. Fifty percent of journal publications and patents are associated with cancer diagnosis or treatment, although lung, liver, and metabolic diseases are also highly researched (**Figure 5**).

There was little correlation between different types of RNA and the disease area, indicating that a range of RNA types have been explored for many disease areas in the research phase.

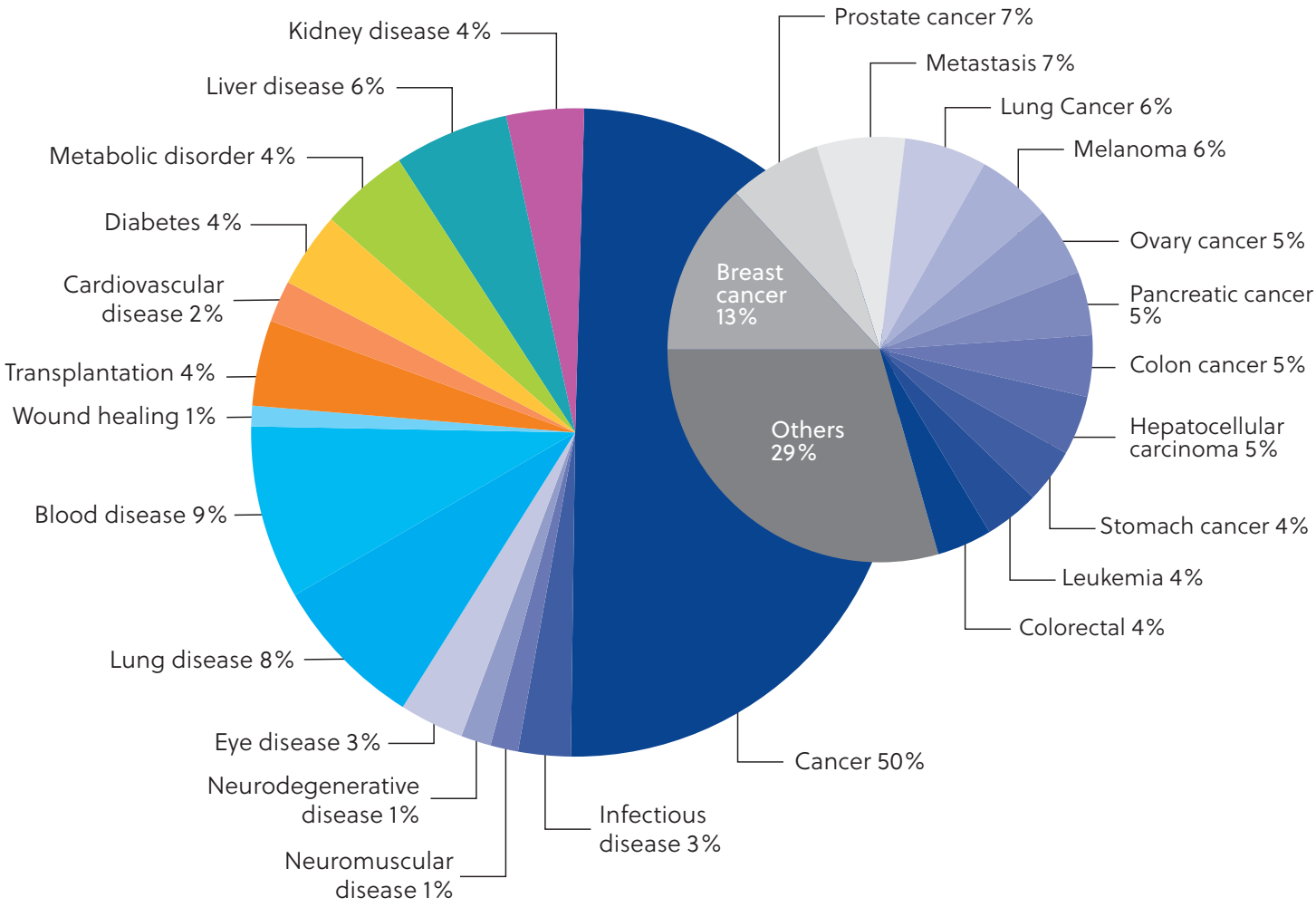


Figure 5. Percentage of publications associated with RNAs in medical applications

In addition to the research and development phase, we also analyzed trends in therapeutic and diagnostic patents for the most common diseases treated by RNA (**Figure 6**). Infectious diseases and cancer are the most frequent diseases treated with RNA, having witnessed the largest growth in the number of patents over the past 20 years (particularly for hepatitis B, influenza, pancreatic neoplasm, melanoma, and non-small cell lung cancer). The number of patents for eye and cardiovascular diseases also grew between 2000–2010 before remaining relatively stable over the following decade.

