Check for updates

ADVANCED THERAPEUTICS
www.advtherap.com

RNA-Based Therapeutics: Cutting-Edge Advances in Clinical Research

Débora Ferreira and Ligia R. Rodrigues*

Over the years, the relevance of gene therapy has increased as an alternative to conventional drugs. Pharmaceutical industries have been directing their efforts toward the creation of novel gene-based therapies for a broad range of diseases. RNA-based therapeutics can efficiently trigger gene silencing and, despite the initial research setbacks, this technology laid the groundwork for advancements in RNA-based drug design that ultimately contributed to the remarkably rapid advance of messenger RNA (mRNA) vaccines to combat the COVID-19 pandemic. In light of this information, RNA medicines are currently well-positioned to have a noteworthy impact in clinical practice. Various RNA medicines have received clinical approval, while others are still in the research phase or undergoing preclinical trials. In this review, an overview of RNA-based therapeutics is aimed to provide, including antisense oligonucleotides and RNA interference-based mechanisms, mRNA-encoded approaches, aptamers, and clustered regularly interspaced short palindromic repeats (CRISPR-Cas)-mediated genome editing. Furthermore, the commonly used chemistries in drug delivery systems and strategies for targeting specific cells, as well as the key nanocarriers used in RNA loading, are explored. The main advances and current challenges will be thoroughly summarized.

1. Introduction

The fast progress of messenger RNA (mRNA) vaccines in response to the COVID-19 crisis has sparked renewed enthusiasm in the potential of RNA as a molecular entity class across diagnostics, prevention, and treatment of diverse illnesses. Indeed, the development timeline of the mRNA-based COVID-19 vaccine stands out as remarkably rapid compared to other approved RNA-based therapeutic classes[1,2] (**Figure 1**). From infectious diseases to cancer, neurodegeneration, metabolic disorders, and rare genetic conditions, RNA holds significant promise in tackling health challenges formerly considered intractable. RNA therapies possess the capability to regulate gene expression or generate therapeutic proteins, rendering them applicable to health conditions with identified genetic targets. Leveraging RNA's distinctive structural and biochemical traits allows precise targeting and programmable applications, given the well-understood principles of complementary base pairing governing RNA binding affinity and specificity. Unlike traditional drug development, which often involves exhaustive screening processes, RNA-based therapies can be meticulously designed once the target is identified. Consequently, RNA therapies are emerging as versatile platform technologies adaptable to diverse disease contexts.

Using RNA as a therapeutic encounters various hurdles, including its inadequate pharmacological characteristics, challenges in intracellular delivery with consequent exposure to off-target cells and immune-related toxicity.[1,3,4] Overcoming these obstacles required chemistry advancements and a deeper knowledge of natural antisense RNA phenomena in order to materialize RNA drugs as a viable option. Moreover, to overcome these barriers and in pursuit of a safe and efficient RNA delivery approach, scientists worldwide have devised delivery systems encompassing both viral-vector and non-viral methodologies. This is pertinent due to the significant negative charge inherent in RNAs, making them susceptible to numerous chemical alterations. The fast progress in nanotechnology and materials science offers abundant opportunities and potential solutions for the hurdles associated with delivering RNA-based drugs.[3]

In this review, we elaborate on the increasing array of therapeutic RNA cargoes, elucidating how the resulting biochemical mechanisms influence delivery. After scrutinizing the commonly utilized chemistries in drug delivery systems and strategies for cell-specific targeting, we spotlight important nanocarriers employed in RNA encapsulation. Furthermore, we summarize the ongoing preclinical and clinical trials concerning RNA therapeutics.

2. RNA-Based Therapeutics and Modes of Action

In theory, RNA holds the potential to target any desired gene by accurately pinpointing its precise nucleotide (nt) sequence. However, it is noteworthy that only a small fraction, ≈0.05% of the human genome, has been targeted by protein-targeted therapeutics

D. Ferreira, L. R. Rodrigues
CEB – Centre of Biological Engineering
University of Minho
Braga 4710-057, Portugal
E-mail: deboraferreira@ceb.uminho.pt
D. Ferreira, L. R. Rodrigues
LABBELS – Associate Laboratory
Braga, Guimarães, Portugal

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/adtp.202500082

DOI: 10.1002/adtp.202500082

 © 2025 Wiley-VCH GmbH

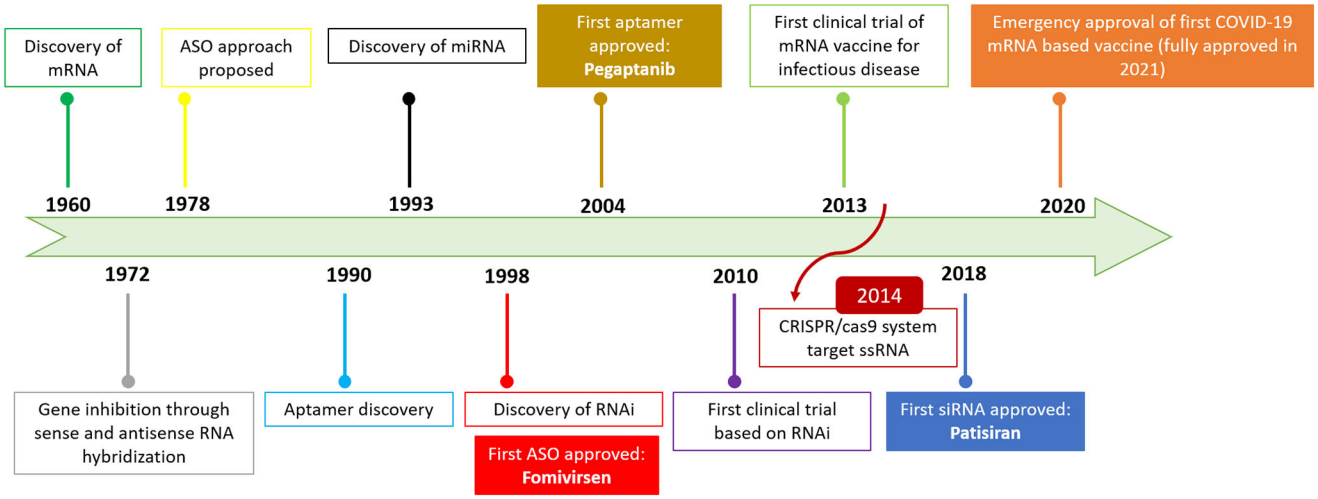


Figure 1. An overview of the major RNA-based technological developments. ASOs – antisense oligonucleotides, CRISPR-Cas9 – clustered regularly interspaced palindromic repeats, mRNA – messenger RNA, miRNA – microRNA, RNAi – RNA interference, siRNA – small interfering RNA, ssRNA – single-stranded RNA.

currently approved, predominantly comprising small-molecule compounds and antibodies. This limitation stems from the fact that a significant portion of the human's genome DNA sequences are transcribed into noncoding transcripts.[5]

RNA therapeutics can be categorized in three primary groups: first, compounds targeting cellular RNA, typically consisting of heavily modified nucleic acid-based antisense oligomers; second, therapies wherein RNA itself acts as the therapeutic agent, introduced into cells to regulate protein expression, as short-interfering RNA (siRNA) molecules for instance; and thirdly, therapeutic genome editing, where RNA acts as a guide for an effector protein modifying cellular DNA sequences (clustered regularly interspaced short palindromic repeats (CRISPR)-Cas9). Numerous RNA-based therapeutic molecules have obtained approval from the US Food and Drug Administration (FDA) approval and have progressed into the clinic, as outlined in **Table 1**. This review aims to offer a comprehensive overview of these diverse strategies, emphasizing their molecular mechanisms. For a deeper understanding, readers are encouraged to explore recent comprehensive reviews focusing on each approach separately, namely antisense technologies,[6–8] RNA interference (RNAi)-based therapeutics,[9] mRNA-based approaches,[10,11] and CRISPR-Cas-mediated genome editing.[12,13]

2.1. Antisense Therapeutics

Antisense oligonucleotides (ASOs) are short single-stranded (ss) nucleic acids typically ranging from 18 to 30 nts in length. They work by complementarily binding to specific RNA sequences through Watson–Crick base pairing.[4] Once bound to the target RNA, ASOs can exert various effects on gene expression, including reducing, restoring, or modifying protein expression levels.[14] There are two main models supporting how ASOs exert their effects after binding to their target RNA, namely the occupancy-mediated degradation model and the occupancy-only model based on different post-hybridization mechanisms.[6] In the first one, ASOs bind to the target RNA and recruit the cellular machinery, such as RNase H, which cleaves the RNA strand in the RNA-DNA heteroduplex formed by the ASO and the target RNA. This cleavage ultimately leads to the degradation of the target RNA molecule, thereby reducing the expression of the corresponding protein. This particular design, often referred to as enzymatic RNA degradation, improves the ASO's affinity to its intended RNA target, thereby enhancing its resistance against nucleases.[6] It has been extensively employed for the purpose of reducing the expression of genes implicated in causing or modifying diseases.[4,15] To date, regulatory approval has been granted for seven RNase H-competent ASOs: fomivirsen,[16] mipomersen,[17] volanesorsen,[18] inotersen,[19] tofersen,[20] eplontersen[21] and olezarsen[22] (Table 1).

Differing from the degradation model, the occupancy-only model suggests that ASOs exhibit high-affinity to target transcripts but do not trigger their degradation due to the absence of RNase H competence. Instead, ASO binding to the target RNA alters its conformation or accessibility, thereby affecting its ability to be translated into protein or undergo other post-transcriptional modifications. The steric hindrance mechanism is another term for this process. The predominant method of employing steric block ASOs involves modifying alternative splicing to selectively exclude (exon skipping) or retain (exon inclusion) specific exons.[23] Various splice-switching ASOs have received FDA approval, including eteplirsen,[24] golodirsen,[25] viltolarsen,[26] casimersen[27] and nusinersen[28] (Table 1).

Furthermore, occupancy-only ASOs have the potential to activate endogenous cellular surveillance mechanisms that eliminate abnormal mRNAs. When ASOs act on pre-mRNAs to induce premature termination codons in mRNAs, the targets may undergo degradation via nonsense-mediated mRNA decay.[29] Additionally, ASOs can modulate translation either by inhibiting or activating it through various mechanisms such as translational arrest,[30] inhibition of 5′cap,[31] or modifications in polyadenylation.[32] Conversely, ASOs can also bind to inhibitory

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 1. Different RNA-based therapeutics approved by the US Food and Drug Administration or the European Medicines Agency.

Name of the therapeutic	Target	Mechanism of action	Disease	Route of administration	Delivery System	Approval Year
Antisense therapeutics						
Fomivirsen	Cytomegalovirus mRNA	Downregulation	Cytomegalovirus retinitis	Intravitreal	Naked	1998 (withdrawn in 2006)
Mipomersen	Apolipoprotein B-100 mRNA	Downregulation	Familial Hypercholesterolemia	Subcutaneous	Naked	2013 (withdrawn in 2019)
Nusinersen	SMN2 mRNA	Splicing modulation	Spinal muscular atrophy / Autosomal recessive neuromuscular disorder	Intrathecal	Naked	2016
Eteplirsen	Exon 51 of dystrophin mRNA	Splicing modulation	Duchenne muscular dystrophy	Intravenous	Naked	2016
Inotersen	Transthyretin mRNA	Downregulation	Hereditary transthyretin-mediated amyloidosis	Subcutaneous	Naked	2018
Golodirsen	Dystrophin mRNA	Splicing modulation	Duchenne muscular dystrophy	Intravenous	Naked	2019
Volanesorsen	Apolipoprotein CIII mRNA	Downregulation	Familial chylomicronemia syndrome	Subcutaneous	Naked	2019
Viltolarsen	Exon 53 of dystrophin mRNA	Splicing modulation	Duchenne muscular dystrophy	Intravenous	Naked	2020
Casimersen	Exon 45 of dystrophin mRNA	Splicing modulation	Duchenne muscular dystrophy	Intravenous	Naked	2021
Tofersen	SOD1 mRNA	Downregulation	Amyotrophic lateral sclerosis associated with SOD1 mutation	Intrathecal	Naked	2023
Eplontersen	Transthyretin mRNA	Downregulation	Hereditary transthyretin-mediated amyloidosis	Subcutaneous	GalNAc	2023
Imetelstat	Telomerase	Inhibition	Lower-risk myelodysplastic syndromes	Intravenous	Naked	2024
Olezarsen	Apolipoprotein CIII mRNA	Downregulation	Familial Chylomicronemia Syndrome	Subcutaneous	GalNAc	2024
Small interfering RNA						
Patisiran	Transthyretin mRNA	Downregulation	Transthyretin-mediated amyloidosis	Intravenous	LNP	2018
Givosiran	ALS1 mRNA	Downregulation	Acute hepatic porphyria	Subcutaneous	GalNAc	2019
Lumasiran	HAO1 mRNA	Downregulation	Primary hyperoxaluria type 1	Subcutaneous	GalNAc	2020
Inclisiran	PCSK9	Downregulation	Atherosclerotic cardiovascular disease	Subcutaneous	GalNAc	2021
Vutrisiran	Transthyretin mRNA	Downregulation	Transthyretin-mediated amyloidosis	Subcutaneous	GalNAc	2022
Nedosiran	Hepatic lactate dehydrogenase mRNA	Downregulation	Primary hyperoxaluria	Subcutaneous	GalNAc	2023

(*Continued*)

elements like upstream open reading frames[33] or other components that inhibit translation,[34] thus leading to the upregulation of target RNAs. Moreover, ASOs possess the ability to interfere with microRNA (miRNA) by directly binding to target mRNAs,[35] or by associating with mRNAs at miRNA-binding sites to impede miRNA interaction.[36]

Occupancy-only ASOs enable greater flexibility for nucleotide modifications compared to RNase H-dependent ASOs, thus enhancing the ASOs properties. It is important to highlight that occupancy-only ASOs should not form RNA-DNA duplexes to prevent the formation of RNase H substrates, thereby avoiding unnecessary cleavage of the target RNA.[3] These two mechanisms offer different strategies for manipulating gene expression levels using ASOs, providing researchers with versatile tools for studying gene function and potentially developing therapeutic interventions for various diseases.

The clinical delivery of ASOs faces several obstacles, such as vulnerability to degradation by serum nucleases, rapid renal

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 1. (Continued)

Name of the therapeutic	Target	Mechanism of action	Disease	Route of administration	Delivery System	Approval Year
Fitusiran	Antithrombin	Downregulation	Hemophilia A or B	Subcutaneous	GalNAc	2025
mRNA therapeutics						
BNT162b2	SARS-CoV-2 Spike mRNA	Expression of SARS-CoV-2 Spike protein	COVID-19	Intramuscular	LNP	2021
mRNA-1273	SARS-CoV-2 Spike mRNA	Expression of SARS-CoV-2 Spike protein	COVID-19	Intramuscular	LNP	2022
Aptamers						
Pegaptanib	VEGF	VEGF antagonist	Age-related macular degeneration	Intravitreal	PEG	2004 (withdrawn in 2011)
Avacincaptad pegol	C5	C5 inhibitor	Geographic atrophy	Intravitreal	PEG	2023

Lipid-based nanoparticles (LNP); Messenger RNA (mRNA); N-acetylgalactosamine (GalNAc); Polyethylene glycol (PEG); Vascular endothelial growth factor (VEGF)

clearance, and inefficient cellular uptake. Furthermore, ASOs are associated with immunogenicity, posing another challenge in their application.

2.2. RNA Interference-Based Therapeutics

RNAi, first discovered in plants and later demonstrated in worms in 1998, was an extraordinary breakthrough in the field of therapeutic treatment of several diseases, such as cancers and gene function research.[37–39] RNAi represents an endogenous cellular mechanism wherein double-stranded (ds) RNAs trigger the targeted degradation of specific RNA targets, serving as an innate defense mechanism against invading viruses and transposable elements.[3,14,40] siRNAs serve as the effector molecules of RNAi. They are small, non-coding, dsRNAs with a specific structure: a 19 + 2mer configuration. This structure consists of a duplex formed by two 21-nt RNA molecules, where 19 bases are complementary and terminal 2-nt 3′ overhangs. These molecules possess the capability to regulate various genomic functions, including RNA processing and stability, chromosome segregation, transcription, and translation.[4] Long double-stranded RNAs are processed by an RNase III like enzyme, denominated Dicer, and cleaved into small fragments. Upon their entry into the cell, the sense strand of siRNA is unwound and cleaved due to the Dicer activity. Then, the antisense strand of single-stranded siRNA acts as a guide for mRNA complementation and the RNA-induced silencing complex (RISC) is formed. If perfect complementarity between siRNA and targeted mRNA is achieved, mRNA is degraded due to the action of a catalytic protein member of the argonaute family (Ago2). On the other hand, imperfect alignment leads to mRNA translational repression and off-target effects.[37–39] The siRNA molecules efficiency depends on several factors, which include target availability, secondary mRNA structures, matching positions, and the intrinsic characteristics of siRNA. The strategy success can be mostly achieved by means of a good siRNA design method.[41] Several modifications to the conventional siRNA design have yielded advantages such as decreased activity of the passenger strand and/or enhanced effectiveness. These adaptations comprise Dicer substrate siRNAs,[42] self-delivering siRNAs (asymmetric and hydrophobic),[43] small internally segmented siRNAs,[44] divalent siRNAs[45] and single-stranded siRNAs.[46]

Various siRNA-based drugs have been FDA-approved, including patisiran,[47] vutrisiran (a successor to patisiran),[48] givosiran,[49] inclisiran,[50] lumasiran,[51] nedosiran[52] and fitusiran[53] (Table 1). Growing evidence indicates that siRNA-based therapeutics might possess additional applications, contributing to the ongoing robust exploration in this area.[54,55] Given such examples, it is reasonable to anticipate an increased use of siRNA-based treatments within clinical settings in the foreseeable future. siRNA drugs encounter comparable hurdles to ASOs concerning delivery, stability, and potential immune responses.

miRNAs play a crucial role as gene regulators, exerting influence over the expression of numerous mRNAs by either inhibiting translation or inducing degradation of their target mRNAs.[56] Partial complementarity enables miRNAs to recognize their targets, allowing a single miRNA to interact with multiple mRNAs. Mimicking miRNA function facilitates the simultaneous modulation of complex gene expression networks.[14] Originally identified in *Caenorhabditis elegans*,[57] miRNAs are transcribed from genomic DNA as primary miRNAs (pri-miRNAs), which adopt loop structures with interspersed mismatches. Following cleavage by Drosha, these pri-miRNAs are converted into precursor miRNAs (pre-miRNAs) spanning 70–100 nt before exiting the nucleus. In the cytoplasm, Dicer processes them into RNA duplexes of 18–25 nt length with two base overhangs in the 3′ region, now recognized as miRNAs. Subsequently, these miRNAs are incorporated into the RISC to form a functional miRISC complex. Upon unwinding of the miRNA duplex, the sense strand is released, while the antisense strand guides the miRISC. Typically, hybridization takes place between the 3′ untranslated region (UTR) of the target mRNA and the 2–7 nt of the 5′ end of the miRNA, leading to the suppression of the target mRNA via translational repression, cleavage, or degradation.[58–60] miRNA-based therapeutics can be classified into two types: miRNA mimics, synthetic dsRNA molecules designed to mimic the function-

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

ality of naturally occurring miRNAs, akin to the siRNA's mechanism of action; and miRNA inhibitors (antimiRs), RNA oligos intended to directly interfere with the mature strand of the targeted miRNA, thereby blocking its function, designed similarly to ASOs.[56] While there are currently no miRNA-based drugs on the market, several promising candidates are undergoing clinical trials.[61]

2.3. Messenger RNA-Based Therapeutics

The RNA therapeutics mentioned earlier function as effectors. However, RNA, specially mRNA, possesses the capability to function as a vehicle of genetic information, thereby allowing it to act as a therapeutic agent through modulation of protein expression. Clinically employed synthetic mRNAs are typically synthesized using in vitro transcription. It comprises a single-stranded open reading frame flanked by untranslated regions, featuring a 5′ cap to facilitate translation and a 3′ poly(A) tail for stability.[62] To optimize translation, modified nucleosides as pseudouridine and N1-methylpseudouridine are frequently incorporated into the mRNA molecule. These modifications not only prevent recognition of the in vitro transcribed mRNA by the innate immune system but also facilitate higher dosing.[63] The applications of mRNA-based therapies can be categorized into two subgroups based on their intended objectives: protein replacement therapies, which entails the administration of mRNA to compensate for defective gene(s) or to provide therapeutic proteins; and vaccination, wherein mRNA encoding specific antigen(s) is delivered to trigger protective immunity against infectious diseases, or to engage the immune system in combating cancer.

