Molecular Therapy
Nucleic Acids
Review

RNA therapeutics in targeting G protein-coupled receptors: Recent advances and challenges

Wanjun Yuan,[1] Xiangyang Shi,[2] and Leo Tsz On Lee[1,3]

[1]Cancer Centre, Faculty of Health Sciences, University of Macau, Taipa 999078, Macau, China; [2]State Key Laboratory for Modification of Chemical Fibers and Polymer Materials, Shanghai Engineering Research Center of Nano-Biomaterials and Regenerative Medicine, College of Biological Science and Medical Engineering, Donghua University, Shanghai 201620, People's Republic of China; [3]Ministry of Education Frontiers Science Center for Precision Oncology, University of Macau, Taipa 999078, Macau, China

G protein-coupled receptors (GPCRs) are the major targets of existing drugs for a plethora of human diseases and dominate the pharmaceutical market. However, over 50% of the GPCRs remain undruggable. To pursue a breakthrough and overcome this situation, there is significant clinical research for developing RNA-based drugs specifically targeting GPCRs, but none has been approved so far. RNA therapeutics represent a unique and promising approach to selectively targeting previously undruggable targets, including undruggable GPCRs. However, the development of RNA therapeutics faces significant challenges in areas of RNA stability and efficient *in vivo* delivery. This review presents an overview of the advances in RNA therapeutics and the diverse types of nanoparticle RNA delivery systems. It also describes the potential applications of GPCR-targeted RNA drugs for various human diseases.

INTRODUCTION

G protein-coupled receptors (GPCRs) are transmembrane proteins that transduce signals from the extracellular environment to modulate cellular responses by regulating the intracellular signaling pathways.[1] The human genome encodes more than 800 GPCRs that respond to a wide variety of signals.[2] GPCRs play significant roles in almost all physiological and pathophysiological processes. Therefore, members of the GPCR superfamily are promising therapeutic targets. However, drugs have been developed successfully for only 110 of the 826 knowns human GPCRs, including ~400 olfactory receptors.[3,4] The major obstacle in developing conventional drugs such as chemical agonists or antagonists for specific GPCRs is the existence of various subtypes and splice variants for each of the known GPCRs and the high structural similarity between members of the GPCR family.[5] Therefore, novel strategies are urgently needed for the development of effective GPCR-targeting drugs with high specificity.

RNA-based therapy has shown significant clinical potential in the last two decades. It is based on the delivery of exogenous therapeutic RNA into cells to correct or modify defective biological pathways that cause diseased conditions.[6] RNA-based drugs show high specificity and potency, low toxicity, and can be synthesized rapidly on a large scale.[7,8] Therefore, the RNA-based drug approach is a promising strategy for developing novel drugs for difficult targets, including the GPCRs. The global RNA-based therapeutics market was valued at USUS$6.16 billion in 2022 and is predicted to achieve an exponential compound annual growth rate (CAGR) of 19.13% between 2022 and 2030.[9]

GPCRs are major drug targets for many human diseases, and RNA-based therapy has shown great promise in targeting GPCRs with high specificity.[10] Therefore, it is expected that GPCR-targeting RNA drugs will elicit successful clinical outcomes. In this review, we summarize and discuss the status and recent advances in the field of GPCR-targeting RNA therapeutics, including mechanistic details, clinical applications, and RNA drug delivery systems.

CURRENT GPCR-TARGETING DRUGS

GPCR is a tractable class of drug targets

GPCRs are activated by a variety of ligands, including ions, hormones, neurotransmitters, proteins, and protons.[3,11] The human GPCR family has been classified into four subfamilies: class A (rhodopsin), B (secretin and adhesion), C (glutamate), and F (Frizzled), which are significantly associated with diabetes,[12] cardiovascular diseases,[13] immunological disorders,[14] infectious diseases,[15] cancer,[16] and neurodegenerative diseases, including Alzheimer's disease,[17] Parkinson's disease,[18] and Huntington's disease.[3,19,20] The extracellular domain of the GPCRs consists of a deep cleft as the orthosteric binding site, which is well suited for the design of small synthetic molecules that inhibit (antagonists) or activate (agonists) the receptors.[21] Therefore, the receptor-binding site for the drugs is easily accessible and does not require membrane permeability, which is an essential criterion for the drugs that target intracellular molecules. Furthermore, GPCRs have highly dynamic structures during activation. When the receptor is activated, the

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Correspondence: Leo Tsz On Lee, Cancer Centre, Ministry of Education Frontiers Science Center for Precision Oncology, Faculty of Health Sciences, University of Macau, Taipa, Macau, China.
E-mail: ltolee@um.edu.mo