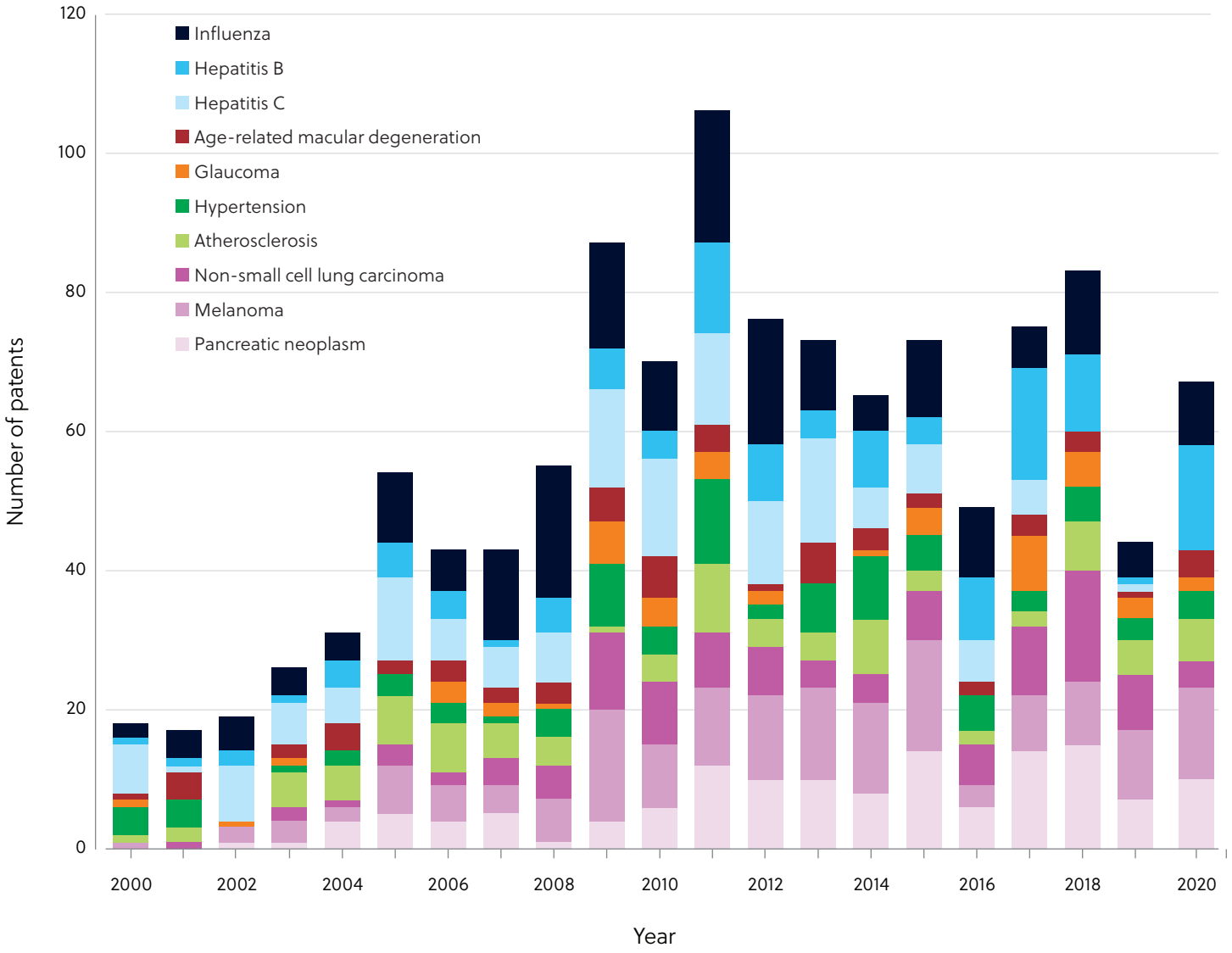


Figure 6. The yearly number of patent publications on specific diseases targeted by RNA therapeutics, vaccines, and diagnostics

Development of RNAs for therapeutics and vaccines: clinical trials and leading companies

After decades of extensive research, the therapeutic potential of RNAs has led to the development of over 250 RNA medicines that are approved or in development. Using the CAS Content Collection,[13] we categorized these therapeutics or vaccines based on their targeting diseases or development status (**Figure 7**)