An evident application of therapeutic mRNA involves expressing proteins that are either absent or dysfunctional in the body. While this approach holds potential for treating a broad spectrum of diseases, it presents additional hurdles, such as the requirement to attain high and prolonged expression of the therapeutic protein in particular cell types, and the complexities associated with delivering mRNA to solid organs. Furthermore, repeated dosing might be needed, which could trigger immune reactions and diminish the efficacy of the therapeutic protein, despite the utilization of modified mRNA and sophisticated delivery systems. Furthermore, if the therapeutic protein is not naturally expressed in the body, it may also provoke an immune reaction. Currently, three prominent biopharmaceutical companies are at the forefront of developing mRNA therapeutics: Moderna (United States), CureVac (Germany), and BioNTech (Germany). Together, they boast a diverse pipeline of gene therapy products covering various areas such as heart diseases, metabolic diseases, and immunomodulators tailored for applications in immuno-oncology. While the mentioned pharmaceutical companies were among the pioneers in mRNA therapeutics, numerous other research groups and biotech start-ups globally are now venturing into this field.[64–66] However, only a limited number of clinical studies have demonstrated promising results regarding the safety and efficacy of this approach. One noteworthy example is the utilization of synthetic mRNA encoding vascular endothelial growth factor (VEGF-A) to facilitate vasculogenesis during cardiac regeneration in patients undergoing coronary artery bypass grafting.[67]

Regarding the second category, the use of mRNA transcripts to act as vaccines, examples include the mRNA rabies vaccine[68] and several other mRNA vaccines targeting various infectious diseases.[10] The effectiveness of mRNA-based vaccines has been unequivocally demonstrated through their utilization as vaccines against COVID-19. The emergence of the COVID-19 pandemic, coupled with the extensive prior development of mRNA vaccines, resulted in a robust existing knowledge base that could be readily adapted to this new challenge. This significantly expedited progress, leading to the approval of mRNA vaccines developed by BioNTech/Pfizer and Moderna within a remarkably short span of less than one year after the release of the genome sequence for the virus in early 2020.[69,70] Moreover, the wealth of efficacy and safety data spanning nearly three decades regarding the use of mRNA in therapeutic applications provided robust support for the rapid approval of mRNA vaccines by health authorities worldwide. These two mRNA vaccines, tozinameran (BNT162b2, Pfizer–BioNTech vaccine) and elasomeran (mRNA-1273, Moderna), as listed in Table 1, are comprised of ionizable lipid nanoparticles (LNPs) and deliver mRNA encoding the viral spike glycoprotein. These vaccines have demonstrated over 90% efficacy in clinical trials. Upon administration via intramuscular injection, this modified spike protein enters cells and facilitates antigen presentation, thereby fostering the development of a specific immune response against SARS-CoV-2 in vaccine recipients. Notably, apart from transient local and systemic reactions, no safety concerns were reported,[71,72] although further evaluation is imperative to assess potential long-term effects.

The development of mRNA-based vaccines for COVID-19 has paved the way for their application in cancer treatment.[73] These vaccines are tailored for each patient and are formulated to instruct the patient's immune system to recognize and eradicate cancer cells.[74] In ongoing clinical investigations, researchers are employing mRNA vaccines to provoke an immune response against particular cancer-associated neoantigens,[75–78] with initial findings suggesting potential clinical advantages.[79] To date, no mRNA-based cancer vaccines have received regulatory approval; however, several phase I-II clinical trials have shown encouraging results, even in tumors that have traditionally been poorly immunogenic (**Table 2**). Furthermore, mRNA vaccines hold promise for the treatment of autoimmune diseases by selectively mitigating autoimmune responses while preserving normal immune function.[80]

In recent years, mRNA therapies have gained attention as a promising strategy for treating rare genetic disorders.[81] mRNA therapies can compensate for defective or deficient proteins by delivering functional mRNA that encodes the defective or deficient proteins to cells. However, because both the mRNA template and the resulting protein have a limited lifespan, these therapies provide only temporary expression of the enzyme that is persistently lacking or deficient. Nevertheless, the transient nature of mRNA can be strategically leveraged for long-lasting effects by delivering CRISPR gene-editing components as mRNA, along with appropriate guide RNAs (gRNAs). In this context, mRNA serves as a set of "come-and-go tools" used to correct disease-causing DNA mutations.[81,82] Several preclinical studies have provided proof-of-concept for mRNA therapies in a variety of rare disease models.[83,84]

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Table 2. Clinical status of mRNA vaccines in different phases of clinical development for the treatment of various types of cancer.

Clinical trial number	Trial phase	Cancer type	Combination [Route of administration]	Target antigen[s]	Sponsor
NCT02316457	I	Breast cancer	None (Intravenous)	BNT-114 plus BNT-122 (personalized set of pre-manufactured non-mutated shared tumour-associated antigens plus a personalized cancer vaccine encoding individual tumour mutations)	BioNTech SE
NCT03948763	I	Non-small-cell lung, pancreatic, and colorectal neoplasms	Pembrolizumab (Intramuscular)	mRNA-5671 (KRAS gene driver mutations)	Merck Sharp and Dohme LLC
NCT03313778	I	Solid tumours	Pembrolizumab (Intramuscular)	mRNA-4157 (personalized cancer vaccine encoding several neoantigens)	ModernaTX, Inc.
NCT04573140	I	High-grade gliomas and glioblastoma	None (Intravenous)	Formulation with pp65 full length LAMP and tumour mRNA	University of Florida
NCT02410733	I	Melanoma	None (Intravenous)	BNT111 (NY-ESO-1 [CTAG1A], tyrosinase, MAGE-A3 and TPTE)	BioNTech SE
NCT05142189	I	Non-small-cell lung cancer	Cemiplimab plus docetaxel (Intravenous)	BNT116 (non-small-cell lung cancer tumour-associated antigens)	BioNTech SE
NCT04163094	I	Ovarian	Carboplatin plus paclitaxel (Intravenous)	BNT115 (ovarian cancer tumour-associated antigens)	University Medical Center Groningen
NCT04161755	I	Pancreatic	Oxaliplatin, irinotecan, fluorouracil, leucovorin, and atezolizumab (Intravenous)	BNT122 (personalized cancer vaccine encoding individual tumour mutations)	Memorial Sloan Kettering Cancer Center
NCT03289962	I	Solid tumours	Atezolizumab (Intravenous)	BNT122 (personalized cancer vaccine encoding individual tumour mutations)	Genentech, Inc.
NCT04382898	I/II	Prostate	Cemiplimab (Intravenous)	BNT112 (PAP, PSA, and three undisclosed antigens)	BioNTech SE
NCT03418480	I/II	HPV16-positive solid tumours	Anti-CD40 antibodies (Intravenous)	BNT113 (HPV16 E6 and E7 oncoproteins)	University of Southampton
NCT04503278	I/II	Solid tumours	Chimeric antigen receptor therapy (Intravenous)	CARVac (CLDN6)	BioNTech Cell and Gene Therapies GmbH
NCT00923312	I/II	Non-small cell lung cancer	None (Intradermal)	CV9201 (NY-ESO-1, MAGE-C1, MAGE-C2, survivin and 5T4)	CureVac
NCT00204607	I/II	Melanoma	None (Intradermal)	Melan-A, Tyrosinase, GP100, MAGE-A1, MAGE-A3, and Survivin	University Hospital Tuebingen
NCT03897881	II	Melanoma	Pembrolizumab (Intramuscular)	mRNA-4157 (personalized cancer vaccine encoding 20 different mutated neoepitopes)	ModernaTX, Inc.
NCT04526899	II	Melanoma	Cemiplimab (Intravenous)	BNT111 (NY-ESO-1, tyrosinase, MAGE-A3, and TPTE)	BioNTech SE
NCT04534205	II	Head and neck squamous cell carcinoma	Pembrolizumab (Intravenous)	BNT113 (HPV16 E6 and E7 oncoproteins)	BioNTech SE
NCT04486378	II	Colorectal	None (Intravenous)	BNT122 (personalized cancer vaccine encoding individual tumour mutations	BioNTech SE
NCT03815058	II	Advanced melanoma	Pembrolizumab (Intravenous)	BNT122 (personalized cancer vaccine encoding individual tumour mutations)	Genentech, Inc.

Lysosomal associated membrane protein (LAMP); Human papillomavirus (HPV)

Numerous mRNA therapies for rare genetic diseases have advanced to early-phase clinical trials (Phase I–II), reflecting the growing translational momentum in the field. Notably, the current clinical trial pipeline for mRNA substitution therapies includes diseases as methylmalonic acidemia (NCT03810690), glycogen storage disease type 1a (NCT05095727), cystic fibrosis (NCT05668741, NCT03375047), ornithine transcarbamylase deficiency (NCT03767270), phenylketonuria (NCT06147856) and propionic acidemia (NCT04159103), each evaluating LNP-formulated mRNA encoding the relevant functional enzymes. Additional clinical investigations into gene-editing therapies include mRNA candidates for hypercholesterolemia (NCT06164730), refractory hyperlipidemia (NCT06451770), and alpha-1 antitrypsin deficiency (NCT06389877), among others.

© 2025 Wiley-VCH GmbH

23963987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

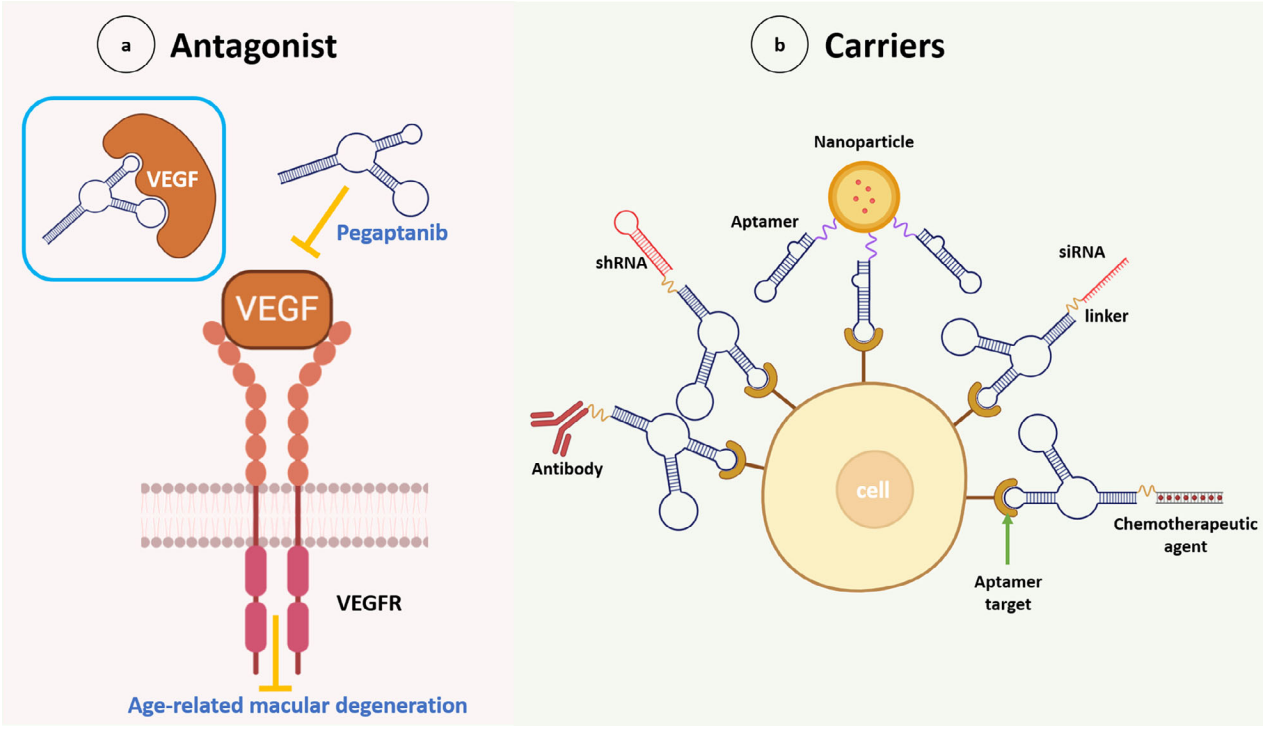


Figure 2. Aptamers can serve as antagonists or carriers for delivery agents. Pegaptanib acts as a selective vascular endothelial growth factor (VEGF) antagonist, inhibiting its interaction with VEGF receptors (VEGFR) and thus treating age-related macular degeneration (a). Cell-specific aptamers can deliver various agents, including siRNA, shRNA, antibodies, chemotherapeutic agents, and can even be conjugated with several classes of nanoparticles (b).

Advances in LNP delivery systems and the refinement of mRNA chemical modification techniques have made stable in vivo delivery of mRNA increasingly feasible. This technological foundation not only underpinned the success of mRNA-based COVID-19 vaccines but also expands the therapeutic potential of mRNA to oncology, rare genetic disorders, and even autoimmune diseases. As the threat of emerging infectious diseases persists and the evident necessity for cost-effective measures to curb their transmission becomes more evident, there is a compelling case for mRNA-based vaccines and therapeutics to emerge as vital tools in combating such outbreaks. Continued innovation and clinical validation are expected to further accelerate their integration into routine clinical practice across a broad spectrum of diseases.

2.4. Aptamers

Aptamers are oligonucleotide molecules known for their unique distinct 3D configurations, which give rise to a variety of secondary structures, such as hairpins, stem loops, bulges, pseudoknots, G-quadruplexes, among others,[85] enabling them to selectively recognize and inhibit a variety of targets, including proteins,[86] pathogens,[87,88] enzymes,[89] toxins,[90] viruses,[91] tissues[92] and even whole cells.[93,94] Nucleic acid aptamers are RNA or ssDNA structures, usually spanning 20 to 80 nt residues. They exhibit high specificity and affinity toward their targets, facilitated by spatial complementarity driven by hydrogen bonding, Van der Waals forces, and electrostatic/ionic interactions.[95,96] The binding affinity between aptamers and their targets is often quantified by their dissociation constant (Kd), typically falling within the picomolar to nanomolar range.[95]

These specific molecules can be screened from combinatorial oligonucleotide libraries, which were first introduced in 1990, through an in vitro iterative process known as Systematic Evolution of Ligands by Exponential Enrichment (SELEX).[97,98] Typically, this process is divided into three main steps: first, the incubation of the oligonucleotide library with the intended target; second, partitioning, which involves discarding the unbound sequences and collecting the bound ones; and finally, amplification through polymerase chain reaction (PCR).[96] Over time, further research has resulted in the creation of various modifications of the traditional SELEX procedure, including cell-SELEX.[93,94] Aptamer-based therapeutics, derived from highly diverse nucleic acid pools, can function as antagonists by disrupting the receptor-ligand interactions[99] or as carriers to deliver other therapeutic agents to target cells/tissues[100] (**Figure 2**).

Although aptamers hold potential as therapeutics, only two FDA-approved aptamer-based drugs are currently available on the market: Pegaptanib, approved in 2004, and avacincaptad pegol, approved in 2023 (Table 1). Pegaptanib is a 27-base RNA antagonist aptamer, selected against VEGF, used in the treatment of age-related macular degeneration, a major contributor to irreversible blindness.[101] In comparison, avacincaptad pegol is a 39-nucleotide RNA aptamer that inhibits complement component C5 for the treatment of geographic atrophy secondary to age-related degeneration.[102] Other aptamer-based drugs are cur-

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

rently undergoing clinical trials and have been comprehensively reviewed elsewhere.[103]

Aptamers offer several advantages over other ligand classes, including enhanced tissue penetration, superior thermal and chemical stability, reduced immunogenicity, high reproducibility, cost-effectiveness compared to antibodies, prolonged shelf-life, and rapid in vitro selection.[85] However, their clinical translation is challenging due to their high sensitivity to nucleases, rapid renal filtration, and complexity of selection techniques.[103]

2.5. CRISPR-Cas9

While mRNA-based therapies rely on short-term expression of a functional copy of a transcript, genome editing presents the potential for a lasting cure by correcting mutations in genomic DNA. The practicality of this method in a clinical setting stems from the identification of ancient bacterial adaptive immune systems,[104,105] and their subsequent evolution into genome editing tools.