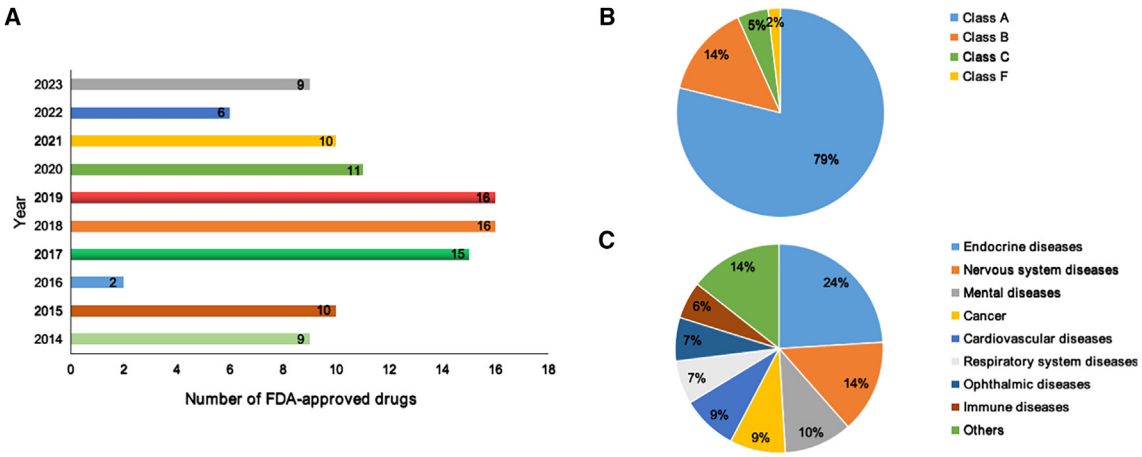

Figure 1. Landscape of FDA-approved GPCR drugs from 2014 to 2023

The trials were further analyzed based on (A) FDA-approved drug numbers, (B) GPCR classes, and (C) disease types. GPCR drugs approved in 2014–2016 were obtained from Hauser et al.[24] Approved GPCR drug numbers from 2017 to 2023 were analyzed based on the data shown in Table 1.

transmembrane helices rearrangement enables binding of the C terminus of the G protein α subunit and stabilizes the activated GPCR.[21,22] The binding of ligands to the GPCR triggers the associated G proteins and/or β-arrestins to stimulate downstream signaling events.[3,23] Amplification of the downstream signaling pathways further enhances the effect of the ligands or the drugs that interact with the GPCRs. Therefore, GPCR-targeted drugs elicit an effective drug response by significantly altering the cellular functions.

Current GPCR drug market

Currently, almost 60% of drugs in the developmental stages and 36% of approved drugs target GPCRs.[24] In 2020, the global market for GPCRs was estimated at US$2.6 billion and is expected to reach US$3.9 billion by 2027, with a CAGR of 5.7% between 2020 and 2027.[25] North America is currently the largest market for GPCR-targeting drugs. In 2021, the market for GPCR-related drugs in the USA was estimated at US$974.6 million. The GPCR drug market has also grown robustly in the Asia-Pacific region because of the increasing population, cancer incidence rates, and research activities. China is expected to reach a market size of US$318.7 million by 2027 for GPCR drugs. The forecast for Japan and Canada is to grow at 4.9% and 5.7%, respectively, for the GPCR drugs. The CAGR is expected to be 5.4% between 2020 and 2027 in the European market for GPCR drugs.[25] In general, the demand from the global market indicated the huge potential market for the drugging of the currently "undruggable" GPCRs.

The increased adoption of GPCR-targeting drugs for the treatment of various human diseases is expected to fuel the growth of the global market for the GPCR-related drugs.[26] As shown in Figure 1A, the US Food and Drug Administration (FDA) has approved 104 GPCR-targeting drugs during the last 10 years (2014–2023), from which 79% drugs were targeted class A, 14% were class B, 5% were class C. However, only 2% of GPCR drugs were class F (Figure 1B). In addition, of these approved GPCR drugs, 24% were used for treatment of endocrine diseases, while 14% were nervous system diseases and 10% were mental diseases (Figure 1C). Table 1 summarizes the FDA-approved GPCR drugs in the last 7 years. However, in recent years, there has been no significant increase of new drugs launched in the market, and only 25 new GPCR-targeting drugs were approved between 2021 and 2023. More than 90% of the agents that enter phase I clinical trials did not obtain FDA approval because of efficacy or safety concerns, unknown prevalence, and impact of genetic variations. Therefore, targeted drugs are available for only less than 13% of the known GPCRs, and there is a great scope for discovering effective and safe drugs for the remaining GPCRs.[21]

Drugs that target different classes of GPCRs

According to Yang et al.[3] and the Drugs@FDA database (accessdata.fda.gov), FDA has approved more than 500 drugs that target class A GPCRs, including 75% for the aminergic receptors and 10% for the peptide ligand receptors. These drugs are used as analgesics or in the treatment of allergies, cardiovascular diseases, hypertension, pulmonary diseases, and cancer-related fatigue.[3,27]