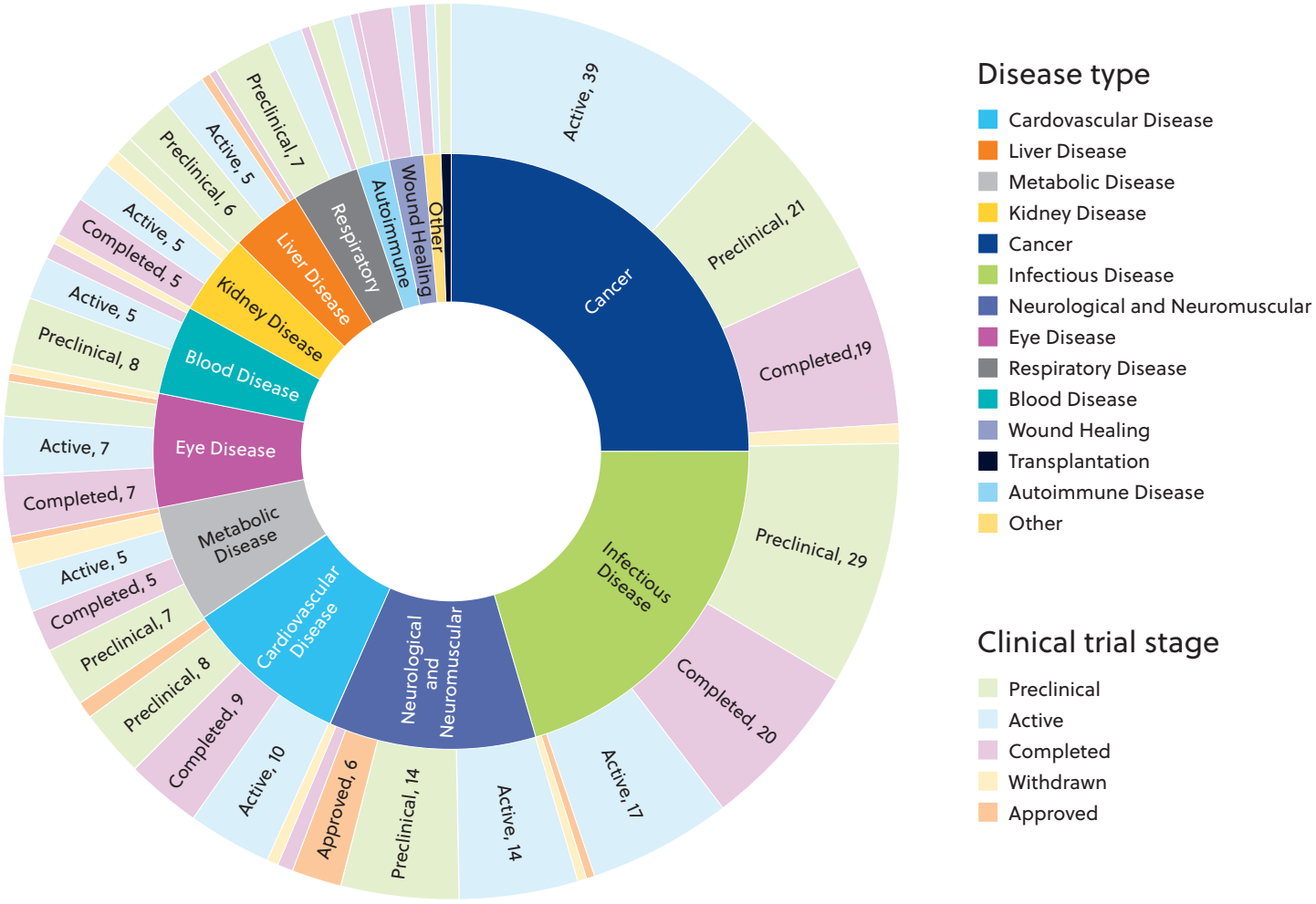


Figure 7. Counts of potential therapeutics and vaccines in different development stages (preclinical, clinical, completed, withdrawn, and approved) for various disease types. (Full list of such clinical trials can be found at https://www.cas.org/rna)

Cancer has the highest number of therapeutics and vaccines in research phases, followed by infectious diseases. However, neurological and neuromuscular diseases currently have the most approved treatment options on the market, followed by cardiovascular disease and infectious diseases. The COVID-19 pandemic has brought RNA medicines to the forefront for infectious disease in both research phases and the number of approved therapeutics, bringing the first mRNA medicines to market.

Figure 8 showcases the top 15 RNA therapeutic and vaccine companies worldwide. Companies typically specialize in one type of RNA but will investigate its applications across multiple diseases. Among the different types of RNAs, mRNA and siRNA are the most utilized by the top 15 companies, followed by ASO, CRISPR, and aptamers.

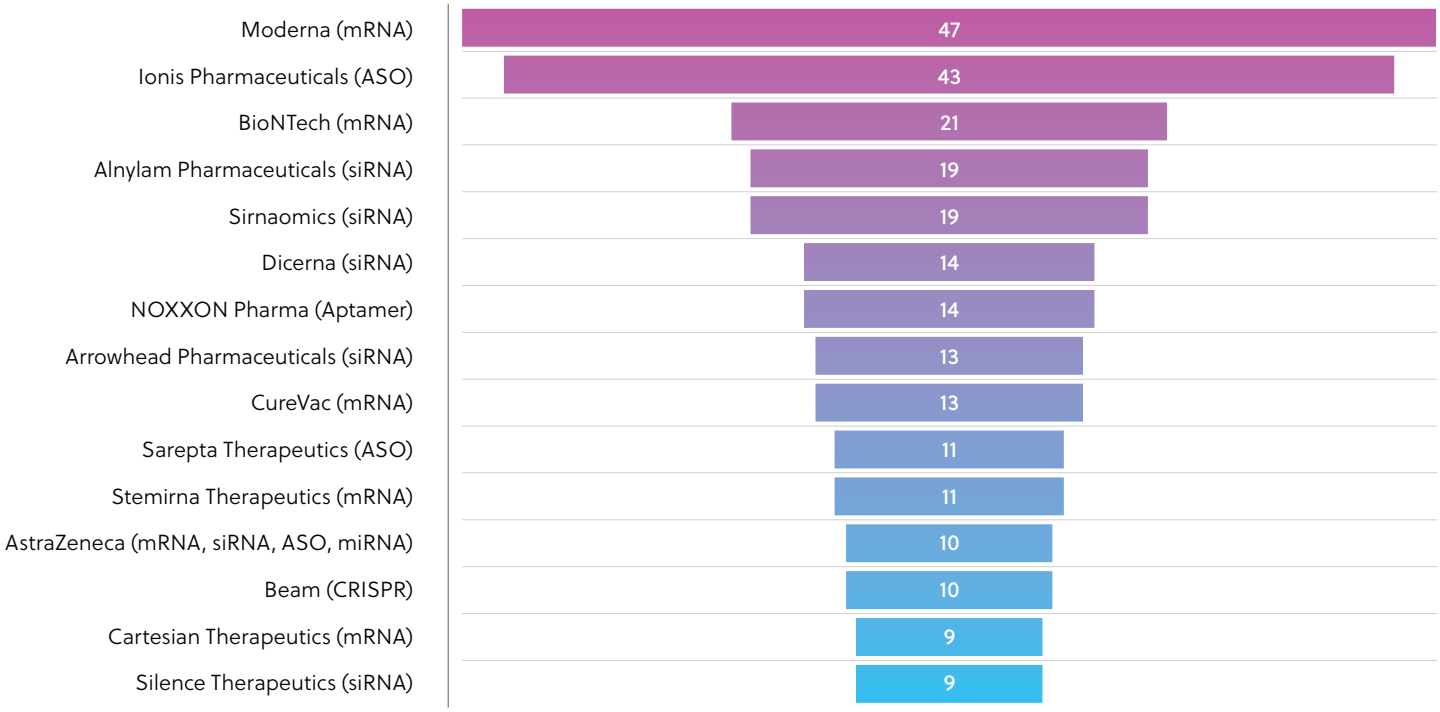


Figure 8. Top pharmaceutical companies ranked by the number of RNA therapeutic and vaccine agents in the development pipeline. Counts include RNA therapeutic or vaccine agents in company-announced preclinical development, in clinical trials, or approved. A single RNA agent can be counted multiple times when applied to multiple diseases