The advent of the CRISPR-Cas9 genome editing system has notably advanced the field of RNA-based therapeutics within the clinical setting.[106,107] The first widely employed genome editing mechanism is the type-II CRISPR-Cas9 system, originating from *Streptococcus pyogenes*.[108] Cas9 acts as an RNA-guided DNA endonuclease, triggering double-strand breaks (DSBs) at precise and specific genomic locations. This enzyme identifies its target sites via a small sgRNA that forms complementary base pairs with regions in the DNA. This customizable RNA can be engineered to direct cas9 to desired genomic regions, with the only sequence requirement being the presence of a short protospacer adjacent motif (PAM), also known as "NGG" sequence, at the 3′ end of the target sequence, restricting editable genomic sites to those harboring PAM sequences.[109–111] Cas9 cleaves the target DNA sequence, generating DSBs that can undergo repair through two mechanisms: non-homologous end joining (NHEJ), and homology-directed repair (HDR). NHEJ, known for its error-prone nature, results in small insertions (indels) or deletions, ultimately disrupting the targeted locus (gene knockout). Conversely, HDR represents a more accurate process, utilizing a short donor DNA sequence for repairing the DSB, thereby enabling gene knock-in.[40] Cas nucleases derived from different bacterial species recognize distinct PAM sequences, thus expanding the scope of targetable regions. Apart from natural Cas variants, laboratory-evolved mutants with alternative PAM recognition have also emerged.[112,113]

Therapeutic strategies employing CRISPR to address hereditary diseases are predominantly in the preclinical phase, although a few have progressed to clinical trials. The first clinical trial involving CRISPR-Cas9 was conducted in China in 2016, marking the world's first human injection of gene-edited cells.[114] In this trial, patient-derived T cells were genetically modified to suppress PD-1 expression and then reinfused into the patients. The results demonstrated successful gene editing with no significant adverse effects, and two patients remained in stable condition. These findings highlighted the feasibility and safety of applying gene-editing technologies in a clinical setting.

CRISPR-mediated gene therapy has demonstrated promising initial clinical outcomes in addressing life-threatening conditions as sickle cell disease and β-thalassemia, both of which are inherited blood disorders stemming from reduced hemoglobin production.[115] Notable examples of successful in vivo delivery of CRISPR-based therapeutics include clinical trials targeting transthyretin amyloidosis (TTR amyloidosis),[116] an autosomal dominant disorder primarily caused by the deposition of amyloid fibrils around cells, which predominantly affects the human nervous system and heart.[117] In 2021, trials of NTLA-2001, a CRISPR-based therapy consisting of a liver-targeting LNP encapsulating sgRNA against the TTR gene, showed promise in reducing the production of the misfolded TTR protein.[116] NTLA-2001 has received orphan drug designation from both the FDA and European Medicines Agency (EMA).

Leber congenital amaurosis type 10 is a severe retinal dystrophy caused by a point mutation within intron 26 of the *CEP290* gene, which plays a role in phototransduction.[118] A candidate genome-editing therapeutic called EDIT-101 has shown promise in restoring normal *CEP290* expression and photoreceptor function.

EBT-101, the first-in-class CRISPR-based gene therapy candidate aimed at functionally curing human immunodeficiency virus (HIV-1), is a dual-cut gene-editing treatment delivered via an adeno-associated virus (AAV).[119] A Phase I/II clinical trial is currently underway to evaluate its safety and tolerability in adults. EBT-101 has received FDA Fast Track designation. Additionally, the FDA has granted investigational new drug clearance to CRD-TMH-001, a CRISPR-based therapy for the treatment of Duchenne muscular dystrophy.

Efficient, safe, and stable delivery of RNA-based therapeutics is a key strategy for advancing disease treatment. To this end, various delivery systems have been engineered to target disease sites based on specific pathological features, demonstrating high loading capacity and promising clinical potential. Future research will focus on enhancing targeting precision and tailoring these systems to meet disease-specific needs.

3. The Pathway to Clinical RNA Delivery

RNA-based therapeutics hold immense promise in targeting a substantial part of undruggable genes and gene products. This potential opens avenues for the creation of entirely novel therapeutic approaches in treating various diseases. The effectiveness of delivering RNA into the cytoplasm while overcoming both extracellular and intracellular obstacles remains pivotal for the success of RNA therapy. Large, hydrophilic, and the negatively-charged nature of RNA inhibits its passive diffusion across lipid bilayers. Moreover, RNA drugs encounter hurdles such as serum nucleases, RNase susceptibility, and macrophage clearance within the reticuloendothelial system. Furthermore, traversing the extracellular matrix and penetrating the cell membrane requires RNA drugs to undergo receptor-mediated endocytosis. Subsequently, liberating RNA from the endosome and ensuring its non-toxic release into the cytoplasm pose significant technical challenges.[120] Consequently, diverse chemical alterations,[121,122] covalent conjugation of various moieties[123,124] and the refinement of delivery formulations[125] have been investigated to address issues pertaining to pharmacodynamics and pharmacokinetics.

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

3.1. Chemical Modifications

The main aims of oligonucleotide chemistry include enhancing resistance to nucleases, boosting affinity to the target, improving pharmacokinetics, and mitigating pro-inflammatory reactions. Due to the limited number of nucleotides in both siRNA and ASOs, researchers can engineer these molecules with site-specific chemical modifications, primarily focusing on the phosphate backbone and sugar rings. The knowledge gained from backbone, base, and sugar modifications initially developed for ASO therapeutics is widely applicable to siRNA therapeutics.[121] However, the molecular prerequisites for efficient recruitment of the RNAi machinery impose limitations on the chemical modification of siRNA. Common modifications to the phosphate backbone, such as phosphorothioates (PS), phosphorodiamidate morpholino oligonucleotides (PMO), peptide nucleic acid oligonucleotides (PNAs), and nucleobase modifications like 5-methylcytosine (m5C), are frequently employed. The PS backbone modification, which involves replacing one of the non-bridging oxygen atoms within the inter-nucleotide phosphate group with sulfur, was the pioneering and widely used chemical modification in ASOs.[126] This modification increases nuclease resistance, enhances cellular uptake and in vivo bioavailability by augmenting hydrophobicity, resisting phosphodiesterases, and promotes the binding of serum proteins.[4,6] Furthermore, PS backbone modification enhances the accumulation and retention of siRNA in multiple tissues, thereby prolonging the duration of silencing in vivo.[127] PMOs, distinguished by a backbone comprising methylenemorpholine rings linked via phosphorodiamidate groups, have shown enhanced target affinity and stability. However, their neutral charge leads to reduced serum protein binding and shortened circulation duration. As a result, they are rapidly cleared from the body after systemic injection and demonstrate limited cellular uptake.[6] PNAs feature a distinctive pseudo-peptide backbone connecting the four natural nucleobases, rendering them resistant to both nucleases and proteases. Similar to PMOs, PNAs lack a charge, resulting in robust binding to complementary sequences. However, they face challenges in cellular delivery as they interact minimally with plasma proteins and are quickly cleared by urinary excretion.[128]

In addition to these alterations, a diverse range of chemistries has been devised for various ribose modifications, including 2′-fluoro (2′-F), 2′-O-methyl (2′-OMe), and 2′-methoxyethyl (2′-MOE). These adjustments enhance oligonucleotide nuclease resistance, leading to improved stability in plasma, prolonged tissue half-lives, and consequently, extended drug effects. The 2′-F and 2′-O-Me modifications mimic the biophysical properties of 2′-OH and can shield siRNAs against RNases while also inhibiting the activation of innate immune receptors by siRNAs. As a result, all therapeutic siRNAs currently undergoing clinical trials incorporate 2′-F or 2′-O-Me modifications (Table 1).[9] Enhanced affinity can also be attained through the use of oligonucleotides modified with locked nucleic acids (LNAs), constrained ethyl oligonucleotides (cEt), and tricyclo-DNA oligonucleotides (tc-DNA).[125,129,130]

mRNA vaccines can be optimized through sequence, nucleoside modification or sequence substitution to enhance translational ability.[131] Chemical modifications like pseudouridine, N1-methylpseudouridine, 5-methyluridine (m5U), 2-thiouridine and others are integrated into mRNA to reduce innate immune activation.[132,133] Similarly, CRISPR systems face delivery and off-target effects challenges, but chemical modifications used in ASOs and siRNAs can enhance gRNA and Cas9 mRNA.[134] These chemical modifications have been shown to enhance genome editing efficiency in human primary T cells and CD34+ hematopoietic stem cells;[135] and reduce off-target DNA cleavage by cas9.[136] Aptamers benefit from chemical modifications to enhance their pharmacokinetic characteristics, including modification with 5′-end polyethylene glycol(PEG)ylation,[137] inverted thymidine capping,[138] and sugar ring substitutions to enhance pharmacokinetic properties and target binding affinity.[139]

By varying the array of chemical modifications, scientists have been able to enhance pharmacokinetic characteristics, modulate the innate immune response, and bolster stability.

3.2. Bioconjugation

ASOs and siRNAs' delivery potential can be improved via direct covalent conjugation of lipids (e.g., cholesterol),[123] peptides,[124] aptamers,[140] antibodies,[141] and sugars (e.g., N-acetylgalactosamine (GalNAc)).[142] These enhance intracellular uptake, cell/tissue targeting, and reduce clearance. Bioconjugates offer precise stoichiometry, enabling simple, high-scale synthesis and well-defined pharmacokinetics. Their small size ensures favorable biodistribution, reaching tissues efficiently beyond those with fenestrated endothelia.

Lipid conjugation enhances siRNA and ASO delivery. Cholesterol-siRNAs enable hepatic gene silencing, including myostatin in skeletal muscle, a task that has historically been difficult to accomplish effectively via RNAi, following systemic delivery.[143] Additionally, other lipid derivatives have been exploited to enhance siRNA delivery, including α-tocopherol, reported to induce a potent silencing of *Apob* in the mouse liver.[144]

N-acetylgalactosamine (GalNAc), a high-affinity ligand for the hepatocyte-specific asialoglycoprotein receptor (ASGR1), enhances the uptake of ASOs and siRNAs into hepatocytes.[145] Its pH-sensitive interaction and enzymatic degradation facilitate oligonucleotide release.[146] GalNAc conjugation boosts ASO potency specifically in the liver, making it a leading strategy for experimental oligonucleotide drugs. Several pharmaceutical companies, most notably Alnylam, employ GalNAc conjugation in drug development for various diseases, including haemophilia and hyperoxaluria. FDA-approved givosiran and late-stage inclisiran demonstrate the efficacy of GalNAc-conjugated siRNAs (Table 1).

Various technologies deliver nucleic acids to hepatic cells, but targeting specific cell surface receptors in other tissues remains a challenge. While antibodies have been used for drug delivery,[147] their application for oligonucleotide delivery is still in the early stages of development. Targeting specific interactions between antibodies and receptors enables for delivery to inaccessible tissues, with success seen in targeting receptors human epidermal growth factor receptor 2 (HER2)[148] and CD7[149] for siRNA delivery. ASOs conjugated with antibodies against CD44 and EphA2[150] have also been explored, allowing targeted release after cellular entry.

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

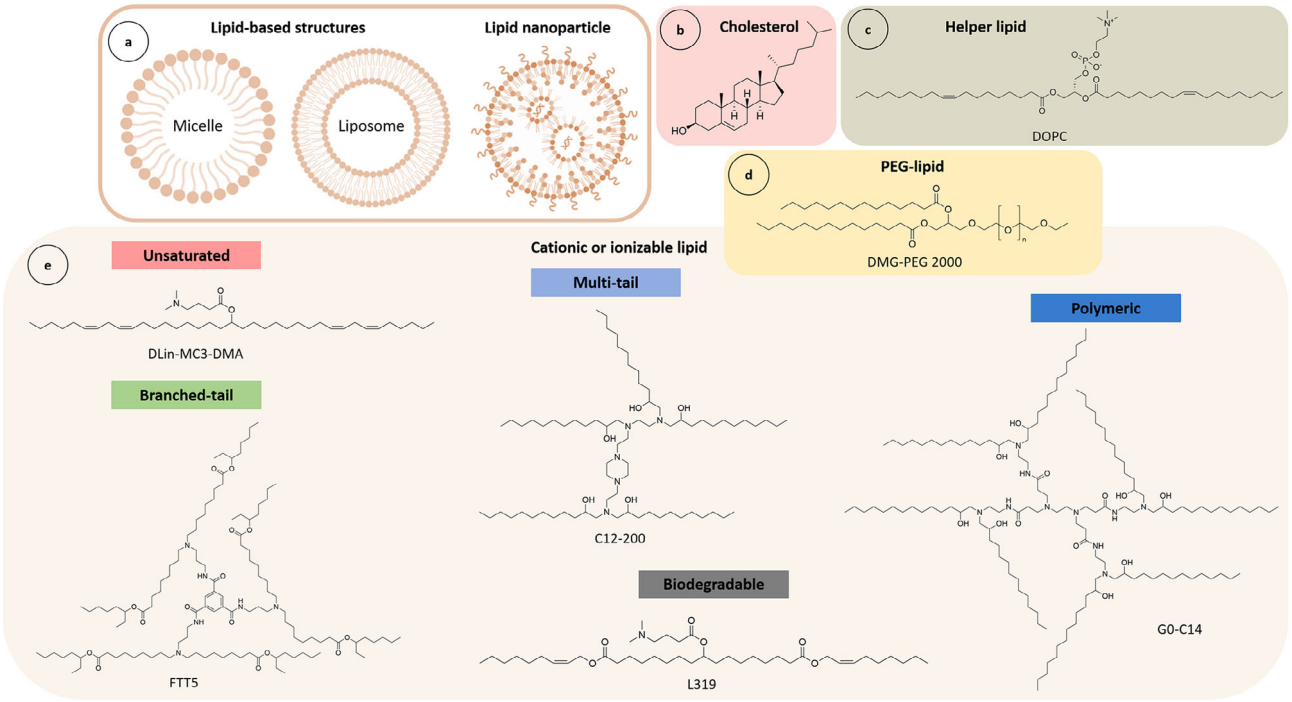


Figure 3. Lipid-based structures can adopt diverse structures, including micelles and liposomes (a). These structures typically comprise cholesterol (b), a helper lipid (c), a PEG-lipid (d), and a cationic or ionizable lipid (e). Based on their structural features, cationic or ionizable lipids can be categorized into five major classes: unsaturated (containing unsaturated bonds), multi-tail (with more than two tails), biodegradable (with biodegradable bonds), polymeric (with a polymer or dendrimer), and branched-tail (with branched lipid tails).

Peptides offer potential for enhancing therapeutic oligonucleotide conjugates. Protein transduction domains, like cell-penetrating peptides (CPPs), derived from protein translocation motifs or basic amino acids, are used for chemical conjugation to ASO chemistries like PMO and PNA. Peptide–PMO (PPMO) conjugates show promise for dystrophin splice switching in Duchenne muscular dystrophy, targeting various tissues, including cardiac muscle.[151,152] PPMOs also demonstrate efficacy in targeting other diseases like myotonic dystrophy type I,[153] X-linked agammaglobulinemia,[154] and spinal muscular atrophy.[155]

3.3. Nanocarriers for RNA Therapeutics

One of the primary challenges in harnessing the full potential of RNA-based drugs is achieving targeted delivery to specific organs and tissues. This precision is essential for maximizing therapeutic efficacy, minimizing side effects, and improving overall treatment outcomes. Moreover, effective carriers are crucial to protect RNA from physiological degradation due to its negative charges and chemical modifications. Nanotechnology and materials science offer solutions for oligonucleotide drug delivery, especially across biological barriers. Nanoparticle (NP) systems provide custom optimization of size, shape, composition, and targeting ligands, allowing for tailored delivery. LNPs, polymer-based nanocarriers, silica NPs, carbon and gold nanomaterials, extracellular vesicles (EVs), and emerging nanocarriers as spherical nucleic acids (SNAs) and DNA nanostructures, are key delivery approaches.