Class B GPCRs are classified into two subfamilies—secretin (B1, with 15 receptors) and adhesion (B2, 33 receptors)—and are therapeutic targets for several human diseases, including obesity, type 2 diabetes mellitus (T2DM), osteoporosis, migraine, depression, and anxiety.[3,28,29] Currently, the FDA has approved 12 drugs that specifically target class B GPCRs.[30] For example, multiple receptor agonists have been developed for the glucagon-like peptide 1 (GLP-1) through selective amino acid substitutions, blocking enzyme cleavage, and/or conjugation to increase the entity bound to plasma proteins.[3] These methods slow down renal clearance of the peptides and extend the half-life of the drug. For example, semaglutide, approved for T2DM in 2017, is the fifth approved GLP-1 receptor agonist, and it has a longer half-life (168 h) than native GLP-1 (1–2 min).[28,31]

Table 1. FDA-approved drugs targeting GPCRs in the 2017 to August 2023

Substance	Brand name	Targets	GPCR class	Indications	Approval year
Abaloparatide	Tymlos	PTHR1	B, parathyroid hormone	osteoporosis	2017
Etelcalcetide	Parsabiv	CaSR	C, calcium-sensing	hyperparathyroidism	2017
Naldemedine	Symproic	OPRM	A, opioid	opioid-induced constipation	2017
Angiotensin II	Giapreza (La Jolla Pharma)	AGTR1	A, angiotensin	septic or other distributive shock	2017
Exenatide	Bydureon BCise	GLP-1	B, glucagon	type 2 diabetes	2017
Oxymetazoline hydrochloride	Rhofade	α_1-AR	A, adrenaline	rosacea	2017
Fluticasone propionate and salmeterol	AirDuo RespiClick	β_2-AR	A, adrenaline	asthma	2017
Macimorelin	Macrilen (Novo Nordisk)	GHSR	A, ghrelin	adult growth hormone deficiency	2017
Semaglutide (injection)	Ozempic (Novo Nordisk)	GLP-1R	B, glucagon	type 2 diabetes, cardiovascular risk reduction	2017
Latanoprostene bunod	Vyzulta (Bausch and Lomb)	PTGFR	A, prostanoid	glaucoma or ocular hypertension	2017
Cetirizine hydrochloride	Zerviate	H1R	A, histamine	conjunctivitis, allergic	2017
Epinephrine	Symjepi	α/β-AR	A, adrenaline	allergic reactions	2017
Triptorelin	Triptodur	GnRH	A, gonadotrophin-releasing hormone	precocious puberty	2017
Buprenorphine	Sublocade	μ-opioid	A, opioid	opioid use disorder	2017
Brimonidine tartrate	Lumify	α_2-AR	A, adrenaline	ocular redness	2017
Gilteritinib	Xospata (Astellas)	Serotonin receptors	A, 5-hydroxytryptamine	relapsed or refractory acute myeloid leukemia	2018
Revefenacin	Yupelri (Mylan Ireland)	CHRM1-CHRM5	A, acetylcholine	chronic obstructive pulmonary disease	2018
Tolvaptan	Jynarque	V2R	A, vasopressin and oxytocin	adults at risk of rapidly progressing autosomal dominant polycystic kidney disease	2018
Lofexidine hydrochloride	Lucemyra	α_2-AR	A, adrenaline	opiate withdrawal	2018
Lutetium 177 dotatate	Lutathera (AAA USA)	SSTR2	A, somatostatin	gastroenteropancreatic neuroendocrine tumors	2018
Fosnetupitant/palonosetron	Akynzeo (Helsinn Healthcare)	NK1R/5-HT3R	A, tachykinin	chemotherapy-associated nausea and vomiting prevention	2018
Elagolix	Orilissa (Abbvie)	GNRHR	A, gonadotropin	endometriosis-associated moderate-to-severe pain	2018
Mogamulizumab-kpkc	Poteligeo (Kyowa Kirin)	CXCR4	A, chemokine	non-Hodgkin lymphoma	2018
Cannabidiol	Epidioloex (GW Research)	CNR1	A, cannabinoid	spasticity related to multiple sclerosis and epilepsy	2018
Prucalopride	Motegrity	5-HT4R	A, hydroxytryptamine	chronic idiopathic constipation	2018
Albuterol sulfate	ProAir Digihaler	β_2-AR	A, adrenaline	asthma and COPD	2018
Galcanezumab-gnlm	Emgality	CGRP	B, calcitonin	migraine prevention, cluster headaches	2018
Fostamatinib	Tavalisse (Rigel Pharma)	Multiple targets, including ADORA3	A, adenosine	chronic immune thrombocytopenia	2018
Mogamulizumab-kpkc	Poteligeo	CCR4	A, chemokine	refractory mycosis fungoides, or Sézary syndrome	2018