Chemical modifications for improving RNA stability and target specificity

RNA consists of a single strand of ribonucleosides (a nucleic acid base attached to a D-ribose sugar) that are linked together by phosphodiester bonds.[16] Stability of RNA therapeutics and vaccines remains a challenge, as RNA is susceptible to degradation by nucleases and hydrolysis by basic compounds in the cellular environment. Chemical modification to the nucleic acid base, the ribose sugar, or the sugar-phosphate backbone can help protect RNA from degradation and improve target specificity, thereby lowering the risk of off-target effects. For those RNAs that act by forming a duplex with a target, thermal destabilizing mutations (mutations that lower the melting temperature of the duplex) can destabilize the complex and improve target specificity by lowering the ability of the RNA to form duplexes with non-target RNA. RNA modification also can improve the delivery of the RNA to the interior of the cell through the plasma membrane, enhancing its activity.[17] Advancements in approaches to modify RNA have helped overcome previous challenges in human RNA medicine, opening the door to a range of innovative medical applications.

Types of Chemical Modifications

Table 1: Types of RNA chemical modifications

Type of modification	Common modifications
RNA base modifications Base modifications that interfere with the formation of hydrogen bonds can thermally destabilize the formation of a duplex with the target and thus improve target specificity by limiting off-target binding.[18] In addition, modification can improve the performance of therapeutic RNA by improving translation, lowering cytotoxic side effects, and immune responses to the mRNA.[19] Additional modified or rare bases are shown in https://www.cas.org/rna	– Methylation of cytidines or uridines (N-5 position) to 5-methylcytidines or 5-methyluridines – Reduction of uridines on the base ring to 5,6-dihydrouridines – Oxygen of cytidines replaced with a sulfur (N-2 position) to become 2-thiocytidines – Oxygen of uridines replaced with a sulfur (N-2 or the N-4 position) to become either 2-thiouridines or 4-thiouridines – Methylation at the N-7 position of the guanosine (which often occurs for the mRNA cap modifications) – Nitrogen of guanosines (N-7 position) converted to a carbon to become 7-deazaguanosine – Similar modifications can happen to adenosines resulting in 7-methyladenosines and 7-deazaadenosines – Pseudouridines and inosines can serve as rare bases
Modifications on ribose The difference between the relatively stable DNA and the less stable RNA is the hydroxyl group on the C-2′ position of the ribose in RNA. Replacing this group can increase stability and reduce off-target effects	– 2′-O-methyl – 2′-fluoro – 2′-O-methoxyethyl (MOE) – 2′-amine – Locked nucleic acid (LNA) (2′-O, 4′-C-methylene bridge connects the 2′ position to the 4′ position on the ribose)[20]
Backbone modifications Modifications at the phosphate group in the sugar-phosphate backbone can improve RNA delivery by neutralizing the negative charge that can interfere with transport across membranes and confer increased resistance to nucleases, thereby improving RNA stability[21]	– Oxygen on phosphate group replaced by a sulfur (phosphorothioate)[22]

Trends in RNA chemical modifications

Using the ~170,000 modified RNA sequences that are annotated and collected in the CAS Content Collection,[13] chemical modifications were further analyzed with specific types of modifications and sequence lengths (**Figures 9 and 10**).