3.3.1. Lipid-Based Nanocarriers

LNPs are a crucial class in drug delivery vehicles that include FDA-approved NPs for liver siRNA[156] and mRNA vaccine delivery.[157,158] Lipids adopt diverse structures, including micelles, lipoplexes, liposomes, and LNPs, which are determined by the relative size of the hydrophilic head group in comparison to the hydrophobic tail(s).[159] LNPs consist of a cationic or ionizable lipid, cholesterol, a helper lipid, and a PEG-lipid (**Figure 3**). It has been demonstrated that different lipid structures affect influence the LNPs-cells interaction.[160] PEG-lipids' structure and molar percentage affect LNPs' pharmacokinetics and delivery efficiency.[161] Modified cholesterol and other lipid substitutions improve LNP delivery to different organs.[75,162]

Lipid formulation enhances nucleic acid delivery by condensing them into NPs, facilitating uptake via endocytosis. Lipoplexes, formed by direct interaction between polyanionic nucleic acids and cationic lipids, are unstable and need immediate use, suitable for local delivery.[163] Contrarily, liposomes, with a lipid bilayer encapsulating nucleic acids, are more stable and complex, often containing cationic or fusogenic lipids and cholesterol-PEGylated lipids.[164] Examples of significant achievements include the suppression of hepatitis B virus[165] and *Apob*[166] via the utilization of siRNAs in preclinical animal investigations, alongside the authorization of patisiran, an siRNA delivered in LNP formulation, and the two COVID-19 mRNA vaccines (Table 1). CT102 utilizes liposomes for ASO delivery, targeting IGF1R mRNA to induce cell death in hepatocellular carcinoma. The formulation optimizes liver concentration through

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

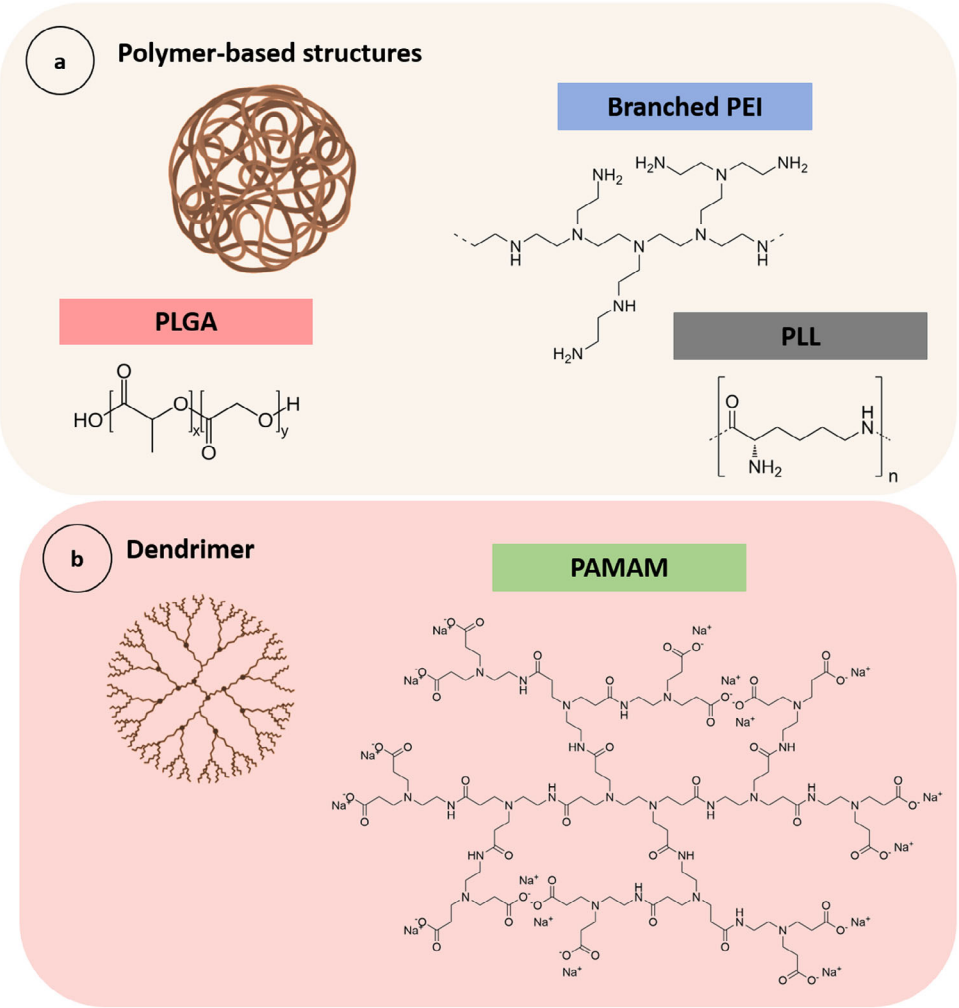


Figure 4. Polymer-based structures, including cationic polymers, such as those derived from poly(ethylenimine) (PEI) and poly(L-lysine) (PLL), bind to the negatively charged RNA backbone via electrostatic interactions involving their amine groups. Poly(lactic-co-glycolic acid) (PLGA)-based polymers are often chemically modified to introduce separate cationic functionalities for RNA binding (a). Dendrimers are highly branched macromolecules with a well-defined architecture, radiating outward from a central core. PAMAM refers to poly(amidoamine), a common dendrimer type (b).

intermolecular interactions like hydrogen bonding and electrostatic interactions.[167]

While these formulations have demonstrated significant value in vaccines and liver-targeted applications, their broader use in gene therapy is limited by poor access to extrahepatic tissues.[168] When LNPs enter the bloodstream, plasma proteins adsorb onto their surface, forming a "protein corona", with some proteins acting as markers for clearance by the mononuclear phagocyte system.[169] The liver, as the body's biological filtration system, sequesters LNPs and clears LNPs. Unintentional accumulation of LNPs in phagocytic nonparenchymal liver cells can hinder therapeutic efficacy by reducing their availability to the target tissue or organ, potentially leading to toxicity and adverse side effects.[170] To fully harness LNPs potential, it is crucial to understand and overcome their liver tropism and to devise strategies that enhance delivery to extrahepatic tissues. Extensive efforts are currently underway to achieve targeted delivery of nucleic acid therapies to extrahepatic tissues using LNPs, which can be broadly categorized into two major strategies: active and passive targeting. Active targeting involves grafting targeting ligands, including antibodies/antibody fragments,[171,172] peptides,[173,174] small sugars/molecules[175] or aptamers,[176] onto LNPs to enable interaction with specific molecular markers on the surface of the target cells. In contrast, passive targeting focuses on modulating the lipid composition to optimize surface characteristics, charge, and LNP size for improved biodistribution.[177,178]

3.3.2. Polymer-Based Nanocarriers

Non-viral RNA delivery systems often employ polymers and polymeric NPs, with cationic and non-cationic varieties being used, offering flexibility in charge, degradability, and molecular weight.[179,180] Poly(lactic-co-glycolic acid) (PLGA) has garnered FDA approval for delivering small-molecule drugs but faces challenges in delivering nucleic acids[181] (**Figure 4**). Thus, to use PLGA as an RNA delivery platform, cationic chemical groups as chitosan, were employed to deliver siRNA in mice, resulting in

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

the inhibition of genes encoding CD98 and TNF-α in the colonic tissue affected by ulcerative colitis.[182]

Cationic polymers like polyethylenimine (PEI) and poly(l-lysine) (PLL) can interact electrostatically with RNA molecules, facilitating their delivery into cells[183] (Figure 4). However, various modifications have been employed to enhance their tolerability and efficacy. Linearly branched PEI derivatives like jetPEI are commonly used for mRNA and siRNA transfection. For instance, NPs composed of PEG-grafted PEI, with various PEG terminal groups and grafting ratios, have been used to deliver mRNA to pulmonary immune cells[184] and cyclodextrin-PEI conjugates have been used to deliver an mRNA vaccine in vivo.[185] Similarly, in vitro release studies revealed that PLL-modified sericin-coated superparamagnetic iron oxide NPs incorporating siRNA exhibited sustained siRNA release kinetics.[186] Fluorination has also proven to be an effective approach for reducing PEI cytotoxicity and improving the biodistribuition control.[187,188] Fluorinated PEI significantly improved performance in siRNA delivery by lowering cell cytotoxicity and enabling greater siRNA acumulation in the liver. In contrast, non-fluorinated PEI primarily delivered siRNA to the lungs following intravenous administration in mice.[187]

Poly(beta-amino ester)s (PBAEs) offer improved biodegradation and lower cytotoxicity compared to PEI and PLL, synthesized with varied chemical structures to enhance drug delivery.[189,190] PBAEs have enabled targeted mRNA vaccine delivery to splenic dendritic cells following systemic administration, without the need for targeting ligands, resuting in a robust antitumor immune response in multiple murine tumor models.[191] Additionally, researchers have also used PBAE-based polymers to deliver mRNA-encoding Cas13 to SARS-CoV-2- infected hamsters via nebulization.[192]

Hybrid lipid-polymer systems, modified to enhance stability and delivery efficiency, also play significant roles in RNA delivery.[193] Another important class of polymers used for RNA delivery is dendrimers, which are characterized by a highly branched architecture.[194] Among them, polyamidoamine (PAMAM) is one of the most commonly used dendrimers in nanomedicine. PAMAM nanodendriplexes have been shown to effectively deliver plasmid DNA encoding short hairpin RNAs (shRNAs) to the brain and spleen,[195] and delivered PAMAM-siRNA conjugates have enabled targeted intracellular delivery in glioblastoma animal models.[196] Researchers have also engineered dendrimer-based lipid NPs to co-encapsulate and deliver a CRISPR-Cas system composed of three components (donor ssDNA, sgRNA and Cas9 mRNA) capable of inducing HDR-mediated correction of single amino acid mutations in vivo.[197] Additionally, novel biodegradable polymers like LODER protect RNA molecules from degradation and have shown promise in clinical trials, particularly for targeting specific mutations like the G12D-mutated KRAS gene.[198]

3.3.3. Silica Nanoparticles

Silica NPs, particularly mesoporous silica NPs (MSNs), offer significant advantages for pharmaceutical delivery due to their customizable surface properties and high loading capacity for nucleic acids.[1,40] MSNs can be modified with different functional groups to enhance biocompatibility and delivery efficiency.[199] They have been used in controlled drug delivery systems, showing promise in gradually releasing antibiotics and other therapeutics.[200,201] To address aggregation and tissue damage concerns, surface PEGylation has been performed, leading to the degradation into non-toxic products that are safely excreted.[202,203] A magnetic MSN-based siRNA delivery vehicle was developed by loading siRNAs into the MSNs mesopores, followed by PEI capping, PEGylation, and modification with the fusogenic peptide KALA. This formulation led to a prolonged half-life in the bloodstream and improved survival rates in lung cancer models.[204] MSNs have also demonstrated high loading capacity and successful delivery of functional survivin siRNA into human colon cancer cells, resulting in significant protein suppression and inhibition of cancer cell growth.[205]

Several studies have also explored the co-delivery of small-molecule drugs and nucleic acids using MSNs as carriers.[203] Notably, a core–shell nanostructure based on hollow-type MSNs (HMSNs) coated with PAMAM-aptamer conjugates was developed to co-deliver sorafenib and a CRISPR-Cas9 system for the synergistic treatment of hepatocellular carcinoma.[206] Moreover, Lee et al. (2018)[207] designed a dual-structured hybrid MSN platform for efficient co-delivery of siRNA and the anticancer drug doxorubicin. Despite their promise, the use of silica NPs presents several safety concerns. Studies have reported adverse effects such as lung damage, immune system dysfunction, and neurotoxicity, attributed to the physicochemical properties of silica NPs. The included impaired cytokine generation, altered phagocytic activity,[208] as well as increased reactive oxygen species levels,[209] emphasize the need for further investigation into their safety profile.

3.3.4. Carbon and Gold Nanomaterials

Gold nanoparticles, quantum dots, nanographene oxide, and carbon nanotubes are synthesized nanostructures capable of harboring RNA, protecting and delivering it to disease sites.[40] Studies have demonstrated their effectiveness in delivering therapeutic nucleic acids, suppressing target genes, and delivering small-molecule anticancer drugs.[210–213]

Gold nanoparticles possess distinctive optical behaviors and can be easily synthesized and surface-functionalized.[214] They have been successfully employed for the topical delivery of therapeutic nucleic acids in both mouse and human skin,[210] as well as for the targeted delivery of Glut1 siRNA in lung cancer starvation therapy.[215] Quantum dots have become essential tools in biomedical research, particularly for enabling simultaneous detection and quantitative, multiplexed, long-term fluorescence imaging.[216] Quantum dot–siRNA complexes have been reported to suppress target genes in HeLa cells.[211] while PEI-modified carboxyl quantum dots mediated the delivery of miR-10a to modulate macrophage polarity in a mouse model of Ashferman's syndrome.[216]

Other nanostructures as nanographene oxide modified with gadolinium, have been successfully employed to deliver small-molecule anticancer drugs and miRNA for the treatment of glioblastoma in mice.[212] Functionalized carbon nanotubes have

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

also been used to deliver siRNA to suppress protein expression in HeLa cells.[213]

3.3.5. Extracellular Vesicles

EVs, natural biological NPs, are gaining nanotechnology interest. These naturally occurring nanovesicles have attracted significant attention as potential carriers for pharmaceuticals and biotherapeutics, owing to their intrinsic ability to encapsulate biologically active cargo. They excel in oligonucleotide drug delivery,[54,55] transversing biological membranes like the blood-brain-barrier (BBB), evading phagocytosis, and exhibiting low toxicity.[217]

Efficient loading of therapeutic cargo into EVs remains a challenge, addressed through both endogenous[218] and exogenous loading methods,[55,219] including electroporation, sonication, among others. For instance, Ferreira et al. (2023) reported the development of an EV-based system for siRNA loading (iExoMEK1). The authors showed that the EV-mediated reduction of *MEK1* expression resulted in tumor regression, accompanied by angiogenesis decrease, in an *ex-vivo* model. Furthermore, EV-mediated intracellular delivery of the CRISPR-Cas system represents a promising strategy for precise and targeted gene editing. Notably, several of these EV-based delivery approaches have reached preclinical trials.[220–222] Further studies should focus on evaluating the safety, efficacy, and long-term stability of gene editing across several diseases, with particular emphasis on *ex vivo* targeting and advanced trial phases. Advances in EV engineering and CRISPR technology will continue to refine these therapies.

Another advantage of EVs is their ability to present target-specific surface ligands, allowing for precise targeting of specific cell types and tissues. These surface ligands can be used to modify EVs biodistribution, enhancing brain penetration[223] or targeting specific cells.[224,225] However, scaling up EV isolation to meet clinical demand remains challenging due to high costs and limited production yields. Additionally, preserving EVs for clinical applications poses a significant concern, highlighting the need for further research into standardized storage conditions.[217] Fortunatey, scalable manufacturing methods for therapeutic EVs have been developed, enabling their potential for clinical applications.[226] Companies like Codiak Biosciences and Evox Therapeutics are actively investigating engineered EVs, which have advanced to the clinical stages. For example, Codiak Biosciences developed exoASO-STAT6, an EV-based therapy engineered to deliver antisense ASOs that disrupt STAT6 signaling in tumor-associated macrophages, thereby inducing an anti-tumor immune response (currently in Phase I).[227]

More recently, to address some of the aforementioned limitations of EVs and to overcome issues such as the lack of endogenous functionality associated with liposomes, their combination to create hybrid EVs has been successfully achieved.[228,229] This approach leverages the natural origin of EVs and the structural versatility of liposomes, thereby accelerating the advancement of novel and efficient RNA delivery systems.

3.3.6. Emerging Novel Approaches

Another NP-based delivery strategy is the SNA strategy. SNA particles utilize hydrophobic core NPs (e.g., gold, silica) decorated with densely packed hydrophilic oligonucleotides via thiol linkages.[3,230] Unlike other designs, SNA-attached oligonucleotides extend outward, protecting oligonucleotides from degradation to some extent.[231] Self-assembling SNAs were developed to activate localized in situ photothermal effects in MCF-7 tumors upon miRNA-21 expression, offering reduced side effects and minimizing non-specific damage through controllable, targeted release.[232]

Building on in-depth research and promising preclinical results, biotechnology companies are actively advancing the clinical translation of SNAs, driven by their outstanding properties, particularly their ability to penetrate various tissues and cell types across a wide range of diseases. The first-in-human phase 0 trial of SNAs (NU-0129) in glioblastoma showed safe brain delivery, siRNA uptake by tumor cells, and reduced Bcl2L12 expression.[233] A phase I trial of cavrotolimod, an immune-stimulating SNA, showed good tolerance and progressed to phase II in combination with PD-1/PD-L1 therapy for skin cancer.[234] Topical SNA treatments for psoriasis targeting TNF-α and IL-17Rα also demonstrated promising safety in early clinical trials. Exicure Inc., a clinical-stage biotechnology company, is broadening the therapeutic scope of SNAs to target a range of diseases, including Angelman syndrome, Batten disease, alopecia, and spinocerebellar ataxia.[230]

DNA-based nanostructures, including DNA origami, DNA hydrogel, and DNA nanotubes, serve as versatile platforms for controlled drug delivery, offering precise shapes for oligonucleotide delivery by self-assembly through base pairing.[3,4] Their modular design has been used to incorporate ASOs,[235] miRNAs[236] or siRNAs,[237] and aptamers are incorporated on their surface for enhanced uptake and degradation resistance.[238] Specialized structures like DNA nanoclews deliver CRISPR-Cas9,[239] while others target tissue macrophages for immunostimulation.[240,241] However, challenges include high costs of DNA and errors in self-assembly, limiting their application in complex structures.[242]

As the NP pipeline continues to improve, next-generation RNA delivery systems can draw valuable insights from clinically approved platforms. Patisiran, for example, provides a well-characterized model, demonstrating stable pharmacokinetics, sustained gene silencing, and clear regulatory pathways.[47] Clinically successful systems tend to share six key features: biodegradable and scalable components, chemically simple structures amenable to large-scale manufacturing, efficient and functional target delivery, a wide therapeutic window with low toxicity, consistent performance across production batches, and the ability to be safely re-dosed. Together, these attributes offer a strategic framework for guiding the development of safer and more effective RNA-loaded NPs.

4. Conclusions and Future Perspectives

RNA therapeutics are rapidly expanding, with over twenty-four approved molecules to date. There has been a consistent rise in patent submissions concerning RNA therapeutics, particularly mRNA, surpassing other technological fields. The top 10 countries, led by the US, account for the majority of RNA therapeutic patent activity.[243] New RNA therapeutic strategies focus on personalized medicine, the integration of artificial intelligence (AI)

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

and bioinformatics, and the anticipated future developments in clinical practice and ongoing research.

Personalized medicine is becoming more viable with RNA technologies, enabling treatments tailored to individual genetic profiles.[244] Genomic and miRNA profiling can guide RNA-based therapies, including customized cancer immunotherapies, gene editing, and RNA vaccines. However, challenges like variable RNA expression across populations and complex biomarker identification require thorough validation across diverse cohorts to ensure clinical effectiveness.[245] Furthermore, circular RNAs (circRNAs) have shown potential as both biomarkers and therapeutic targets across a range of diseases.[246,247] Their inherent stability, resistance to degradation, and capacity to enhance protein expression make them promising candidates for various therapeutic applications, including vaccines and gene therapies.[248] Integrating circRNA research into the field of RNA therapeutics may unveil new insights and treatment strategies. Future efforts should prioritize understanding their roles in disease and advancing circRNA-based therapies to enhance patient outcomes.