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Table 1. *Continued*

Substance	Brand name	Targets	GPCR class	Indications	Approval year
Glasdegib	Daurismo	SMO	F, smoothened	acute myeloid leukemia	2018
Erenumab-aooe	Aimovig (Amgen)	CALCRL	B, calcitonin	migraine (prevention)	2018
Semaglutide (oral)	Rybelsus (Novo Nordisk)	GLP-1R	B, glucagon	type 2 diabetes mellitus	2019
Sumatriptan	Tosymra	5-HT1B/1D	A, hydroxytryptamine	migraine	2019
Brexanolone	Zulresso	GABA	C, GABA	postpartum depression	2019
Aclidinium bromide and formoterol fumarate	Duaklir Pressair	muscarinic acetylcholine receptor (mAChR)/β_2-AR	A, acetylcholine/adrenaline	COPD	2019
Lasmiditan	Reyvow (Eli Lilly)	HTR1F	A, 5-hydroxytryptamine	migraine	2019
Pitolisant	Wakix (Harmony)	HRH3	A, histamine	narcolepsy, excessive daytime sleepiness	2019
Lumateperone	Caplyta (Intra-Cellular)	HTR2A, DRD1, DRD2	A, dopamine, 5-hydroxytryptamine	schizophrenia	2019
Ubrogepant	Ubrelvy	CGRP	B, calcitonin	migraine	2019
Bremelanotide	Vyleesi	MC1R	A, melanocortin	sexual arousal disorder	2019
Afamelanotide	Scenesse	MC1R	A, melanocortin	prevention of phototoxicity in erythropoietic protoporphyria	2019
Lemborexant	Dayvigo (Eisai)	HCRTR1	A, orexin	insomnia	2019
Cetirizine hydrochloride	Quzyttir	H1R	A, histamine	urticaria	2019
Gallium 68 dotatoc	NA (UIHC-PET Imaging Center)	SSTR2	A, somatostatin	diagnostic agent for neuroendocrine tumors	2019
Phenylephrine hydrochloride	Biorphen	α_1-AR	A, adrenaline	hypotension	2019
Siponimod	Mayzent (Novartis)	S1PR1, S1PR5	A, lysophospholipid	relapsing forms of multiple sclerosis	2019
Istradefylline	Nourianz (Kyowa Kirin)	ADORA2A	A, adenosine	Parkinson's disease	2019
Cysteamine	Procysbi (Horizon Pharma)	NPY2R	A, neuropeptide	radiation sickness	2020
Ozanimod	Zeposia (Celgene)	S1PR1, S1PR5	A, lysophospholipid	relapsing forms of multiple sclerosis	2020
Oliceridine	Olinvyk	OPRM1	A, opioid	moderate-to-severe acute pain	2020
Rimegepant	Nurtec ODT (Biohaven Pharm)	CALCRL	B, calcitonin	migraine	2020
Setmelanotide	Imcivree	mACh4R	A, acetylcholine	weight loss (obesity/overweight)	2020
Eptinezumab-jjmr	Vyepti (Lundbeck)	CALCRL	B, calcitonin	migraine (prevention)	2020
Relugolix	Orgovyx	GnRH	A, gonadotrophin-releasing hormone	prostate cancer	2020
Amisulpride	Barhemsys	D2R	A, dopamine	nausea/vomiting, postoperative	2020
Clascoterone	Winlevi	mAChR	A, acetylcholine	acne	2020
Tramadol hydrochloride	Qdolo	μ-opioid	A, opioid	pain	2020
ozanimod	Zeposia	S1P	A, lysophospholipid	multiple sclerosis, ulcerative colitis	2020
Difelikefalin	Korsuva	κ-opioid receptor	A, opioid	pain following abdominal surgery	2021
Avacopan	Tavneos	C5aR	A, complement peptide	severe active anti-neutrophil cytoplasmic autoantibody-associated vasculitis	2021
Ponesimod	Ponvory	S1P	A, lysophospholipid	multiple sclerosis	2021
Varenicline solution	Tyrvaya	mAChR	A, acetylcholine	dry eye disease	2021
pilocarpine ophthalmic	Vuity	mAChR	A, acetylcholine	presbyopia	2021

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Table 1. *Continued*

Substance	Brand name	Targets	GPCR class	Indications	Approval year
naloxone hydrochloride	Zimhi	opioid	A, opioid	opioid emergency	2021
Atogepant	Qulipta	CGRP	B, calcitonin	prevent migraine headache	2021
Relugolix	Myfembree	GnRH	A, gonadotrophin-releasing hormone	premenopausal women to control heavy menstrual bleeding due to uterine fibroids	2021
Leuprolide	Camcevi	GnRH	A, gonadotrophin-releasing hormone	adult patients with advanced prostate cancer	2021
Baclofen	Lyvispah	GABBR1	C, GABA	spasticity	2021
Daridorexant	Quviviq	OXR	A, orexin	insomnia	2022
Ganaxolone	ZTALMY	GABA	C, GABA	CDKL5 deficiency disorder	2022
Dexmedetomidine	Igalmi	α_2-AR	A, adrenaline	agitation	2022
Tirzepatide	Mounjaro	GLP-1	B, glucagon	type 2 diabetes in adults to decrease blood sugar levels	2022
Terlipressin	Terlivaz	V1R/V2R	A, vasopressin and oxytocin	hepatorenal syndrome	2022
Omidenepag isopropyl	Omlonti	EP2	A, prostanoid	glaucoma and ocular hypertension	2022
Naloxone	RiVive	μ-opioid receptor	A, opioid	the reversal of an opioid overdose or suspected opioid overdose	2023
Zuranolone	Zurzuvae	GABAA	C, GABA	postpartum depression	2023
Albuterol and budesonide	Airsupra	β_2-AR	A, adrenaline	asthma	2023
Sparsentan	Filspari	AT1R/ETAR	A, angiotensin/endothelin	primary immunoglobulin A nephropathy	2023
Zavegepant	Zavzpret	CGRP	B, calcitonin	migraine	2023
Rizatriptan	RizaFilm	5-HT1B/1DR	A, hydroxytryptamine	migraine	2023
Phenylephrine hydrochloride and tropicamide	Mydcombi	α_1-AR	A, adrenaline	pupillary dilation	2023
Fezolinetant	Veozah	NK3R	A, tachykinin	menopausal disorders, hot flashes	2023
Nalmefene hydrochloride	Opvee	μ-opioid receptor	A, opioid	opioid overdose	2023