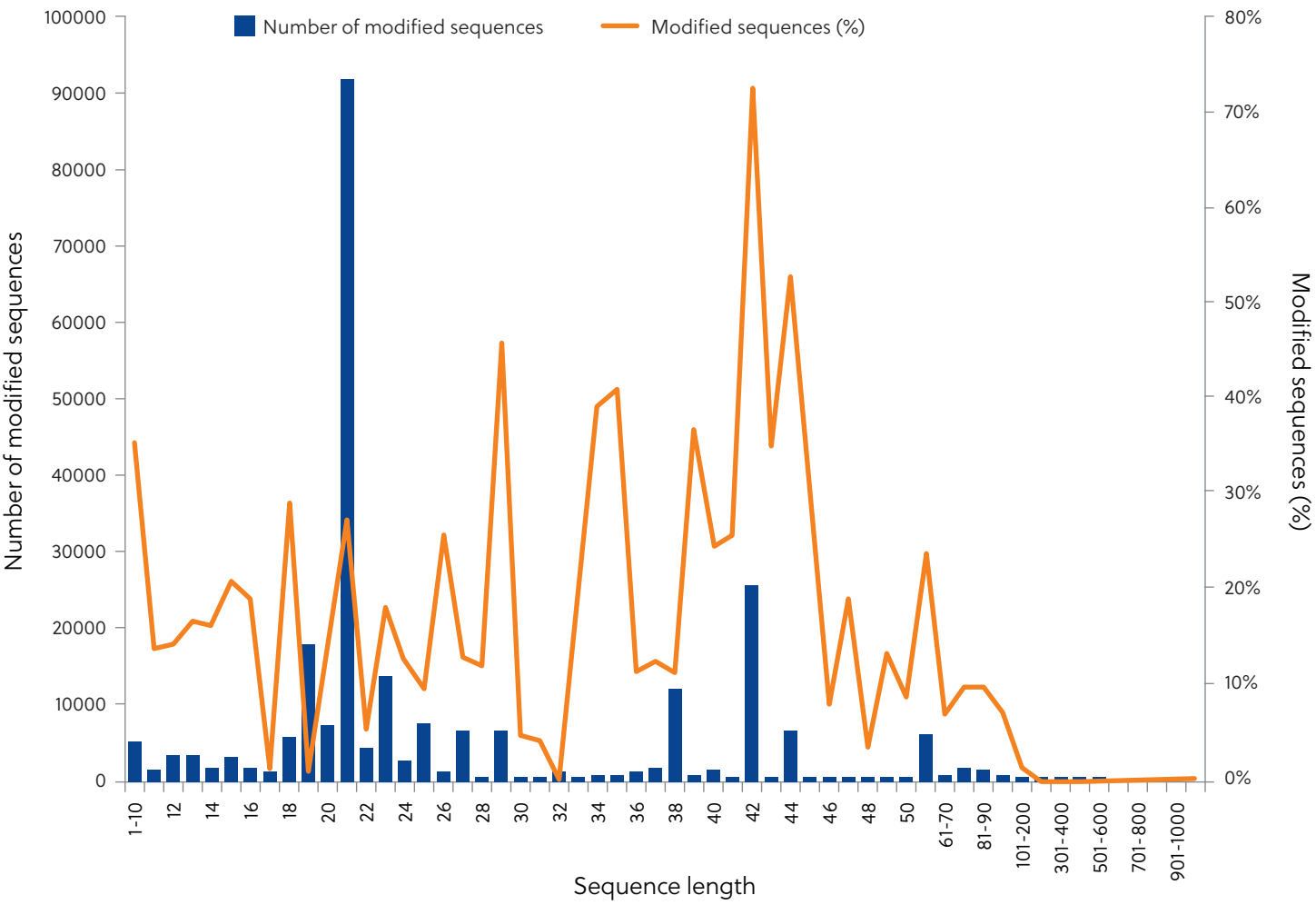
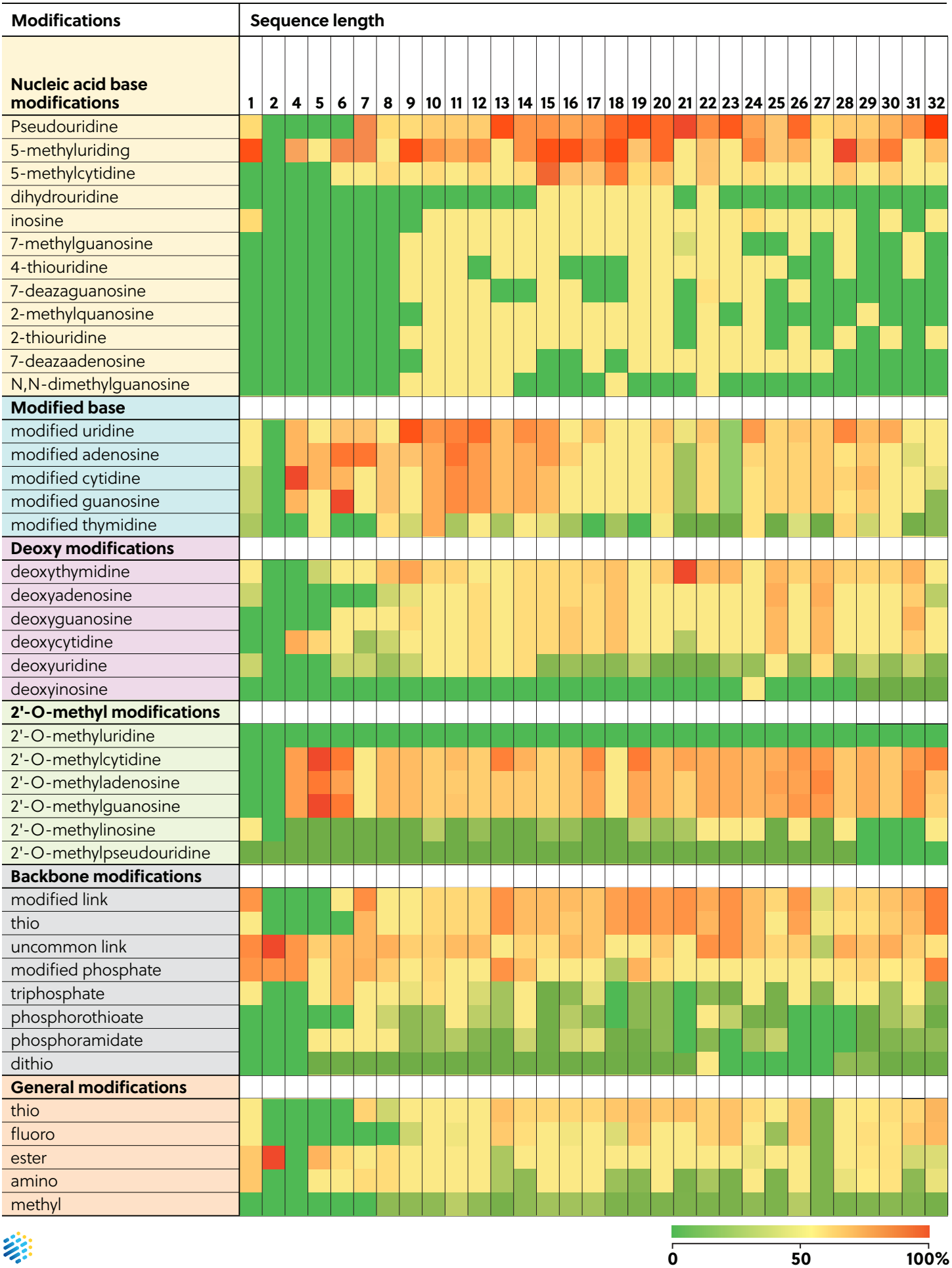