The integration of AI and bioinformatics into RNA therapeutic development holds great promise for advancing both research and clinical applications.[249] AI can analyze large-scale genomic data to identify patterns and predict responses,[250] while machine learning facilitates the discovery of novel miRNA targets.[251] Bioinformatics tools are likewise crucial for decoding complex RNA regulatory networks involved in disease processes.[252] Moreover, AI-assisted design has significantly enhanced the accuracy and efficiency of RNA sequence design and screening processes, while also enabling adaptation to personalized therapeutic needs.[253,254] These advancements are particularly valuable in addressing one of the key challenges in RNA therapeutics: off-target effects, which remain a major concern for both siRNAs and gRNAs.[255] Although progress has been made in optimizing sequence specificity, strategies such as using multiple siRNAs or gRNAs targeting the same sequence at lower individual doses have shown promise in reducing off-target activity.[256] In CRISPR-Cas9 systems, engineering of nuclease variants has led to reduced off-target cleavage,[257] and computational tools for predicting off-target sites are rapidly evolving, offering increasingly reliable guidance for precise sequence design.[255] However, biologically validating AI-driven predictions remains a key challenge. Experimental confirmation and close collaboration between computational and clinical researchers are essential to ensure clinical relevance and successful translation.[258] With remarkable progress in RNA-based discoveries expected over the next five years, this is a pivotal moment to integrate RNA technologies with AI, an approach that could significantly accelerate the development and clinical adoption of RNA therapies.

RNA-based therapeutics, including ASOs, siRNAs, aptamers, mRNA, and CRISPR-Cas9, have emerged as pivotal tools for addressing untargeted diseases, propelling biopharmaceutical sectors, and offering new treatment strategies. Challenges like off-target binding, stability, sequence-induced toxicity, and delivery inefficiencies hinder widespread use. Chemical modifications, such as backbone and ribose modifications, play a key role in improving the stability and cellular uptake of RNA-based therapeutics. These modifications aim to reduce the RNA's negative charge, thereby enhancing its interaction with the cell surface and facilitating cellular entry. Common strategies include incorporating LNA bases and the conjugation of biomolecules such as GalNAc.

A major challenge in RNA therapeutics is ensuring effective delivery to target sites, given RNA's inherent physicochemical instability. No single chemical modification or delivery system can address all barriers. Therapeutic RNA must resist nucleases, evade immune clearance, reach specific tissues, and be efficiently released into the cytoplasm with high biocompatibility. Design strategies should be disease- and molecule-specific. From the approved RNA-based therapeutics, ASOs often rely solely on chemical modifications, such as phosphorothioate backbones or 2′-position substitutions, not requiring appropriate carriers. In contrast, siRNA and mRNA typically need both chemical modifications and delivery vectors. siRNA commonly uses conjugates, while mRNA relies on LNPs. Understanding the structure–activity relationship between RNA molecules and their delivery platforms, including hydrophobic, electrostatic, and covalent interactions, is key for optimizing stability and efficacy. Because RNA interacts with complex biological environments, combining chemical modifications with advanced delivery systems is often necessary. Smart, stimuli-responsive nanocarriers, triggered by pH, redox conditions, or external stimuli like light or magnetic fields, offer promising improvements in targeted delivery and reduced toxicity, though they increase technical complexity and production costs.[259]

An additional limitation in RNA therapeutics development is the gap between in vitro and in vivo models, which contributes to the high failure rate of candidate drugs. To address this, screening platforms are evolving from traditional high-throughput methods toward high-content approaches that enable detailed imaging and analysis of multiple features within multicellular, 3D cell culture systems, which more faithfully replicate in vivo cellular behavior.

In summary, RNA therapeutics have rapidly progressed from abstract scientific concepts to transformative clinical tools, propelled by breakthroughs in molecular biology, chemical engineering, and delivery technologies. Yet, significant hurdles remain before their full potential is realized in everyday clinical practice. Looking ahead, the smart integration of precisely engineered nucleic acids, tailored chemical modifications, and next-generation delivery systems promises to unlock a new era of personalized medicine that is poised to tackle some of the most pressing unmet needs in modern healthcare.

Acknowledgements

This study was supported by the Portuguese Foundation for Science and Technology (FCT) under the scope of the strategic funding of UIDB/04469/ unit, and by LABBELS – Associate Laboratory in Biotechnology, Bioengineering and Microelectromechanical Systems, LA/P/0029/2020. Débora Ferreira acknowledge FCT for the Scientific Employment Stimulus (https://doi.org/10.54499/2022.01681.CEECIND/CP1718/CT0013).

Conflict of Interest

The authors declare no conflict of interest.

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Author Contributions

D.F. performed in writing – original draft, writing – review and editing, conceptualization. L.R.R. performed in writing – review and editing, supervision, conceptualization

Keywords

antisense oligonucleotides, aptamers, CRISPR-Cas, mRNA-encoded approaches, RNA therapeutics, RNA-interference mechanisms

Received: February 19, 2025
Revised: June 4, 2025
Published online:

[1] S. Chatterjee, M. Bhattacharya, S.-S. Lee, C. Chakraborty, *Curr. Res. Biotechnol.* **2023**, *6*, 100150.
[2] H. Parhiz, E. N. Atochina-Vasserman, D. Weissman, *Lancet* **2024**, *403*, 1192.
[3] Y. Zhu, L. Zhu, X. Wang, H. Jin, *Cell Death Dis.* **2022**, *13*, 644.
[4] T. C. Roberts, R. Langer, M. J. A. Wood, *Nat. Rev. Drug Discovery* **2020**, *19*, 673.
[5] J. P. Falese, A. Donlic, A. E. Hargrove, *Chem. Soc. Rev.* **2021**, *50*, 2224.
[6] S. T. Crooke, B. F. Baker, R. M. Crooke, X. Liang, *Nat. Rev. Drug Discovery* **2021**, *20*, 427.
[7] S. T. Crooke, X.-H. Liang, B. F. Baker, R. M. Crooke, *J. Biol. Chem.* **2021**, *296*, 100416.
[8] M. Egli, M. Manoharan, *Nucleic Acids Res.* **2023**, *51*, 2529.
[9] R. L. Setten, J. J. Rossi, S. Han, *Nat. Rev. Drug Discovery* **2019**, *18*, 421.
[10] N. Chaudhary, D. Weissman, K. A. Whitehead, *Nat. Rev. Drug Discovery* **2021**, *20*, 817.
[11] E. Rohner, R. Yang, K. S. Foo, A. Goedel, K. R. Chien, *Nat. Biotechnol.* **2022**, *40*, 1586.
[12] A. Raguram, S. Banskota, D. R. Liu, *Cell* **2022**, *185*, 2806.
[13] J. Y. Wang, J. A. Doudna, *Science* **2023**, *379*, add8643.
[14] A. Sparmann, J. Vogel, *EMBO J.* **2023**, *42*, 114760.
[15] S. T. Crooke, *Nucleic Acid Ther.* **2017**, *27*, 70.
[16] C. M. Perry, J. A. Barman Balfour, *Drugs* **1999**, *57*, 375.
[17] F. J. Raal, R. D. Santos, D. J. Blom, A. D. Marais, M.-J. Charng, W. C. Cromwell, R. H. Lachmann, D. Gaudet, J. L. Tan, S. Chasan-Taber, D. L. Tribble, J. D. Flaim, S. T. Crooke, *Lancet* **2010**, *375*, 998.
[18] J. Paik, S. Duggan, *Drugs* **2019**, *79*, 1349.
[19] V. Mathew, A. K. Wang, *Drug Des., Dev. Ther.* **2019**, *13*, 1515.
[20] T. M. Miller, M. E. Cudkowicz, A. Genge, P. J. Shaw, G. Sobue, R. C. Bucelli, A. Chiò, P. Van Damme, A. C. Ludolph, J. D. Glass, J. A. Andrews, S. Babu, M. Benatar, C. J. McDermott, T. Cochrane, S. Chary, S. Chew, H. Zhu, F. Wu, I. Nestorov, D. Graham, P. Sun, M. McNeill, L. Fanning, T. A. Ferguson, S. Fradette, *N. Engl. J. Med.* **2022**, *387*, 1099.
[21] T. Coelho, W. Marques, N. R. Dasgupta, C.-C. Chao, Y. Parman, M. C. França, Y.-C. Guo, J. Wixner, L.-S. Ro, C. R. Calandra, P. A. Kowacs, J. L. Berk, L. Obici, F. A. Barroso, M. Weiler, I. Conceição, S. W. Jung, G. Buchele, M. Brambatti, J. Chen, S. G. Hughes, E. Schneider, N. J. Viney, A. Masri, M. R. Gertz, Y. Ando, J. D. Gillmore, S. Khella, P. J. B. Dyck, M. Waddington Cruz, et al., *JAMA, J. Am. Med. Assoc.* **2023**, *330*, 1448.
[22] E. S. G. Stroes, V. J. Alexander, E. Karwatowska-Prokopczuk, R. A. Hegele, M. Arca, C. M. Ballantyne, H. Soran, T. A. Prohaska, S. Xia, H. N. Ginsberg, J. L. Witztum, S. Tsimikas, *N. Engl. J. Med.* **2024**, *390*, 1781.
[23] J. Desterro, P. Bak-Gordon, M. Carmo-Fonseca, *Nat. Rev. Drug Discovery* **2020**, *19*, 112.
[24] K. R. Lim, R. Maruyama, T. Yokota, *Drug Des., Dev. Ther.* **2017**, *11*, 533.
[25] Y.-A. Heo, *Drugs* **2020**, *80*, 329.
[26] P. R. Clemens, V. K. Rao, A. M. Connolly, A. D. Harper, J. K. Mah, C. M. McDonald, E. C. Smith, C. M. Zaidman, T. Nakagawa, E. P. Hoffman, *J. Neuromuscular Dis.* **2022**, *9*, 493.
[27] K. R. Wagner, N. L. Kuntz, E. Koenig, L. East, S. Upadhyay, B. Han, P. B. Shieh, *Muscle Nerve* **2021**, *64*, 285.
[28] E. E. Neil, E. K. Bisaccia, *J. Pediatr. Pharmacol. Ther.* **2019**, *24*, 194.
[29] A. J. Ward, M. Norrbom, S. Chun, C. F. Bennett, F. Rigo, *Nucleic Acids Res.* **2014**, *42*, 5871.
[30] D. A. Melton, *Proc. Natl. Acad. Sci.* **1985**, *82*, 144.
[31] B. Baker, *Nucleic Acids Res.* **1999**, *27*, 1547.
[32] T. A. Vickers, J. R. Wyatt, T. Burckin, C. F. Bennett, S. M. Freier, *Nucleic Acids Res.* **2001**, *29*, 1293.
[33] X. Liang, W. Shen, H. Sun, M. T. Migawa, T. A. Vickers, S. T. Crooke, *Nat. Biotechnol.* **2016**, *34*, 875.
[34] X. Liang, H. Sun, W. Shen, S. Wang, J. Yao, M. T. Migawa, H.-H. Bui, S. S. Damle, S. Riney, M. J. Graham, R. M. Crooke, S. T. Crooke, *Nucleic Acids Res.* **2017**, *45*, 9528.
[35] Z. Li, T. M. Rana, *Nat. Rev. Drug Discovery* **2014**, *13*, 622.
[36] W.-Y. Choi, A. J. Giraldez, A. F. Schier, *Science* **2007**, *318*, 271.
[37] H. Dana, G. M. Chalbatani, H. Mahmoodzadeh, R. Karimloo, O. Rezaiean, A. Moradzadeh, N. Mehmandoost, F. Moazzen, A. Mazraeh, V. Marmari, M. Ebrahimi, M. M. Rashno, S. J. Abadi, E. Gharagouzlo, *Int. J. Biomed. Sci.* **2017**, *13*, 48.
[38] J. G. Parvani, M. W. Jackson, *Endocr.-Relat. Cancer* **2017**, *24*, R81.
[39] G. Shan, *Int. J. Biochem. Cell Biol.* **2010**, *42*, 1243.
[40] T. R. Damase, R. Sukhovershin, C. Boada, F. Taraballi, R. I. Pettigrew, J. P. Cooke, *Front. Bioeng. Biotechnol.* **2021**, *9*, 628137.
[41] E. Fakhr, F. Zare, L. Teimoori-Toolabi, *Cancer Gene Ther.* **2016**, *23*, 73.
[42] D.-H. Kim, M. A. Behlke, S. D. Rose, M.-S. Chang, S. Choi, J. J. Rossi, *Nat. Biotechnol.* **2005**, *23*, 222.
[43] M. Byrne, R. Tzekov, Y. Wang, A. Rodgers, J. Cardia, G. Ford, K. Holton, L. Pandarinathan, J. Lapierre, W. Stanney, K. Bulock, S. Shaw, L. Libertine, K. Fettes, A. Khvorova, S. Kaushal, P. Pavco, *J. Ocul. Pharmacol. Ther.* **2013**, *29*, 855.
[44] J. B. Bramsen, M. B. Laursen, C. K. Damgaard, S. W. Lena, B. Ravindra Babu, J. Wengel, J. Kjems, *Nucleic Acids Res.* **2007**, *35*, 5886.
[45] J. F. Alterman, B. M. D. C. Godinho, M. R. Hassler, C. M. Ferguson, D. Echeverria, E. Sapp, R. A. Haraszti, A. H. Coles, F. Conroy, R. Miller, L. Roux, P. Yan, E. G. Knox, A. A. Turanov, R. M. King, G. Gernoux, C. Mueller, H. L. Gray-Edwards, R. P. Moser, N. C. Bishop, S. M. Jaber, M. J. Gounis, M. Sena-Esteves, A. A. Pai, M. DiFiglia, N. Aronin, A. Khvorova, *Nat. Biotechnol.* **2019**, *37*, 884.
[46] W. F. Lima, T. P. Prakash, H. M. Murray, G. A. Kinberger, W. Li, A. E. Chappell, C. S. Li, S. F. Murray, H. Gaus, P. P. Seth, E. E. Swayze, S. T. Crooke, *Cell* **2012**, *150*, 883.
[47] X. Zhang, V. Goel, G. J. Robbie, *J. Clin. Pharmacol.* **2020**, *60*, 573.
[48] D. Adams, I. L. Tournev, M. S. Taylor, T. Coelho, V. Planté-Bordeneuve, J. L. Berk, A. González-Duarte, J. D. Gillmore, S.-C. Low, Y. Sekijima, L. Obici, C. Chen, P. Badri, S. M. Arum, J. Vest, M. Polydefkis, *Amyloid* **2023**, *30*, 18.
[49] M. Balwani, E. Sardh, P. Ventura, P. A. Peiró, D. C. Rees, U. Stölzel, D. M. Bissell, H. L. Bonkovsky, J. Windyga, K. E. Anderson, C. Parker, S. M. Silver, S. B. Keel, J.-D. Wang, P. E. Stein, P. Harper, D. Vassiliou, B. Wang, J. Phillips, A. Ivanova, J. G. Langendonk, R. Kauppinen, E. Minder, Y. Horie, C. Penz, J. Chen, S. Liu, J. J. Ko, M. T. Sweetser, P. Garg, et al., *N. Engl. J. Med.* **2020**, *382*, 2289.
[50] Y. N. Lamb, *Drugs* **2021**, *81*, 389.