COPD, chronic obstructive pulmonary disease.

Class C GPCRs include 22 receptors that are subdivided into five subfamilies, including eight orphan receptors, one calcium-sensing receptor (CaSR), two gamma-aminobutyric acid (GABA) type B receptors, three taste 1 sensory receptor (TS1R1–3), and eight metabotropic glutamate receptors (mGluR1–8).[3,32] To date, 16 drugs that target the class C GPCRs have been approved by the FDA. At present, 15 drug candidates targeting the metabotropic glutamate receptor subtypes (mGluRs) are undergoing clinical trials for pain, migraine, and Parkinson's disease.[3] For example, acamprosate is an antagonist of mGluR5 and was approved by FDA in 2004 as an anti-neoplastic agent.[33] Furthermore, allosteric modulators of class C have also attracted significant drug development efforts. For example, cinacalcet is a small-molecule positive allosteric modulator (PAM) of the CaSR and a calcimimetic that was approved by the FDA in 2004 for hyperparathyroidism.[34] Class F GPCRs include 11 members and are characterized as coiled-coil receptors (FZD1–10) that mediate Wnt signaling and are essential for the embryonic development.[3] Only one of the class F GPCRs (smoothened receptor [SMO]) has been validated as a drug target.[3] In 2012, the FDA approved the small-molecule antagonist of SMO, glasdegib, for the treatment of patients with acute myeloid leukemia (AML).[35]

Biased ligands

Recent studies on biased GPCR signaling have transformed the understanding of GPCR signaling and opened a new area in GPCR-targeted drug development. "Biased signaling" occurs when a selective ligand preferentially activates one signaling pathway over others that use the same receptor in a single cellular system.[23,36] The biased GPCR ligands offer the potential for highly targeted GPCR therapeutics while avoiding side effects.[36,37] Biased GPCR signaling involves angiotensin II receptor type 2 (AT2R), μ-opioid receptor (OR), κ-OR, β-adrenergic receptors (βARs), dopamine receptor D2 (DRD2), calcitonin receptor (CTR), chemokine receptors (CCRs), and adenosine receptors (ARs). In 2020, the FDA approved oliceridine (Olinvyk), a μ-OR-biased ligand, for restricted use to manage moderate to severe acute pain in adults.[38] Oliceridine acts as a "biased

agonist" of the μ-OR and preferentially activates the G-protein-dependent pathway with minimal receptor phosphorylation to trigger the b-arrestin dependent (or G-protein-independent) pathway. Compared to the traditional opioids, oliceridine shows significantly reduced risk of opioid-related adverse effects, such as constipation and respiratory depression.[38] Additionally, a recent study has shown that MIPS521, a PAM of the adenosine A1 receptor (A1R), exhibits analgesic efficacy in animal neuropathic pain model through the stabilization of the adenosine-receptor-G-protein complex bound to its endogenous agonist. This study elucidates the A1R is a potential agent of non-opioid analgesia on neuropathic pain management.[39]

GPCR dimerization

The signaling function of GPCRs involves formation of homo- or heterodimers and oligomers.[40] Therefore, therapeutic strategies involve modulation of receptor dimerization and/or oligomerization.[41] For example, pre-eclampsia is associated with altered GPCR heterodimerization. In patients with pre-eclampsia, significant increase in the formation of the angiotensin II receptor type I (AGT1R)/bradykinin B2 receptor (B2R) heterodimers promotes angiotensin II (Ang II)-stimulated activation of Gαq/11 on the membranes and upregulates Ang II-related hypersensitivity.[42] However, there are limited approaches for targeting GPCR dimerization. Previous studies have demonstrated that transmembrane peptides can be used to inhibit GPCR dimerization. For example, in an animal model, drinking behavior was affected by inhibiting dimerization of the secretin receptor and AGTR1 using a peptide from transmembrane domain 4 (TM4).[43] The TM5/TM6 cannabinoid receptor (CB1R) peptide inhibits dimerization of the CB_1R and serotonin receptor (5HT2AR) and is an ideal candidate for cannabis-based pain management.[44]