Figure 9. RNA sequences containing modifications and their distribution with respect to sequence lengths (from the CAS Content Collection). Blue bars: the absolute number of modified RNA sequences; orange line: the percentage of modified RNA sequences in the total RNA sequences with the same sequence length

According to the data in the CAS Content Collection,[13,23] the use of RNA modification began to take off in 1995 and is associated with smaller sequence lengths (**Figure 9**). Modified 18–27-nucleotide RNAs are predominant, reflecting the fact that this sequence length is commonly used in siRNAs and ASOs. Single strands making up the double-stranded siRNA are commonly 21 or 23 nucleotides in length—the length of the processed, naturally occurring siRNAs. 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. There is a sharp contrast in the types and frequency of modifications in sequences <100 nucleotides versus >100 nucleotides (**Figure 10**). Sequences that are <100 nucleotides in length are modified much more frequently than the longer sequences, which contain 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 of these longer sequences might provide steric hindrance that could inhibit translation, thus limiting the efficacy of the RNA medicine.


Modifications
Sequence length
Nucleic acid base modifications
1 2 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25 26 27 28 29 30 31 32
Pseudouridine
5-methyluriding
5-methylcytidine
dihydrouridine
inosine
7-methylguanosine
4-thiouridine
7-deazaguanosine
2-methylquanosine
2-thiouridine
7-deazaadenosine
N,N-dimethylguanosine
Modified base
modified uridine
modified adenosine
modified cytidine
modified guanosine
modified thymidine
Deoxy modifications
deoxythymidine
deoxyadenosine
deoxyguanosine
deoxycytidine
deoxyuridine
deoxyinosine
2'-O-methyl modifications
2'-O-methyluridine
2'-O-methylcytidine
2'-O-methyladenosine
2'-O-methylguanosine
2'-O-methylinosine
2'-O-methylpseudouridine
Backbone modifications
modified link
thio
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thio
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50
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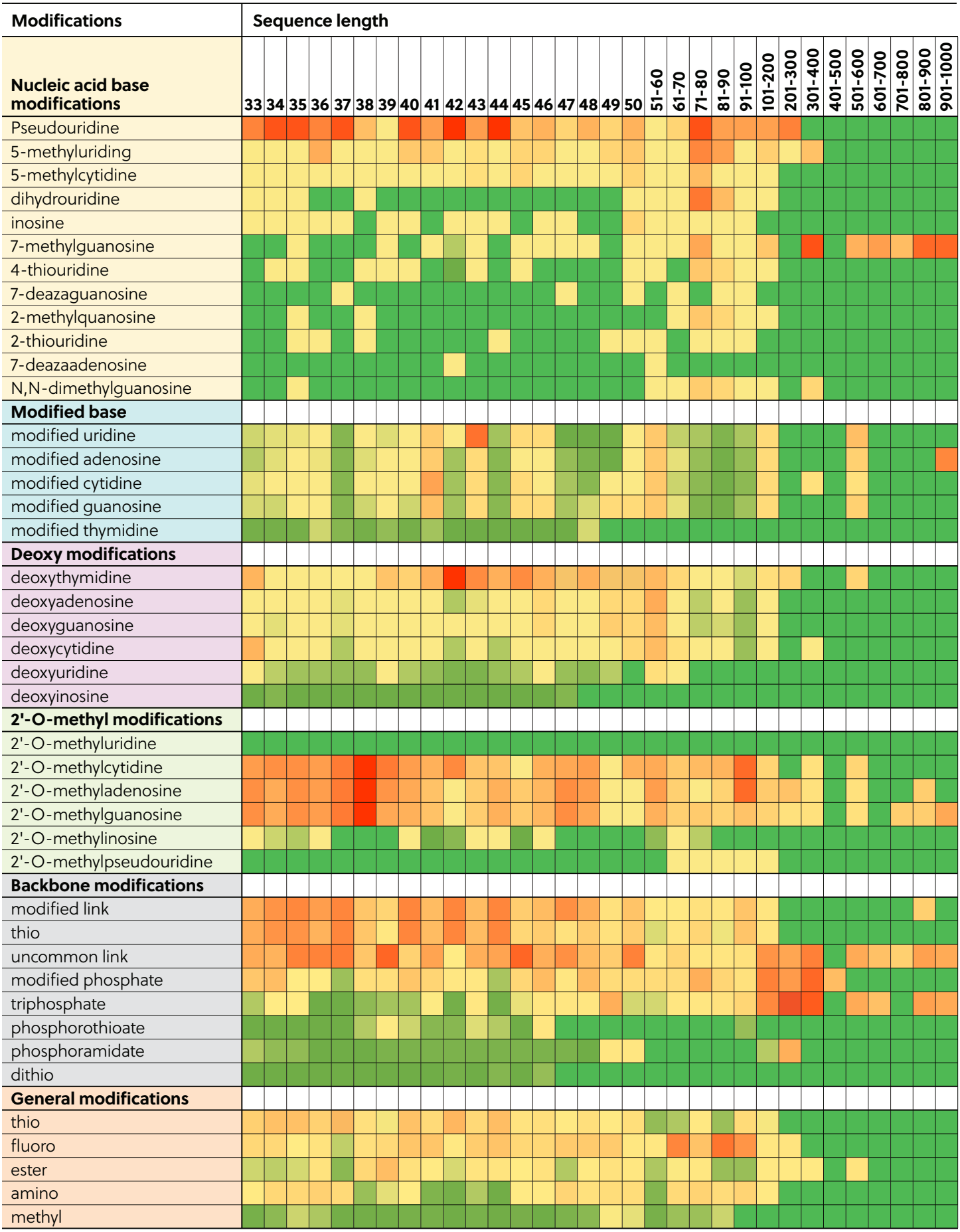