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

[51] S. F. Garrelfs, Y. Frishberg, S. A. Hulton, M. J. Koren, W. D. O'Riordan, P. Cochat, G. Deschênes, H. Shasha-Lavsky, J. M. Saland, W. G. van't Hoff, D. G. Fuster, D. Magen, S. H. Moochhala, G. Schalk, E. Simkova, J. W. Groothoff, D. J. Sas, K. A. Meliambro, J. Lu, M. T. Sweetser, P. P. Garg, A. K. Vaishnaw, J. M. Gansner, T. L. McGregor, J. C. Lieske, *N. Engl. J. Med.* **2021**, *384*, 1216.
[52] Y. Y. Syed, *Drugs* **2023**, *83*, 1729.
[53] G. Young, A. Srivastava, K. Kavakli, C. Ross, J. Sathar, C.-W. You, H. Tran, J. Sun, R. Wu, S. Poloskey, Z. Qiu, S. Kichou, S. Andersson, B. Mei, S. Rangarajan, *Lancet* **2023**, *401*, 1427.
[54] D. Ferreira, C. Santos-Pereira, M. Costa, J. Afonso, S. Yang, J. Hensel, K. M. McAndrews, A. Longatto-Filho, R. Fernandes, J. B. Melo, F. Baltazar, J. N. Moreira, R. Kalluri, L. R. Rodrigues, *Biomater. Adv.* **2023**, *154*, 213643.
[55] R. Silva, D. Ferreira, L. R. Rodrigues, *J. Drug Delivery Sci. Technol.* **2022**, *78*, 103931.
[56] R. Rupaimoole, F. J. Slack, *Nat. Rev. Drug Discovery* **2017**, *16*, 203.
[57] R. C. Lee, R. L. Feinbaum, V. Ambros, *Cell* **1993**, *75*, 843.
[58] J. K. W. Lam, M. Y. T. Chow, Y. Zhang, S. W. S. Leung, *Mol. Ther.–Nucleic Acids* **2015**, *4*, 252.
[59] S. Lou, T. Sun, H. Li, Z. Hu, *Biotechnol. Biofuels* **2018**, *11*, 244.
[60] J. O'Brien, H. Hayder, Y. Zayed, C. Peng, *Front. Endocrinol.* **2018**, *9*, 402.
[61] A. A. Seyhan, *Int. J. Mol. Sci.* **2024**, *25*, 1469.
[62] B. Jani, R. Fuchs, *J. Visualized Exp.* **2012**, *26*, 3702.
[63] K. Karikó, M. Buckstein, H. Ni, D. Weissman, *Immunity* **2005**, *23*, 165.
[64] B. Connolly, C. Isaacs, L. Cheng, K. H. Asrani, R. R. Subramanian, *J. Nucleic Acids* **2018**, *2018*, 1.
[65] J. Cao, D. An, M. Galduroz, J. Zhuo, S. Liang, M. Eybye, A. Frassetto, E. Kuroda, A. Funahashi, J. Santana, C. Mihai, K. E. Benenato, E. S. Kumarasinghe, S. Sabnis, T. Salerno, K. Coughlan, E. J. Miracco, B. Levy, G. Besin, J. Schultz, C. Lukacs, L. Guey, P. Finn, T. Furukawa, P. H. Giangrande, T. Saheki, P. G. V. Martini, *Mol. Ther.* **2019**, *27*, 1242.
[66] X. Zhu, L. Yin, M. Theisen, J. Zhuo, S. Siddiqui, B. Levy, V. Presnyak, A. Frassetto, J. Milton, T. Salerno, K. E. Benenato, J. Milano, A. Lynn, S. Sabnis, K. Burke, G. Besin, C. M. Lukacs, L. T. Guey, P. F. Finn, P. G. V. Martini, *Am. J. Hum. Genet.* **2019**, *104*, 625.
[67] V. Anttila, A. Saraste, J. Knuuti, P. Jaakkola, M. Hedman, S. Svedlund, M. Lagerström-Fermér, M. Kjaer, A. Jeppsson, L.-M. Gan, *Mol. Ther.–Methods Clin. Dev.* **2020**, *18*, 464.
[68] M. Alberer, U. Gnad-Vogt, H. S. Hong, K. T. Mehr, L. Backert, G. Finak, R. Gottardo, M. A. Bica, A. Garofano, S. D. Koch, M. Fotin-Mleczek, I. Hoerr, R. Clemens, F. von Sonnenburg, *Lancet* **2017**, *390*, 1511.
[69] L. A. Jackson, E. J. Anderson, N. G. Rouphael, P. C. Roberts, M. Makhene, R. N. Coler, M. P. McCullough, J. D. Chappell, M. R. Denison, L. J. Stevens, A. J. Pruijssers, A. McDermott, B. Flach, N. A. Doria-Rose, K. S. Corbett, K. M. Morabito, S. O'Dell, S. D. Schmidt, P. A. Swanson, M. Padilla, J. R. Mascola, K. M. Neuzil, H. Bennett, W. Sun, E. Peters, M. Makowski, J. Albert, K. Cross, W. Buchanan, R. Pikaart-Tautges, et al., *N. Engl. J. Med.* **2020**, *383*, 1920.
[70] M. J. Mulligan, K. E. Lyke, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, K. Neuzil, V. Raabe, R. Bailey, K. A. Swanson, P. Li, K. Koury, W. Kalina, D. Cooper, C. Fontes-Garfias, P.-Y. Shi, Ö. Türeci, K. R. Tompkins, E. E. Walsh, R. Frenck, A. R. Falsey, P. R. Dormitzer, W. C. Gruber, U. Şahin, K. U. Jansen, *Nature* **2020**, *586*, 589.
[71] F. P. Polack, S. J. Thomas, N. Kitchin, J. Absalon, A. Gurtman, S. Lockhart, J. L. Perez, G. Pérez Marc, E. D. Moreira, C. Zerbini, R. Bailey, K. A. Swanson, S. Roychoudhury, K. Koury, P. Li, W. V. Kalina, D. Cooper, R. W. Frenck, L. L. Hammitt, Ö. Türeci, H. Nell, A. Schaefer, S. Ünal, D. B. Tresnan, S. Mather, P. R. Dormitzer, U. Şahin, K. U. Jansen, W. C. Gruber, *N. Engl. J. Med.* **2020**, *383*, 2603.
[72] L. R. Baden, H. M. El Sahly, B. Essink, K. Kotloff, S. Frey, R. Novak, D. Diemert, S. A. Spector, N. Rouphael, C. B. Creech, J. McGettigan, S. Khetan, N. Segall, J. Solis, A. Brosz, C. Fierro, H. Schwartz, K. Neuzil, L. Corey, P. Gilbert, H. Janes, D. Follmann, M. Marovich, J. Mascola, L. Polakowski, J. Ledgerwood, B. S. Graham, H. Bennett, R. Pajon, C. Knightly, et al., *N. Engl. J. Med.* **2021**, *384*, 403.
[73] E. J. Sayour, D. Boczkowski, D. A. Mitchell, S. K. Nair, *Nat. Rev. Clin. Oncol.* **2024**, *21*, 489.
[74] F. Lang, B. Schrörs, M. Löwer, Ö. Türeci, U. Sahin, *Nat. Rev. Drug Discovery* **2022**, *21*, 261.
[75] L. M. Kranz, M. Diken, H. Haas, S. Kreiter, C. Loquai, K. C. Reuter, M. Meng, D. Fritz, F. Vascotto, H. Hefesha, C. Grunwitz, M. Vormehr, Y. Hüsemann, A. Selmi, A. N. Kuhn, J. Buck, E. Derhovanessian, R. Rae, S. Attig, J. Diekmann, R. A. Jabulowsky, S. Heesch, J. Hassel, P. Langguth, S. Grabbe, C. Huber, Ö. Türeci, U. Sahin, *Nature* **2016**, *534*, 396.
[76] U. Sahin, E. Derhovanessian, M. Miller, B.-P. Kloke, P. Simon, M. Löwer, V. Bukur, A. D. Tadmor, U. Luxemburger, B. Schrörs, T. Omokoko, M. Vormehr, C. Albrecht, A. Paruzynski, A. N. Kuhn, J. Buck, S. Heesch, K. H. Schreeb, F. Müller, I. Ortseifer, I. Vogler, E. Godehardt, S. Attig, R. Rae, A. Breitkreuz, C. Tolliver, M. Suchan, G. Martic, A. Hohberger, P. Sorn, et al., *Nature* **2017**, *547*, 222.
[77] C. D. Palmer, A. R. Rappaport, M. J. Davis, M. G. Hart, C. D. Scallan, S.-J. Hong, L. Gitlin, L. D. Kraemer, S. Kounlavouth, A. Yang, L. Smith, D. Schenk, M. Skoberne, K. Taquechel, M. Marrali, J. R. Jaroslavsky, C. N. Nganje, E. Maloney, R. Zhou, D. Navarro-Gomez, A. C. Greene, G. Grotenbreg, R. Greer, W. Blair, M. D. Cao, S. Chan, K. Bae, A. I. Spira, S. Roychowdhury, D. P. Carbone, et al., *Nat. Med.* **2022**, *28*, 1619.
[78] L. A. Rojas, Z. Sethna, K. C. Soares, C. Olcese, N. Pang, E. Patterson, J. Lihm, N. Ceglia, P. Guasp, A. Chu, R. Yu, A. K. Chandra, T. Waters, J. Ruan, M. Amisaki, A. Zebboudj, Z. Odgerel, G. Payne, E. Derhovanessian, F. Müller, I. Rhee, M. Yadav, A. Dobrin, M. Sadelain, M. Łuksza, N. Cohen, L. Tang, O. Basturk, M. Gönen, S. Katz, et al., *Nature* **2023**, *618*, 144.
[79] E. Dolgin, *Nat. Rev. Drug Discovery* **2023**, *22*, 607.
[80] C. Krienke, L. Kolb, E. Diken, M. Streuber, S. Kirchhoff, T. Bukur, Ö. Akilli-Öztürk, L. M. Kranz, H. Berger, J. Petschenka, M. Diken, S. Kreiter, N. Yogev, A. Waisman, K. Karikó, Ö. Türeci, U. Sahin, *Science* **2021**, *371*, 145.
[81] P. J. L. Schürmann, S. P. E. van Breda Vriesman, J. A. Castro-Alpízar, S. A. A. Kooijmans, E. E. S. Nieuwenhuis, R. M. Schiffelers, S. A. Fuchs, *Wiley Interdiscip. Rev.:Nanomed. Nanobiotechnol.* **2025**, *17*, 70019.
[82] G. Shen, J. Liu, H. Yang, N. Xie, Y. Yang, *J. Controlled Release* **2024**, *369*, 696.
[83] L. Jiang, J.-S. Park, L. Yin, R. Laureano, E. Jacquinet, J. Yang, S. Liang, A. Frassetto, J. Zhuo, X. Yan, X. Zhu, S. Fortucci, K. Hoar, C. Mihai, C. Tunkey, V. Presnyak, K. E. Benenato, C. M. Lukacs, P. G. V. Martini, L. T. Guey, *Nat. Commun.* **2020**, *11*, 5339.
[84] D. Jericó, K. M. Córdoba, L. Jiang, C. Schmitt, M. Morán, A. Sampedro, M. Alegre, M. Collantes, E. Santamaría, E. Alegre, C. Culerier, A. E.-H. de Mendoza, J. Oyarzabal, M. A. Martín, I. Peñuelas, M. A. Ávila, L. Gouya, P. G. V. Martini, A. Fontanellas, *Mol. Ther.–Nucleic Acids* **2021**, *25*, 207.
[85] D. Sousa, D. Ferreira, J. L. Rodrigues, L. R. Rodrigues, *Applications of Targeted Nano Drugs and Delivery Systems*, Elsevier, Amsterdam, Netherlands, **2019**.
[86] A. C. Pereira, A. F. Pina, D. Sousa, D. Ferreira, C. Santos-Pereira, J. L. Rodrigues, L. D. R. Melo, G. Sales, S. F. Sousa, L. R. Rodrigues, *Mol. Syst. Des. Eng.* **2022**, *7*, 637.
[87] M. G. Sande, D. Ferreira, J. L. Rodrigues, L. D. R. Melo, A. Saragliadis, D. Linke, F. T. C. Moreira, M. G. F. Sales, L. R. Rodrigues, *Bioengineering* **2023**, *10*, 178.

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

[88] M. G. Sande, D. Ferreira, J. L. Rodrigues, L. D. R. Melo, D. Linke, C. J. Silva, F. T. C. Moreira, M. G. F. Sales, L. R. Rodrigues, *Biosensors* **2022**, *12*, 614.
[89] L. Guo, Y. Song, Y. Yuan, J. Chen, H. Liang, F. Guo, Z. Yu, P. Liang, Y. Wang, P. Wang, *Anal. Bioanal. Chem.* **2021**, *413*, 4427.
[90] X. Zhang, Y. Gao, B. Deng, B. Hu, L. Zhao, H. Guo, C. Yang, Z. Ma, M. Sun, B. Jiao, L. Wang, *Toxins* **2022**, *14*, 195.
[91] C. Bai, Z. Lu, H. Jiang, Z. Yang, X. Liu, H. Ding, H. Li, J. Dong, A. Huang, T. Fang, Y. Jiang, L. Zhu, X. Lou, S. Li, N. Shao, *Biosens. Bioelectron.* **2018**, *110*, 162.
[92] G. S. Zamay, T. I. Ivanchenko, T. N. Zamay, V. L. Grigorieva, Y. E. Glazyrin, O. S. Kolovskaya, I. V. Garanzha, A. A. Barinov, A. V. Krat, G. G. Mironov, A. Gargaun, D. V. Veprintsev, S. S. Bekuzarov, A. K. Kirichenko, R. A. Zukov, M. M. Petrova, A. A. Modestov, M. V. Berezovski, A. S. Zamay, *Mol. Ther.–Nucleic Acids* **2017**, *6*, 150.
[93] K. Tsogtbaatar, D. A. Sousa, D. Ferreira, A. Tevlek, H. M. Aydın, E. Çelik, L. Rodrigues, *Invest. New Drugs* **2022**, *40*, 172.
[94] D. Ferreira, J. Barbosa, D. A. Sousa, C. Silva, L. D. R. Melo, M. Avci-Adali, H. P. Wendel, L. R. Rodrigues, *Sci. Rep.* **2021**, *11*, 8614.
[95] J. Byun, *Life* **2021**, *11*, 193.
[96] Z. Fu, J. Xiang, *Int. J. Mol. Sci.* **2020**, *21*, 2793.
[97] A. D. Ellington, J. W. Szostak, *Nature* **1990**, *346*, 818.
[98] C. Tuerk, L. Gold, *Science* **1990**, *249*, 505.
[99] J. Zhou, J. Rossi, *Nat. Rev. Drug Discovery* **2017**, *16*, 181.
[100] J. Zhou, J. J. Rossi, *Mol. Ther.–Nucleic Acids* **2014**, *3*, 169.
[101] E. W. M. Ng, D. T. Shima, P. Calias, E. T. Cunningham, D. R. Guyer, A. P. Adamis, *Nat. Rev. Drug Discovery* **2006**, *5*, 123.
[102] A. M. Khanani, S. S. Patel, G. Staurenghi, R. Tadayoni, C. J. Danzig, D. A. Eichenbaum, J. Hsu, C. C. Wykoff, J. S. Heier, D. R. Lally, J. Monés, J. S. Nielsen, V. S. Sheth, P. K. Kaiser, J. Clark, L. Zhu, H. Patel, J. Tang, D. Desai, G. J. Jaffe, A. Alezzandrini, A. A. Francone, J. Bafalluy, S. Bainttein, J. Luna Pinto, M. Saravia, M. Vidosevich, C. Zeolite, F. Furno Sola, A. Chang, et al., *Lancet* **2023**, *402*, 1449.
[103] A. M. Sousa, D. Ferreira, L. R. Rodrigues, M. O. Pereira, *J. Controlled Release* **2024**, *367*, 522.
[104] S. J. J. Brouns, M. M. Jore, M. Lundgren, E. R. Westra, R. J. H. Slijkhuis, A. P. L. Snijders, M. J. Dickman, K. S. Makarova, E. V. Koonin, J. van der Oost, *Science* **2008**, *321*, 960.
[105] F. J. M. Mojica, C. Díez-Villasenor, J. García-Martínez, E. Soria, *J. Mol. Evol.* **2005**, *60*, 174.
[106] N. Dammes, D. Peer, *Trends Pharmacol. Sci.* **2020**, *41*, 755.
[107] G. Sharma, A. R. Sharma, M. Bhattacharya, S.-S. Lee, C. Chakraborty, *Mol. Ther.* **2021**, *29*, 571.
[108] M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna, E. Charpentier, *Science* **2012**, *337*, 816.
[109] M. Jinek, A. East, A. Cheng, S. Lin, E. Ma, J. Doudna, *Elife* **2013**, *2*, 00471.
[110] P. Mali, L. Yang, K. M. Esvelt, J. Aach, M. Guell, J. E. DiCarlo, J. E. Norville, G. M. Church, *Science* **2013**, *339*, 823.
[111] F. Jiang, J. A. Doudna, *Annu. Rev. Biophys.* **2017**, *46*, 505.
[112] J. H. Hu, S. M. Miller, M. H. Geurts, W. Tang, L. Chen, N. Sun, C. M. Zeina, X. Gao, H. A. Rees, Z. Lin, D. R. Liu, *Nature* **2018**, *556*, 57.
[113] S. M. Miller, T. Wang, P. B. Randolph, M. Arbab, M. W. Shen, T. P. Huang, Z. Matuszek, G. A. Newby, H. A. Rees, D. R. Liu, *Nat. Biotechnol.* **2020**, *38*, 471.
[114] Y. Lu, J. Xue, T. Deng, X. Zhou, K. Yu, L. Deng, M. Huang, X. Yi, M. Liang, Y. Wang, H. Shen, R. Tong, W. Wang, L. Li, J. Song, J. Li, X. Su, Z. Ding, Y. Gong, J. Zhu, Y. Wang, B. Zou, Y. Zhang, Y. Li, L. Zhou, Y. Liu, M. Yu, Y. Wang, X. Zhang, L. Yin, et al., *Nat. Med.* **2020**, *26*, 732.
[115] H. Frangoul, D. Altshuler, M. D. Cappellini, Y.-S. Chen, J. Domm, B. K. Eustace, J. Foell, J. de la Fuente, S. Grupp, R. Handgretinger, T. W. Ho, A. Kattamis, A. Kernytsky, J. Lekstrom-Himes, A. M. Li, F. Locatelli, M. Y. Mapara, M. de Montalembert, D. Rondelli, A. Sharma, S. Sheth, S. Soni, M. H. Steinberg, D. Wall, A. Yen, S. Corbacioglu, *N. Engl. J. Med.* **2021**, *384*, 252.
[116] J. D. Gillmore, E. Gane, J. Taubel, J. Kao, M. Fontana, M. L. Maitland, J. Seitzer, D. O'Connell, K. R. Walsh, K. Wood, J. Phillips, Y. Xu, A. Amaral, A. P. Boyd, J. E. Cehelsky, M. D. McKee, A. Schiermeier, O. Harari, A. Murphy, C. A. Kyratsous, B. Zambrowicz, R. Soltys, D. E. Gutstein, J. Leonard, L. Sepp-Lorenzino, D. Lebwohl, *N. Engl. J. Med.* **2021**, *385*, 493.
[117] J. Wen, T. Cao, J. Wu, Y. Chen, S. Zhi, Y. Huang, P. Zhen, G. Wu, L. Aagaard, J. Zhong, P. Liang, J. Huang, *Mol. Ther.* **2022**, *30*, 164.
[118] M. L. Maeder, M. Stefanidakis, C. J. Wilson, R. Baral, L. A. Barrera, G. S. Bounoutas, D. Bumcrot, H. Chao, D. M. Ciulla, J. A. DaSilva, A. Dass, V. Dhanapal, T. J. Fennell, A. E. Friedland, G. Giannoukos, S. W. Gloskowski, A. Glucksmann, G. M. Gotta, H. Jayaram, S. J. Haskett, B. Hopkins, J. E. Horng, S. Joshi, E. Marco, R. Mepani, D. Reyon, T. Ta, D. G. Tabbaa, S. J. Samuelsson, S. Shen, et al., *Nat. Med.* **2019**, *25*, 229.
[119] P. Mancuso, C. Chen, R. Kaminski, J. Gordon, S. Liao, J. A. Robinson, M. D. Smith, H. Liu, I. K. Sariyer, R. Sariyer, T. A. Peterson, M. Donadoni, J. B. Williams, S. Siddiqui, B. A. Bunnell, B. Ling, A. G. MacLean, T. H. Burdo, K. Khalili, *Nat. Commun.* **2020**, *11*, 6065.
[120] S. F. Dowdy, *Nat. Biotechnol.* **2017**, *35*, 222.
[121] A. Khvorova, J. K. Watts, *Nat. Biotechnol.* **2017**, *35*, 238.
[122] J. B. Bramsen, J. Kjems, *Front. Genet.* **2012**, *3*, 154.
[123] J. Soutschek, A. Akinc, B. Bramlage, K. Charisse, R. Constien, M. Donoghue, S. Elbashir, A. Geick, P. Hadwiger, J. Harborth, M. John, V. Kesavan, G. Lavine, R. K. Pandey, T. Racie, K. G. Rajeev, I. Röhl, I. Toudjarska, G. Wang, S. Wuschko, D. Bumcrot, V. Koteliansky, S. Limmer, M. Manoharan, H.-P. Vornlocher, *Nature* **2004**, *432*, 173.
[124] C. Ämmälä, W. J. Drury, L. Knerr, I. Ahlstedt, P. Stillemark-Billton, C. Wennberg-Huldt, E.-M. Andersson, E. Valeur, R. Jansson-Löfmark, D. Janzén, L. Sundström, J. Meuller, J. Claesson, P. Andersson, C. Johansson, R. G. Lee, T. P. Prakash, P. P. Seth, B. P. Monia, S. Andersson, *Sci. Adv.* **2018**, *4*, aat3386.
[125] K. Paunovska, D. Loughrey, J. E. Dahlman, *Nat. Rev. Genet.* **2022**, *23*, 265.
[126] T. P. Prakash, *Chem. Biodivers.* **2011**, *8*, 1616.
[127] R. A. Haraszti, L. Roux, A. H. Coles, A. A. Turanov, J. F. Alterman, D. Echeverria, B. M. D. C. Godinho, N. Aronin, A. Khvorova, *Nucleic Acids Res.* **2017**, *45*, 7581.
[128] S. P. Pradeep, S. Malik, F. J. Slack, R. Bahal, *RNA* **2023**, *29*, 434.
[129] H. Adachi, M. Hengesbach, Y.-T. Yu, P. Morais, *Biomedicines* **2021**, *9*, 550.
[130] S. C. Humphreys, M. B. Thayer, J. Campbell, W. L. K. Chen, D. Adams, J. M. Lade, B. M. Rock, *J. Med. Chem.* **2020**, *63*, 6407.
[131] E. Kon, U. Elia, D. Peer, *Curr. Opin. Biotechnol.* **2022**, *73*, 329.
[132] K. Karikó, H. Muramatsu, F. A. Welsh, J. Ludwig, H. Kato, S. Akira, D. Weissman, *Mol. Ther.* **2008**, *16*, 1833.
[133] H. Kwon, M. Kim, Y. Seo, Y. S. Moon, H. J. Lee, K. Lee, H. Lee, *Biomaterials* **2018**, *156*, 172.
[134] Q. Chen, Y. Zhang, H. Yin, *Adv. Drug Delivery Rev.* **2021**, *168*, 246.
[135] A. Hendel, R. O. Bak, J. T. Clark, A. B. Kennedy, D. E. Ryan, S. Roy, I. Steinfeld, B. D. Lunstad, R. J. Kaiser, A. B. Wilkens, R. Bacchetta, A. Tsalenko, D. Dellinger, L. Bruhn, M. H. Porteus, *Nat. Biotechnol.* **2015**, *33*, 985.
[136] C. R. Cromwell, K. Sung, J. Park, A. R. Krysler, J. Jovel, S. K. Kim, B. P. Hubbard, *Nat. Commun.* **2018**, *9*, 1448.
[137] S. Hoffmann, J. Hoos, S. Klussmann, S. Vonhoff, *Curr. Protoc. Nucleic Acid Chem.* **2011**, *4*, 46.
[138] H. Shi, X. He, W. Cui, K. Wang, K. Deng, D. Li, F. Xu, *Anal. Chim. Acta* **2014**, *812*, 138.
[139] K. E. Maier, M. Levy, *Mol. Ther.–Methods Clin. Dev.* **2016**, *3*, 16014.