Peptide drugs

FDA had approved ∼50 GPCR peptide drugs up to 2021 and more than 10 potential peptide therapeutics are in the pipeline.[45] A majority of the GPCR-targeting peptide drugs functionally mimic the structure of endogenous peptides and target class A or class B GPCRs. Peptide drugs targeting various class A GPCRs, such as μ-OR and κ-OR, and vasopressin receptors are used to relieve pain, induce labor, and treat cardiovascular diseases; these peptides are, however, highly unstable and demonstrate low plasma protein binding.[31] Therefore, chemical modifications are required to enhance pipette circulation time and to improve their biological activity, selectivity, and stability for reducing blood clearance. For example, selepressin is a novel selective vasopressin V1a receptor agonist with a longer plasma half-life because of the introduction of non-natural amino acids at the proteolysis site.[46,47] Semaglutide is a GLP-1 receptor agonist with a half-life of 168 h that is approved for the treatment of T2DM. Semaglutide contains a free fatty acid linker that allows the molecule to form a non-covalent reversible interaction with serum albumin, which significantly enhances its half-life.[31] Furthermore, tirzepatide (Mounjaro), a dual GIP/GLP-1, is a key regulator of insulin secretion and has also been used for T2DM treatments in adults.[48]

Monoclonal antibodies

GPCR-targeted monoclonal antibodies (mAbs) are another significant area of drug development because of the high specificity, affinity, and ease of purification.[49] GPCR-targeted mAbs have been developed for the treatment of cancers, inflammation, and metabolic disorders.[3] Currently, three GPCR-targeting mAbs have been approved by the FDA. Erenumab (Aimovig), a calcitonin gene-related peptide receptor (CGRP-R) antagonist, was the first GPCR-targeted mAb approved by the FDA in 2018 for the treatment of migraine.[50] Mogamulizumab (Potilegeo), a chemokine receptor CCR4 binder, was approved by the FDA in 2018 for the treatment of mycosis fungoides or Sézary syndrome and acts by targeting the CCR4-positive T cell lymphomas.[51] In 2020, the FDA approved eptinezumab (Vyepti), an anti-CGRP monoclonal antibody for the preventive treatment of migraine in adults.[52] To date, 57 GPCR-targeting monoclonal antibodies are in preclinical and clinical trials.[53] In addition to mAbs, bispecific antibodies, nanobodies, antibody-drug conjugates, and antibody-peptide conjugates are potential variants for future drug development.[54]

Photopharmacology

Photopharmacology is another new approach to targeting GPCRs without genetic manipulation, wherein GPCR activity is modulated by small molecules that are regulated by light.[55] Photopharmacology has great therapeutic potential because it allows precise spatiotemporal control of receptor activation. For example, Taura et al. developed a caged light-sensitive A2A adenosine receptor (A2AR) antagonist, MRS7145, to treat movement disorders. The active antagonist is locally released upon illumination by violet light (405 nm) and activates the A2AR. MRS7145 induces accumulation of cyclic AMP (cAMP) mediated by A2AR in a light- and dose-dependent manner.[55,56]

RNA THERAPEUTICS

Advances in RNA-based therapy has shown great promise in the drug development industry because they can theoretically target genetic components based on Watson-Crick base pairing, and demonstrate high precision, relatively predictable performance, high thermostability, and lower cost of synthesis compared to the protein- or peptide-based drugs.[57,58] At present, there are six distinct categories of RNA therapeutics: (1) antisense oligonucleotides (ASOs), (2) aptamers, (3) small interfering RNAs (siRNAs), (4) small activating RNAs (saRNAs), (5) microRNAs (miRNAs), and (6) messenger RNAs (mRNAs). The RNA-based drugs approved by the FDA in the six categories are listed in Table 2 and Figure 2, and those undergoing clinical trials are listed in Table 3. Currently, there are 21 FDA-approved RNA drugs that use ASO, siRNA, mRNA, and aptamer approaches. Moreover, there are significant basic research and preclinical publications that have demonstrated the potential of RNA therapeutics that are not yet in clinical trials.[59] The mechanisms and recent developments in each of these six categories are summarized in Figure 3, and the details are discussed in the following sections.

Antisense oligonucleotides

Antisense oligonucleotides (ASOs) are short, single-stranded oligonucleotides that downregulate the synthesis of their target proteins. ASOs