Figure 10. Frequencies of modifications of RNA and their distributions based on sequence lengths (CAS Content Collection[13])

In summary, chemical modifications protect RNA medicines from nuclease degradation and the cellular environment, and they improve target specificity to enhance pharmaceutical activity. There are several opportunities for chemical modification that can be used to tailor a therapeutic RNA for its dedicated target: RNA base modifications can improve stability and translation of therapeutic RNAs; ribose modifications can mitigate off-target effects by lowering the thermal stability to enhance target-specific binding; and the choice of the backbone determines whether the RNA blocks cellular processes such as translation, transcription, or splicing or targets an RNA for nuclease digestion. A major innovation for all RNAs is that the type and degree of modification can be adjusted to enhance the effectiveness of the RNA medicine.

Nanocarrier-related research

RNA delivery systems face numerous challenges; they need to protect the RNA against nuclease degradation, bypass the immune system, avoid non-specific interactions with serum proteins, and block renal clearance.[11] While these biological barriers are usually addressed by modifying the chemical structure of the RNA, additional strategies are necessary to overcome other barriers in the body; RNA nanocarriers provide a successful way to protect and deliver RNA medicines.[24]

Trends in research on RNA delivery systems

Currently, there are nearly 7,000 scientific publications in the CAS Content Collection[13] related to the RNA delivery systems, including patents and non-patents (journal articles, books, dissertations, meeting abstracts, etc.).[13,25] RNA carrier-related studies are dominated by the lipid nanoparticles, closely followed by the polymeric nanocarriers (**Figure 11**).

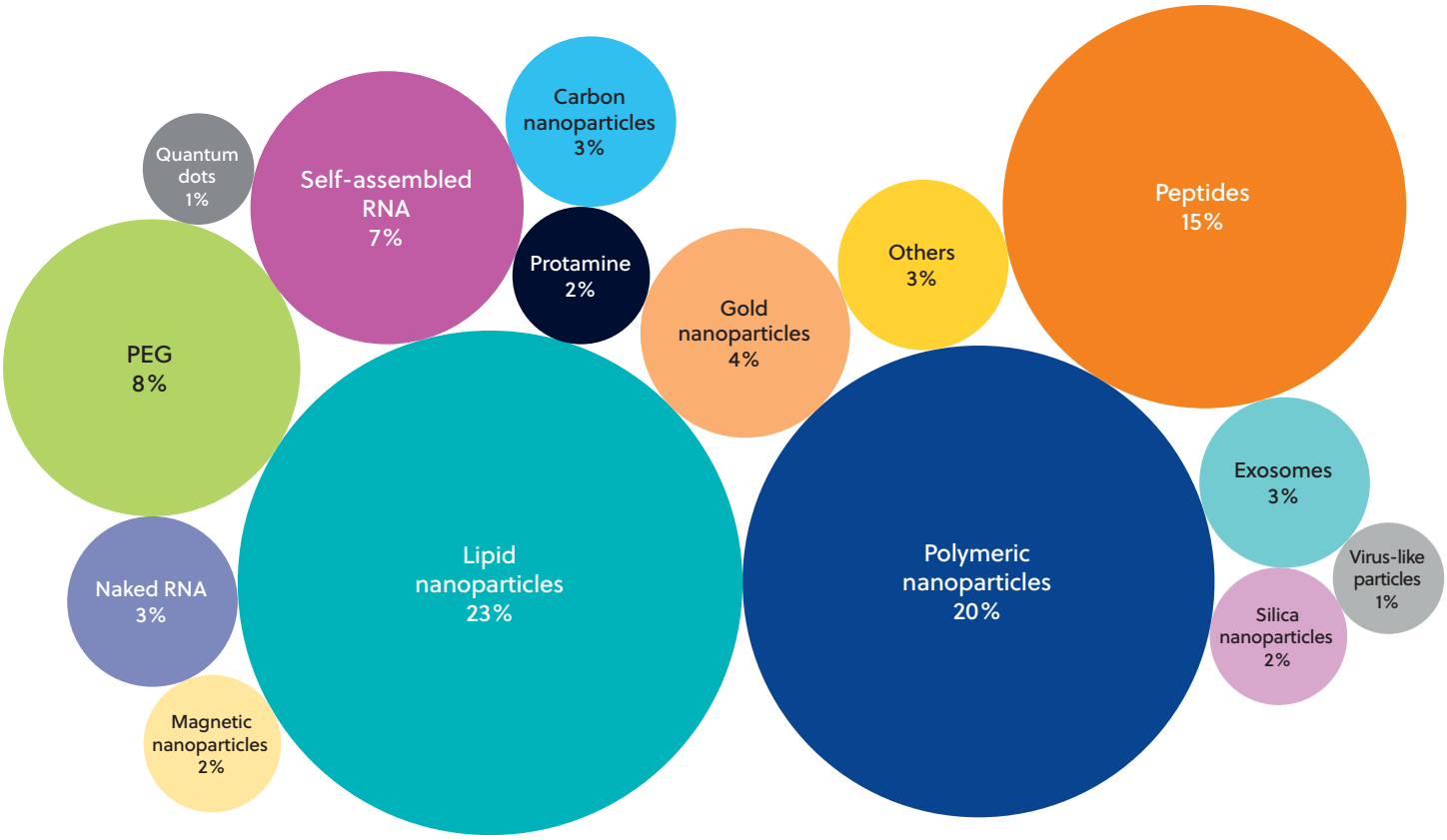

Figure 11. Percentage distribution of RNA nanocarrier-related documents in the CAS Content Collection[13]

While many vehicles have been used for the delivery of RNA medicines, an ideal RNA-carrier should have low toxicity, biodegradability, and biocompatibility. Potential candidates include lipids, chitosan, cyclodextrin, polyethyleneimine (PEI), poly(lactic-co-glycolic acid), dendrimers, magnetic nanoparticles, carbon nanotubes, gold nanoparticles, silica nanoparticles, and others.