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

[140] J. O. McNamara, E. R. Andrechek, Y. Wang, K. D. Viles, R. E. Rempel, E. Gilboa, B. a Sullenger, P. H. Giangrande, *Nat. Biotechnol.* **2006**, *24*, 1005.
[141] E. Song, P. Zhu, S.-K. Lee, D. Chowdhury, S. Kussman, D. M. Dykxhoorn, Y. Feng, D. Palliser, D. B. Weiner, P. Shankar, W. A. Marasco, J. Lieberman, *Nat. Biotechnol.* **2005**, *23*, 709.
[142] J. K. Nair, J. L. S. Willoughby, A. Chan, K. Charisse, M. R. Alam, Q. Wang, M. Hoekstra, P. Kandasamy, A. V. Kel'in, S. Milstein, N. Taneja, J. O'Shea, S. Shaikh, L. Zhang, R. J. van der Sluis, M. E. Jung, A. Akinc, R. Hutabarat, S. Kuchimanchi, K. Fitzgerald, T. Zimmermann, T. J. C. van Berkel, M. A. Maier, K. G. Rajeev, M. Manoharan, *J. Am. Chem. Soc.* **2014**, *136*, 16958.
[143] T. Khan, H. Weber, J. DiMuzio, A. Matter, B. Dogdas, T. Shah, A. Thankappan, J. Disa, V. Jadhav, L. Lubbers, L. Sepp-Lorenzino, W. R. Strapps, M. Tadin-Strapps, *Mol. Ther.–Nucleic Acids* **2016**, *5*, 342.
[144] K. Nishina, T. Unno, Y. Uno, T. Kubodera, T. Kanouchi, H. Mizusawa, T. Yokota, *Mol. Ther.* **2008**, *16*, 734.
[145] J. Winkler, *Ther. Delivery* **2013**, *4*, 791.
[146] A. D. Springer, S. F. Dowdy, *Nucleic Acid Ther.* **2018**, *28*, 109.
[147] E. L. Sievers, P. D. Senter, *Annu. Rev. Med.* **2013**, *64*, 15.
[148] Y. Yao, T. Sun, S. Huang, S. Dou, L. Lin, J. Chen, J. Ruan, C. Mao, F. Yu, M. Zeng, J. Zang, Q. Liu, F. Su, P. Zhang, J. Lieberman, J. Wang, E. Song, *Sci. Transl. Med.* **2012**, *4*, 130ra48.
[149] P. Kumar, H.-S. Ban, S.-S. Kim, H. Wu, T. Pearson, D. L. Greiner, A. Laouar, J. Yao, V. Haridas, K. Habiro, Y.-G. Yang, J.-H. Jeong, K.-Y. Lee, Y.-H. Kim, S. W. Kim, M. Peipp, G. H. Fey, N. Manjunath, L. D. Shultz, S.-K. Lee, P. Shankar, *Cell* **2008**, *134*, 577.
[150] A. E. Arnold, E. Malek-Adamian, P. U. Le, A. Meng, S. Martínez-Montero, K. Petrecca, M. J. Damha, M. S. Shoichet, *Mol. Ther.–Nucleic Acids* **2018**, *11*, 518.
[151] H. Yin, H. M. Moulton, Y. Seow, C. Boyd, J. Boutilier, P. Iverson, M. J. A. Wood, *Hum. Mol. Genet.* **2008**, *17*, 3909.
[152] B. Wu, H. M. Moulton, P. L. Iversen, J. Jiang, J. Li, J. Li, C. F. Spurney, A. Sali, A. D. Guerron, K. Nagaraju, T. Doran, P. Lu, X. Xiao, Q. L. Lu, *Proc. Natl. Acad. Sci.* **2008**, *105*, 14814.
[153] A. F. Klein, M. A. Varela, L. Arandel, A. Holland, N. Naouar, A. Arzumanov, D. Seoane, L. Revillod, G. Bassez, A. Ferry, D. Jauvin, G. Gourdon, J. Puymirat, M. J. Gait, D. Furling, M. J. A. Wood, *J. Clin. Invest.* **2019**, *129*, 4739.
[154] B. Bestas, P. M. D. Moreno, K. E. M. Blomberg, D. K. Mohammad, A. F. Saleh, T. Sutlu, J. Z. Nordin, P. Guterstam, M. O. Gustafsson, S. Kharazi, B. Piątosa, T. C. Roberts, M. A. Behlke, M. J. A. Wood, M. J. Gait, K. E. Lundin, S. El Andaloussi, R. Månsson, A. Berglöf, J. Wengel, C. I. E. Smith, *J. Clin. Invest.* **2014**, *124*, 4067.
[155] S. M. Hammond, G. Hazell, F. Shabanpoor, A. F. Saleh, M. Bowerman, J. N. Sleigh, K. E. Meijboom, H. Zhou, F. Muntoni, K. Talbot, M. J. Gait, M. J. A. Wood, *Proc. Natl. Acad. Sci.* **2016**, *113*, 10962.
[156] D. Adams, A. Gonzalez-Duarte, W. D. O'Riordan, C.-C. Yang, M. Ueda, A. V. Kristen, I. Tournev, H. H. Schmidt, T. Coelho, J. L. Berk, K.-P. Lin, G. Vita, S. Attarian, V. Planté-Bordeneuve, M. M. Mezei, J. M. Campistol, J. Buades, T. H. Brannagan, B. J. Kim, J. Oh, Y. Parman, Y. Sekijima, P. N. Hawkins, S. D. Solomon, M. Polydefkis, P. J. Dyck, P. J. Gandhi, S. Goyal, J. Chen, A. L. Strahs, et al., *N. Engl. J. Med.* **2018**, *379*, 11.
[157] M. G. Thompson, J. L. Burgess, A. L. Naleway, H. L. Tyner, S. K. Yoon, J. Meece, L. E. W. Olsho, A. J. Caban-Martinez, A. Fowlkes, K. Lutrick, J. L. Kuntz, K. Dunnigan, M. J. Odean, K. T. Hegmann, E. Stefanski, L. J. Edwards, N. Schaefer-Solle, L. Grant, K. Ellingson, H. C. Groom, T. Zunie, M. S. Thiese, L. Ivacic, M. G. Wesley, J. M. Lamberte, X. Sun, M. E. Smith, A. L. Phillips, K. D. Groover, Y. M. Yoo, et al., *MMWR. Morb. Mortal. Wkly. Rep.* **2021**, *70*, 495.
[158] C. Dobrowolski, K. Paunovska, M. Z. C. Hatit, M. P. Lokugamage, J. E. Dahlman, *Adv. Healthcare Mater.* **2021**, *10*, 2002022.
[159] J. N. Israelachvili, D. J. Mitchell, B. W. Ninham, *Biochim. Biophys. Acta – Biomembr.* **1977**, *470*, 185.
[160] S. C. Semple, A. Akinc, J. Chen, A. P. Sandhu, B. L. Mui, C. K. Cho, D. W. Y. Sah, D. Stebbing, E. J. Crosley, E. Yaworski, I. M. Hafez, J. R. Dorkin, J. Qin, K. Lam, K. G. Rajeev, K. F. Wong, L. B. Jeffs, L. Nechev, M. L. Eisenhardt, M. Jayaraman, M. Kazem, M. A. Maier, M. Srinivasulu, M. J. Weinstein, Q. Chen, R. Alvarez, S. A. Barros, S. De, S. K. Klimuk, T. Borland, et al., *Nat. Biotechnol.* **2010**, *28*, 172.
[161] B. L. Mui, Y. K. Tam, M. Jayaraman, S. M. Ansell, X. Du, Y. Y. C. Tam, P. J. Lin, S. Chen, J. K. Narayanannair, K. G. Rajeev, M. Manoharan, A. Akinc, M. A. Maier, P. Cullis, T. D. Madden, M. J. Hope, *Mol. Ther.–Nucleic Acids* **2013**, *2*, 139.
[162] K. J. Kauffman, M. A. Oberli, J. R. Dorkin, J. E. Hurtado, J. C. Kaczmarek, S. Bhadani, J. Wyckoff, R. Langer, A. Jaklenec, D. G. Anderson, *Mol. Ther.–Nucleic Acids* **2018**, *10*, 55.
[163] P. Kumar, H. Wu, J. L. McBride, K.-E. Jung, M. Hee Kim, B. L. Davidson, S. Kyung Lee, P. Shankar, N. Manjunath, *Nature* **2007**, *448*, 39.
[164] A. R. de Fougerolles, *Hum. Gene Ther.* **2008**, *19*, 125.
[165] D. V Morrissey, J. A. Lockridge, L. Shaw, K. Blanchard, K. Jensen, W. Breen, K. Hartsough, L. Machemer, S. Radka, V. Jadhav, N. Vaish, S. Zinnen, C. Vargeese, K. Bowman, C. S. Shaffer, L. B. Jeffs, A. Judge, I. MacLachlan, B. Polisky, *Nat. Biotechnol.* **2005**, *23*, 1002.
[166] T. S. Zimmermann, A. C. H. Lee, A. Akinc, B. Bramlage, D. Bumcrot, M. N. Fedoruk, J. Harborth, J. A. Heyes, L. B. Jeffs, M. John, A. D. Judge, K. Lam, K. McClintock, L. V. Nechev, L. R. Palmer, T. Racie, I. Röhl, S. Seiffert, S. Shanmugam, V. Sood, J. Soutschek, I. Toudjarska, A. J. Wheat, E. Yaworski, W. Zedalis, V. Koteliansky, M. Manoharan, H.-P. Vornlocher, I. MacLachlan, *Nature* **2006**, *441*, 111.
[167] Y. Pan, J. Guan, Y. Gao, Y. Zhu, H. Li, H. Guo, Q. He, Z. Guan, Z. Yang, *Mol. Ther.–Nucleic Acids* **2023**, *32*, 807.
[168] M. Hosseini-Kharat, K. E. Bremmell, C. A. Prestidge, *Mol. Ther.–Methods Clin. Dev.* **2025**, *33*, 101436.
[169] M. Jeong, Y. Lee, J. Park, H. Jung, H. Lee, *Adv. Drug Delivery Rev.* **2023**, *200*, 114990.
[170] W. Ngo, S. Ahmed, C. Blackadar, B. Bussin, Q. Ji, S. M. Mladjenovic, Z. Sepahi, W. C. W. Chan, *Adv. Drug Delivery Rev.* **2022**, *185*, 114238.
[171] L. Breda, T. E. Papp, M. P. Triebwasser, A. Yadegari, M. T. Fedorky, N. Tanaka, O. Abdulmalik, G. Pavani, Y. Wang, S. A. Grupp, S. T. Chou, H. Ni, B. L. Mui, Y. K. Tam, D. Weissman, S. Rivella, H. Parhiz, *Science* **2023**, *381*, 436.
[172] J. G. Rurik, I. Tombácz, A. Yadegari, P. O. Méndez Fernández, S. V. Shewale, L. Li, T. Kimura, O. Y. Soliman, T. E. Papp, Y. K. Tam, B. L. Mui, S. M. Albelda, E. Puré, C. H. June, H. Aghajanian, D. Weissman, H. Parhiz, J. A. Epstein, *Science* **2022**, *375*, 91.
[173] L. E. Swart, M. H. A. M. Fens, A. van Oort, P. Waranecki, L. D. Mata Casimiro, D. Tuk, M. Hendriksen, L. van den Brink, E. Schweighart, C. Seinen, R. Nelson, A. Krippner-Heidenreich, T. O'Toole, R. M. Schiffelers, S. Kooijmans, O. Heidenreich, *Pharmaceutics* **2023**, *15*, 1603.
[174] E. L. Han, S. Tang, D. Kim, A. M. Murray, K. L. Swingle, A. G. Hamilton, K. Mrksich, M. S. Padilla, R. Palanki, J. J. Li, M. J. Mitchell, *Nano Lett.* **2025**, *25*, 800.
[175] R. Goswami, D. Chatzikleanthous, G. Lou, F. Giusti, A. Bonci, M. Taccone, M. Brazzoli, S. Gallorini, I. Ferlenghi, F. Berti, D. T. O'Hagan, C. Pergola, B. C. Baudner, R. Adamo, *ACS Infect. Dis.* **2019**, *5*, 1546.
[176] C. Liang, B. Guo, H. Wu, N. Shao, D. Li, J. Liu, L. Dang, C. Wang, H. Li, S. Li, W. K. Lau, Y. Cao, Z. Yang, C. Lu, X. He, D. W. T. Au, X. Pan, B.-T. Zhang, C. Lu, H. Zhang, K. Yue, A. Qian, P. Shang, J. Xu, L. Xiao, Z. Bian, W. Tan, Z. Liang, F. He, L. Zhang, et al., *Nat. Med.* **2015**, *21*, 288.
[177] M. H. Y. Cheng, Y. Zhang, K. Fox, J. Leung, C. Strong, E. Kang, Y. Chen, M. Tong, H. Bommadevara, E. Jan, O. Y. L. Ip, C.