Table 2. FDA-approved RNA drugs

RNA drugs	Brand name	Targets	Diseases	Company	Approval year
ASOs					
Fomivirsen	Vitravene	CMV mRNA	CMV retinitis	Ionis Pharmaceutical, Novartis	1998
Mipomersen	Kynamro	Apo-B-100 mRNA	homozygous familial hypercholesterolemia	Kastle Therapeutics, Ionis Pharmaceuticals, Genzyme	2013
Nusinersen	Spinraza	SMN2 pre-mRNA	spinal muscular atrophy	Ionis Pharmaceuticals, Biogen	2016
Eteplirsen	Exondys 51	exon 51 of DMD	DMD	Sarepta Therapeutics	2016
Inotersen	Tegsedi	TTR mRNA	familial amyloid polyneuropathy	Ionis Pharmaceuticals	2018
Milasen	N/A	CLN7	Mila Makovec's CLN7 gene associated with Batten disease	Boston Children's Hospital	2018
Golodirsen	Vyondys 53	exon 53 of DMD	DMD	Sarepta Therapeutics	2019
Viltolarsen	Viltepso	exon 53 of DMD	DMD	NS Pharma	2020
Casimersen	Amondys 45	exon 45 of DMD	DMD	Sarepta Therapeutics	2021
Tofersen	Qalsody	SOD-1	amyotrophic lateral sclerosis	Biogen	2023
Aptamers					
Pegaptanib	Macugen	heparin- binding domain of VEGF-165	neovascular age-related macular degeneration	OSI Pharmaceuticals	2004
Defibrotide	Defitelio	adenosine A1/A2receptor	veno-occlusive disease in liver	Jazz Pharmaceuticals	2016
siRNAs					
Patisiran	Onpattro	TTR mRNA	polyneuropathy caused by hATTR amyloidosis	Alnylam	2018
Givosiran	Givlaari	ALS1 mRNA	acute hepatic porphyria	Alnylam	2020
Lumasiran	Oxlumo	HAO1 mRNA	primary hyperoxaluria type 1	Alnylam	2020
Inclisiran	Leqvio	PCSK9	atherosclerotic cardiovascular disease	Novartis	2021
Vutrisiran	Amvuttra	TTR mRNA	polyneuropathy caused by hATTR amyloidosis	Alnylam	2022
Nedosiran	Rivfloza	LDH mRNA	primary hyperoxaluria type 1	Novo Nordisk	2023
mRNAs					
BNT162b2	Comirnaty	SARS-CoV-2 S antigens	COVID-19 vaccine	Pfizer- BioNTech	2020
mRNA-1273	Spikevax	SARS-CoV-2 S antigens	COVID-19 vaccine	Moderna	2020

were first reported in 1978[60] and are widely used for studying target gene functions and as a viable therapeutic approach for specific human diseases.[61] ASOs silence gene expressions through several mechanisms: (1) inhibition of 5′ cap formation, (2) alteration of the splicing process, (3) ribonuclease H (RNase H)-dependent activation, and (4) RNase H-independent ASO activation (steric blocker).[62,63] ASO targeting to the 5′ UTR sequence could inhibit 5′ cap formation, thus leading to gene silencing.[63] In addition, pre-mRNAs splice noncoding introns and exons to form mature mRNA. This slicing process could be altered by ASO for disease treatments. For example, ASOs are utilized to delete certain sequences in Duchenne muscular dystrophy (DMD)/dystrophin pre-mRNA that can repair RNA by facilitating splicing, thus effectively treating DMD.[63,64] ASO could also work through RNase H-dependent and -independent (steric-blocker) actions. RNase H-dependent ASOs form a duplex with their target RNAs. Subsequently, RNase H recognizes the central region of the RNA-DNA hybrid and cleaves the corresponding RNA strand, thus decreasing target gene transcript levels.[65] The binding of the RNase H-independent ASOs to the target RNA generates a steric block that prevents pre-mRNA maturation or mRNA translation without RNA degradation. The steric-blocker ASOs impede protein-RNA binding interactions between the splicing machinery components and the pre-mRNAs. Therefore, steric-blocker ASOs can be used to regulate the target protein levels by interfering with the splicing of the target RNAs (pre-mRNAs or mRNAs).[66] Typically, steric-blocking ASOs could prevent recognition and cleavage by RNase H with sugar modification, including 2′-O-2-methoxyethyl (2′-O-MOE), 2′-O-methyl (2′-O-Me), 2′-fluoro (2′-F), and locked nucleic acid (LNA).[61,67,68]

In 1998, the FDA approved fomivirsen (Vitravene), the first drug with phosphorothioate (PS) linkages within the ASO backbone, for the treatment of cytomegalovirus retinitis (CMV). Fomivirsen blocked

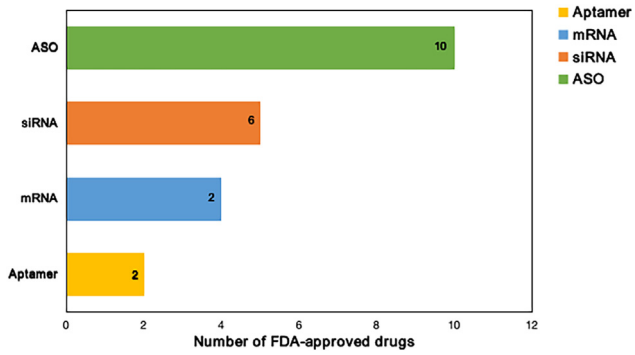

Figure 2. Summary of FDA-approved RNA drugs
Number of RNA drugs was analyzed based on data in Table 2.