Table 2: The function and advantages of types of RNA nanocarriers

RNA delivery system	Function	Advantages
Lipid nanoparticles	Synthetic cationic lipids facilitate cell membrane adsorption and fusion to deliver anionic nucleic acids to target cells	– Ease of production[26] – Biodegradability[26] – Increased RNA stability[26] – Enhanced cellular uptake and intracellular release[26]
Polymeric nanoparticles	Cationic polymers (e.g., proteins, polysaccharides) form a protective 'shell' to encapsulate anionic nucleic acids for cellular delivery[27]	– Versatile, adaptable, and scalable[27] – Low immunogenicity and cellular toxicity[27]
Peptides	Short cell-penetrating peptides with variable sequences, lengths, and polarities to facilitate cellular uptake via multiple pathways	– Versatile – Biocompatible – High target specificity
Gold nanoparticles	The dense shell of oligonucleotides on the surface of the gold nanoparticles protects the conjugated RNA (covalent attachment or supramolecular assembly)	– Adaptable[28,29] – Biocompatible[28,29] – Protects RNA from degradation[28,29]
Exosomes	Natural transporters[30] that are secreted by most cells and may be loaded with RNAs for cellular uptake and delivery	– Lower toxicity and immunogenicity – Unique membrane composition enhances ability to enter target cells – No accumulation of therapeutic RNAs in the liver[31,32]
Magnetic nanoparticles	Deliver nucleic acids through the application of a magnetic field	– Highly efficient transfection efficiency
Carbon nanotubes	Tubes made of carbon with nanosized diameters (carbon nanotubes) provide a strong material. They can promote internalization in the cell and gene silencing[35]	– Strong, yet highly flexible with high tensile strength
Virus-like particles	Either naturally occurring empty virus shells or synthetic virus-like protein complexes are used to carry and delivery RNA to target cells	– Biocompatibility[36] – Biodegradability[36] – High target specificity[36]

RNA delivery system	Function	Advantages
Protamine	Naturally occurring cationic peptides form electrostatic complexes with nucleic acids	– Protects RNA from degradation
Silica nanoparticles	Hollow particles that can encapsulate therapeutics and allow slow release of the cargo as they degrade in the body	– Large surface area and tunable pore size[37] – Simple surface modifications[37] – Efficient encapsulation of cargo molecules[37]
Quantum dots	Semiconductor crystalline nanoparticles with unique tunable optical properties that emit narrow wavelength bands under a wide excitation range. Quantum dots can be used to deliver RNAs to target cells or track RNA distribution within cells[38]	– High stability – Adaptable – Most desirable for imaging-guided therapies
PEGylation	The covalent attachment of polyethylene glycol (PEG) helps form a micelle-like structure to deliver RNAs efficiently to target cells	– Reduced immune response[39] – Improves pharmacokinetic properties[40] – Enhanced efficacy[40] – Increased RNA stability[41]
Self-assembled RNA	Supramolecular assembly of individual molecules driven by non-covalent interactions; includes lipid and polymer nanoparticles	– Highly customizable
Naked RNA	RNA delivered without the use of a nanocarrier	– Can provide a more efficient translation than mRNA-loaded nanoparticles for certain routes of drug administration[42]

Conclusion

Over the last 50 years, our understanding of the types and functions of RNA has vastly expanded. The ability to leverage the wide range of biological functions of RNA opens vast possibilities for the development of new therapeutic molecules, diagnostic biomarkers, or clinical targets. Even more, innovative approaches in chemical modifications and RNA nanocarriers have started to mitigate the classical challenges faced in human RNA medicine, including instability, insufficient delivery or efficacy, and off-target effects. After the recent success of the lipid nanoparticle-based mRNA vaccines against COVID-19, the enthusiasm around RNA medicines has been reignited, and the continued development of novel technologies in RNA modification and delivery systems present exciting avenues of exploration and evaluation.

With these advancements, there are now approved RNA medicines in cardiovascular, metabolic, liver, infectious, neurological, neuromuscular, kidney, and eye diseases—with the hope of many more that are currently in the research pipeline. With the cost-effectiveness, ease in manufacturing, and opportunity to tailor RNAs to previously 'undruggable' sites, RNA medicines now hold promising potential to treat a wide range of diseases from the most common to the extremely rare.

In this white paper, the CAS Content Collection[13] search has highlighted current research trends in RNA research and development, including a notable explosion in circRNA, exosome RNA, lncRNA, and CRISPR-related research. In addition, cancer and infectious diseases such as COVID-19 were identified as key therapeutic areas for RNA medicines. We also describe the different types of chemical modifications and their applications in medicine and identify lipid nanoparticles and polymeric nanocarriers as the predominant players in RNA-delivery research.

Our expanded understanding of RNA in its many forms has allowed us to develop more stable and effective RNA medicines. These developments, combined with innovative nanotechnology delivery systems, have resulted in an explosion of new therapeutic options for diseases ranging from viral infections to cancer. This arsenal of adaptable and targeted RNA medicines has the potential to revolutionize the treatment of human disease.

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