© 2025 Wiley-VCH GmbH

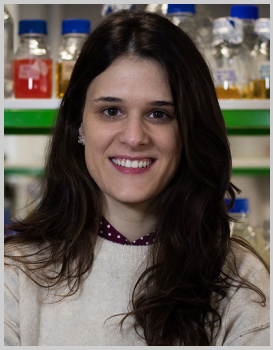
23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Rodríguez-Rodríguez, K. Saatchi, U. O. Häfeli, A. Abdolahzadeh, D. Witzigmann, P. R. Cullis, *Nat. Commun.* **2025**, *16*, 4135.
[178] E. Álvarez-Benedicto, Z. Tian, S. Chatterjee, D. Orlando, M. Kim, E. D. Guerrero, X. Wang, D. J. Siegwart, *Angew. Chem. Int. Ed.* **2023**, *62*, 202310395.
[179] C. I. C. Crucho, M. T. Barros, *Mater. Sci. Eng. C* **2017**, *80*, 771.
[180] N. Kamaly, B. Yameen, J. Wu, O. C. Farokhzad, *Chem. Rev.* **2016**, *116*, 2602.
[181] H. Zhong, G. Chan, Y. Hu, H. Hu, D. Ouyang, *Pharmaceutics* **2018**, *10*, 263.
[182] B. Xiao, Z. Zhang, E. Viennois, Y. Kang, M. Zhang, M. K. Han, J. Chen, D. Merlin, *Theranostics* **2016**, *6*, 2250.
[183] M. Harada-Shiba, K. Yamauchi, A. Harada, I. Takamisawa, K. Shimokado, K. Kataoka, *Gene Ther.* **2002**, *9*, 407.
[184] X. Ke, L. Shelton, Y. Hu, Y. Zhu, E. Chow, H. Tang, J. L. Santos, H.-Q. Mao, *ACS Appl. Mater. Interfaces* **2020**, *12*, 35835.
[185] L. Tan, T. Zheng, M. Li, X. Zhong, Y. Tang, M. Qin, X. Sun, *Drug Deliv. Transl. Res.* **2020**, *10*, 678.
[186] G. Kara, S. Malekghasemi, B. Ozpolat, E. B. Denkbas, *Colloids Surf., A* **2021**, *630*, 127622.
[187] L. Xue, Y. Yan, P. Kos, X. Chen, D. J. Siegwart, *Drug Deliv. Transl. Res.* **2021**, *11*, 255.
[188] E. Hamada, T. Kurosaki, J. Hashizume, H. Harasawa, H. Nakagawa, T. Nakamura, Y. Kodama, H. Sasaki, *Pharmaceutics* **2021**, *13*, 126.
[189] H. Yin, R. L. Kanasty, A. A. Eltoukhy, A. J. Vegas, J. R. Dorkin, D. G. Anderson, *Nat. Rev. Genet.* **2014**, *15*, 541.
[190] J. R. Magaña Rodriguez, M. Guerra-Rebollo, S. Borrós, C. Fornaguera, *Drug Deliv. Transl. Res.* **2024**, *14*, 3477.
[191] E. Ben-Akiva, J. Karlsson, S. Hemmati, H. Yu, S. Y. Tzeng, D. M. Pardoll, J. J. Green, *Proc. Natl. Acad. Sci. USA* **2023**, *120*, 2301606120.
[192] E. L. Blanchard, D. Vanover, S. S. Bawage, P. M. Tiwari, L. Rotolo, J. Beyersdorf, H. E. Peck, N. C. Bruno, R. Hincapie, F. Michel, J. Murray, H. Sadhwani, B. Vanderheyden, M. G. Finn, M. A. Brinton, E. R. Lafontaine, R. J. Hogan, C. Zurla, P. J. Santangelo, *Nat. Biotechnol.* **2021**, *39*, 717.
[193] J. C. Kaczmarek, A. K. Patel, K. J. Kauffman, O. S. Fenton, M. J. Webber, M. W. Heartlein, F. DeRosa, D. G. Anderson, *Angew. Chem. Int. Ed.* **2016**, *55*, 13808.
[194] S. Das, B. Chanchlani, S. P. S., V. Are, S. Biswas, *Nanomedicine* **2025**, *20*, 835.
[195] K. Mayilsamy, E. Markoutsa, M. Das, P. Chopade, D. Puro, A. Kumar, D. Gulick, A. E. Willing, S. S. Mohapatra, S. Mohapatra, *Nanomed. Nanotechnol., Biol. Med.* **2020**, *29*, 102247.
[196] W. Liyanage, T. Wu, S. Kannan, R. M. Kannan, *ACS Appl. Mater. Interfaces* **2022**, *14*, 46290.
[197] L. Farbiak, Q. Cheng, T. Wei, E. Álvarez-Benedicto, L. T. Johnson, S. Lee, D. J. Siegwart, *Adv. Mater.* **2021**, *33*, 2006619.
[198] Y. Ramot, S. Rotkopf, R. M. Gabai, E. Z Khvalevsky, S. Muravnik, G. A. Marzoli, A. J. Domb, A. Shemi, A. Nyska, *Toxicol. Pathol.* **2016**, *44*, 856.
[199] B. Dunn, J. I. Zink, *Acc. Chem. Res.* **2007**, *40*, 729.
[200] S. C. Abeylath, E. Turos, *Expert Opin. Drug Delivery* **2008**, *5*, 931.
[201] J. Hsiao, C. Tsai, T. Chung, Y. Hung, M. Yao, H. Liu, C. Mou, C. Yang, Y. Chen, D. Huang, *Small* **2008**, *4*, 1445.
[202] K. Möller, K. Müller, H. Engelke, C. Bräuchle, E. Wagner, T. Bein, *Nanoscale* **2016**, *8*, 4007.
[203] J. L. Paris, M. Vallet-Regí, *Pharmaceutics* **2020**, *12*, 526.
[204] Y. Chen, H. Gu, D. S.-Z. Zhang, F. Li, T. Liu, W. Xia, *Biomaterials* **2014**, *35*, 10058.
[205] A. K. Meka, Y. Niu, S. Karmakar, S. B. Hartono, J. Zhang, C. X. C. Lin, H. Zhang, A. Whittaker, K. Jack, M. Yu, C. Yu, *ChemNanoMat* **2016**, *2*, 220.
[206] B.-C. Zhang, B.-Y. Luo, J.-J. Zou, P.-Y. Wu, J.-L. Jiang, J.-Q. Le, R.-R. Zhao, L. Chen, J.-W. Shao, *ACS Appl. Mater. Interfaces* **2020**, *12*, 57362.
[207] J. Lee, S. Kang, M. Ahn, H. Jang, D. Min, *Small* **2018**, *14*, 1702564.
[208] S. Bancos, D. L. Stevens, K. M. Tyner, *Int. J. Nanomed.* **2015**, *10*, 183.
[209] Q. Yang, S. W. Jones, C. L. Parker, W. C. Zamboni, J. E. Bear, S. K. Lai, *Mol. Pharmaceutics* **2014**, *11*, 1250.
[210] D. Zheng, D. A. Giljohann, D. L. Chen, M. D. Massich, X.-Q. Wang, H. Iordanov, C. A. Mirkin, A. S. Paller, *Proc. Natl. Acad. Sci.* **2012**, *109*, 11975.
[211] J.-M. Li, M.-X. Zhao, H. Su, Y.-Y. Wang, C.-P. Tan, L.-N. Ji, Z.-W. Mao, *Biomaterials* **2011**, *32*, 7978.
[212] H.-W. Yang, C.-Y. Huang, C.-W. Lin, H.-L. Liu, C.-W. Huang, S.-S. Liao, P.-Y. Chen, Y.-J. Lu, K.-C. Wei, C.-C. M. Ma, *Biomaterials* **2014**, *35*, 6534.
[213] N. W. S. Kam, Z. Liu, H. Dai, *J. Am. Chem. Soc.* **2005**, *127*, 12492.
[214] B. B. Mendes, J. Conniot, A. Avital, D. Yao, X. Jiang, X. Zhou, N. Sharf-Pauker, Y. Xiao, O. Adir, H. Liang, J. Shi, A. Schroeder, J. Conde, *Nat. Rev. Methods Prim.* **2022**, *2*, 24.
[215] J. Li, J. Yu, Q. Fang, Y. Du, X. Zhang, *ChemBioChem* **2024**, *25*.
[216] J. E. Won, M. Park, S.-H. Hong, Y. S. Kim, H. Song, *npj Regen. Med.* **2025**, *10*, 15.
[217] D. Ferreira, J. N. Moreira, L. R. Rodrigues, *Crit. Rev. Oncol. Hematol.* **2022**, *172*, 103628.
[218] R. C. Lai, F. Arslan, M. M. Lee, N. S. K. Sze, A. Choo, T. S. Chen, M. Salto-Tellez, L. Timmers, C. N. Lee, R. M. El Oakley, G. Pasterkamp, D. P. V. de Kleijn, S. K. Lim, *Stem Cell Res.* **2010**, *4*, 214.
[219] T. N. Lamichhane, A. Jeyaram, D. B. Patel, B. Parajuli, N. K. Livingston, N. Arumugasaamy, J. S. Schardt, S. M. Jay, *Cell. Mol. Bioeng.* **2016**, *9*, 315.
[220] J. Zhuang, J. Tan, C. Wu, J. Zhang, T. Liu, C. Fan, J. Li, Y. Zhang, *Nucleic Acids Res.* **2020**, *48*, 8870.
[221] C. He, D. Jaffar Ali, Y. Li, Y. Zhu, B. Sun, Z. Xiao, *PeerJ* **2020**, *8*, 9524.
[222] K. M. McAndrews, F. Xiao, A. Chronopoulos, V. S. LeBleu, F. G. Kugeratski, R. Kalluri, *Life Sci. Alliance* **2021**, *4*, 202000875.
[223] L. Alvarez-Erviti, Y. Seow, H. Yin, C. Betts, S. Lakhal, M. J. A. Wood, *Nat. Biotechnol.* **2011**, *29*, 341.
[224] S. Ohno, M. Takanashi, K. Sudo, S. Ueda, A. Ishikawa, N. Matsuyama, K. Fujita, T. Mizutani, T. Ohgi, T. Ochiya, N. Gotoh, M. Kuroda, *Mol. Ther.* **2013**, *21*, 185.
[225] F. Pi, D. W. Binzel, T. J. Lee, Z. Li, M. Sun, P. Rychahou, H. Li, F. Haque, S. Wang, C. M. Croce, B. Guo, B. M. Evers, P. Guo, *Nat. Nanotechnol.* **2017**, *13*, 82.
[226] M. Mendt, S. Kamerkar, H. Sugimoto, K. M. McAndrews, C.-C. Wu, M. Gagea, S. Yang, E. V. R. Blanko, Q. Peng, X. Ma, J. R. Marszalek, A. Maitra, C. Yee, K. Rezvani, E. Shpall, V. S. LeBleu, R. Kalluri, *JCI Insight* **2018**, *3*, 99263.
[227] S. Kamerkar, C. Leng, O. Burenkova, S. C. Jang, C. McCoy, K. Zhang, K. Dooley, S. Kasera, T. Zi, S. Sisó, W. Dahlberg, C. L. Sia, S. Patel, K. Schmidt, K. Economides, T. Soos, D. Burzyn, S. Sathyanarayanan, *Sci. Adv.* **2022**, *8*, abj7002.
[228] G. Son, J. Song, J. C. Park, H. N. Kim, H. Kim, *Nat. Commun.* **2025**, *16*, 4799.
[229] S. Wu, J. Yun, W. Tang, G. Familiari, M. Relucenti, J. Wu, X. Li, H. Chen, R. Chen, *ACS Nano* **2023**, *17*, 11838.
[230] Z. Tao, H. Zhang, S. Wu, J. Zhang, Y. Cheng, L. Lei, Y. Qin, H. Wei, C.-Y. Yu, *Nanoscale* **2024**, *16*, 4392.
[231] C. H. Kapadia, J. R. Melamed, E. S. Day, *BioDrugs* **2018**, *32*, 297.
[232] L. Huang, J. Zhang, L. Pang, S. Hu, L. Zhang, S. Zhao, *Chem. Commun.* **2021**, *57*, 11617.
[233] P. Kumthekar, C. H. Ko, T. Paunesku, K. Dixit, A. M. Sonabend, O. Bloch, M. Tate, M. Schwartz, L. Zuckerman, R. Lezon, R. V. Lukas, B.

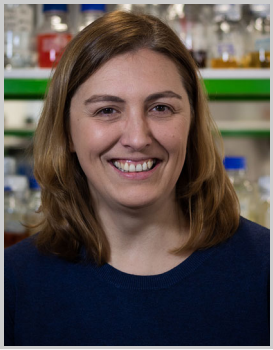
© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Jovanovic, K. McCortney, H. Colman, S. Chen, B. Lai, O. Antipova, J. Deng, L. Li, S. Tommasini-Ghelfi, L. A. Hurley, D. Unruh, N. V. Sharma, M. Kandpal, F. M. Kouri, R. V. Davuluri, D. J. Brat, M. Muzzio, M. Glass, V. Vijayakumar, et al., *Sci. Transl. Med.* **2021**, *13*, abb3945.
[234] W. L. Daniel, U. Lorch, S. Mix, A. S. Bexon, *Front. Immunol.* **2022**, *13*, 1073777.
[235] J. Zhao, J. Wu, Y. Fan, C. Yu, L. Yu, F. Shao, *Biomolecules* **2025**, *15*, 537.
[236] M. Lee, J. Hwang, Y. Song, S. Kim, N. Park, *Biomater. Adv.* **2025**, *169*, 214160.
[237] T. Xu, S. Yu, Y. Sun, S. Wu, D. Gao, M. Wang, Z. Wang, Y. Tian, Q. Min, J. Zhu, *Small* **2021**, *17*.
[238] M. Safarkhani, S. Ahmadi, H. Ipakchi, M. R. Saeb, P. Makvandi, M. E Warkiani, N. Rabiee, Y. Huh, *Adv. Sci.* **2024**, *11*, 2401617.
[239] W. Sun, W. Ji, J. M. Hall, Q. Hu, C. Wang, C. L. Beisel, Z. Gu, *Angew. Chem., Int. Ed.* **2015**, *54*, 12029.
[240] S. Sellner, S. Kocabey, K. Nekolla, F. Krombach, T. Liedl, M. Rehberg, *Biomaterials* **2015**, *53*, 453.
[241] K. Mohri, M. Nishikawa, N. Takahashi, T. Shiomi, N. Matsuoka, K. Ogawa, M. Endo, K. Hidaka, H. Sugiyama, Y. Takahashi, Y. Takakura, *ACS Nano* **2012**, *6*, 5931.
[242] A. V. Pinheiro, D. Han, W. M. Shih, H. Yan, *Nat. Nanotechnol.* **2011**, *6*, 763.
[243] Y. Chen, S.-H. Xiong, F. Li, X.-J. Kong, D.-F. Ouyang, Y. Zheng, H. Yu, Y.-J. Hu, *Mol. Ther.–Nucleic Acids* **2022**, *29*, 150.
[244] H. Tani, *Int. J. Mol. Sci.* **2024**, *25*, 12284.
[245] M. Winkle, S. M. El-Daly, M. Fabbri, G. A. Calin, *Nat. Rev. Drug Discovery* **2021**, *20*, 629.
[246] G. Pisignano, D. C. Michael, T. H. Visal, R. Pirlog, M. Ladomery, G. A. Calin, *Oncogene* **2023**, *42*, 2783.
[247] S. Puri, J. Hu, Z. Sun, M. Lin, T. D. Stein, L. A. Farrer, B. Wolozin, X. Zhang, *Alzheimer's Dement.* **2023**, *19*, 3389.
[248] Z. Gong, W. Hu, C. Zhou, J. Guo, L. Yang, B. Wang, *npj Vaccines* **2025**, *10*, 41.
[249] Y. Luo, L. Liu, Z. He, S. Zhang, P. Huo, Z. Wang, Q. Jiaxin, L. Zhao, Y. Wu, D. Zhang, D. Bu, R. Chen, Y. Zhao, *Comput. Struct. Biotechnol. J.* **2022**, *20*, 5680.
[250] Z. Bereczki, B. Benczik, O. M. Balogh, S. Marton, E. Puhl, M. Pétervári, M. Váczy-Földi, Z. T. Papp, A. Makkos, K. Glass, F. Locquet, G. Euler, R. Schulz, P. Ferdinandy, B. Ágg, *Br. J. Pharmacol.* **2025**, *182*, 340.
[251] J. Wei, P. Lotfy, K. Faizi, S. Baungaard, E. Gibson, E. Wang, H. Slabodkin, E. Kinnaman, S. Chandrasekaran, H. Kitano, M. G. Durrant, C. V. Duffy, A. Pawluk, P. D. Hsu, S. Konermann, *Cell Syst.* **2023**, *14*, 1087.
[252] H. V. Thi, T.-N. Hoang, N. Q. K. Le, D.-T. Chu, *Prog. Mol. Biol. Transl. Sci.* **2024**, *203*, 83.
[253] K. Leppek, G. W. Byeon, W. Kladwang, H. K. Wayment-Steele, C. H. Kerr, A. F. Xu, D. S. Kim, V. V. Topkar, C. Choe, D. Rothschild, G. C. Tiu, R. Wellington-Oguri, K. Fujii, E. Sharma, A. M. Watkins, J. J. Nicol, J. Romano, B. Tunguz, F. Diaz, H. Cai, P. Guo, J. Wu, F. Meng, S. Shi, E. Participants, P. R. Dormitzer, A. Solórzano, M. Barna, R. Das, *Nat. Commun.* **2022**, *13*, 1536.
[254] S. Jain, J. Kaur, S. Prasad, I. Roy, *Expert Opin. Drug Discovery* **2021**, *16*, 255.
[255] H.-H. Wessels, A. Stirn, A. Méndez-Mancilla, E. J. Kim, S. K. Hart, D. A. Knowles, N. E. Sanjana, *Nat. Biotechnol.* **2024**, *42*, 628.
[256] M. Hannus, M. Beitzinger, J. C. Engelmann, M.-T. Weickert, R. Spang, S. Hannus, G. Meister, *Nucleic Acids Res.* **2014**, *42*, 8049.
[257] A. Casini, M. Olivieri, G. Petris, C. Montagna, G. Reginato, G. Maule, F. Lorenzin, D. Prandi, A. Romanel, F. Demichelis, A. Inga, A. Cereseto, *Nat. Biotechnol.* **2018**, *36*, 265.
[258] S. K. Niazi, *Biomedicines* **2023**, *11*, 1275.
[259] H. Zhou, D. S. Chen, C. J. Hu, X. Hong, J. Shi, Y. Xiao, *Adv. Sci.* **2023**, *10*, 2303597.



Débora Ferreira is a Postdoctoral researcher at the Centre of Biological Engineering at University of Minho. She holds a PhD in Bioengineering Systems from the MIT-Portugal Program. Her research focuses on the use of engineered nanoplatforms as drug delivery vehicles for a wide range of different therapeutics, including RNAi-based therapies and chemical compounds, for the treatment of triple negative breast cancer.



Ligia R. Rodrigues is group leader at the Centre of Biological Engineering at the University of Minho, and tenured Associate professor with habilitation within the field of Synthetic Biology and Cancer Research. Her scientific activity has focused on engineering novel targeting and drug delivery systems towards solid tumors. She has co-authored over 100 publications in peer-reviewed journals, book chapters, and delivered several invited lectures.

© 2025 Wiley-VCH GmbH

23663987, 0, Downloaded from https://advanced.onlinelibrary.wiley.com/doi/10.1002/adtp.202500082 by Universidade Do Minho, Wiley Online Library on [22/07/2025]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License