the translation of a key CMV protein, UL123, by binding to the complementary sequence of its mRNA.[69] However, this drug was removed from the market in 2006 because of the success of anti-retroviral therapy, which significantly reduced the probability of opportunistic infections in subjects with HIV. Since then, several ASO therapeutics have been successfully marketed in the US, including mipomersen (Kynamro) and inotersen (Tegsedi). These are also known as ASO gapmers because they contain chemically modified RNA bases flanking both sides of a central 8- to 10-base DNA "gap."[70] Mipomersen (Kynamro) is a 20-mer gapmer and is approved by the FDA for the treatment of homozygous familial hypercholesterolemia (HoFH) because it significantly reduces the levels of apolipoprotein B (*ApoB*) mRNAs and low-density lipoprotein (LDL).[71,72] Inotersen (Tegsedi) is approved for the treatment of adult subjects with hereditary transthyretin-mediated amyloidosis (hATTR amyloidosis) and acts by selectively suppressing the transthyretin (TTR) mRNA levels.[73] Nusinersen is a 2′-MOE-modified 18-mer ASO that modulates alternative splicing of *SMN2* and was approved by the FDA in 2016 for the treatment of spinal muscular atrophy (SMA).[74] Milasen is a personalized ASO that was specifically designed for a 6-year-old child with a rare Batten disease, which was caused by premature translational termination of the ceroid lipofuscinosis 7 (*CLN7*) pre-mRNA because of improper exon splicing.[59] Milasen restores normal exon 6–7 splicing of the *CLN7* pre-mRNA.[61] Eteplirsen (Exondys 51), golodirsen (Vyondys 53), viltolarsen (Viltepso), and casimersen (Amondys 45) are ASO drugs with phosphorodiamidate morpholino oligomer (PMO) modifications that were approved by the FDA in 2016,[75] 2019,[76] 2020,[77] and 2021,[78] respectively, for the treatment of DMD, a lethal neuromuscular disorder that is caused by genetic mutations that alter the reading frame of the X-linked dystrophin gene.[73] Additionally, tofersen (Qalsody) is an ASO that targets the production *SOD1* for the treatment of amyotrophic lateral sclerosis (ALS). It was approved by FDA in April 2023.[79] Currently, several new ASOs are undergoing clinical trials (https://www.clinicaltrials.gov/.) for the treatment of specific human diseases (Table 3).

RNA aptamers

Aptamers were first developed by the Gold and Szostak groups in 1990. They are short, synthetic, single-stranded RNA or DNA oligonucleotides or their modified analogs that form complex three-dimensional (3D) structures by specifically binding to their target molecules.[80] Aptamers are selected from large libraries of random oligonucleotide libraries (>1,015 random sequences) using the systematic evolution of ligands by exponential enrichment (SELEX) method.[81] These aptamers show high affinity and specificity and interact with a wide variety of specific molecular targets, such as proteins, DNA, RNA, small molecules, and ions.[82] RNA aptamer functions like a nucleic acid antibody or a chemical inhibitor and modulates target protein functions.[73,83] RNA aptamer also serves as a targeting ligand to modify and bind nanocarriers that improve the specificity of target drug delivery in clinical therapy.[84] The metabolic stability and pharmacokinetic properties of the RNA aptamers can be improved through chemical modifications, as described for the ASOs.[72]

Pegaptanib (Macugen) was the first FDA-approved aptamer in 2004 for the treatment of neovascular age-related macular degeneration.[85] Pegaptanib binds to vascular endothelial growth factor (VEGF) with high affinity and prevents its binding with the VEGF receptor.[86] In 2016, defibrotide (Defitelio) was approved by the FDA for the treatment of hepatic veno-occlusive disease/sinusoidal obstruction syndrome (VOD/SOS). Defibrotide protected the endothelium lining of the blood vessels during chemotherapy with fludarabine by increasing the levels of prostaglandins and prostacyclin, altering the activity of platelets, and decreasing the activity of the plasminogen activator inhibitor-1 (*PAI-1*).[87] However, the molecular mechanism of defibrotide is poorly understood.[87,88] Both pegaptanib and defibrotide show minimal side effects and highlight the potential of aptamer-based therapies. Currently, several aptamers are being evaluated in clinical trials. For example, NOX-A12 is an anti- C-X-C motif chemokine ligand 12 (*CXCL12*) aptamer that increases the number of circulating tumor-infiltrating T cells by neutralizing *CXCL12* and is proposed for the treatment of pancreatic cancer (NCT01521533), colorectal cancer (NCT01521533), and multiple myeloma (NCT03168139).[89–91] Zimur is an anti-C5 aptamer that is undergoing a clinical trial for the treatment of geographic atrophy secondary to age-related macular degeneration (NCT02686658).[92]

siRNA

siRNAs regulate gene expression in eukaryotic cells through a phenomenon known as RNA interference (RNAi). The first step of RNA interference occurs in the cytoplasm, wherein Dicer, an endoribonuclease, produces mature 21- to 23-base siRNAs by cleaving long double-stranded RNAs or short hairpin RNAs.[93] After processing, mature siRNAs are incorporated into the RNA-induced silencing complex (RISC), which then interacts with the Argonaute 2 (Ago2) protein; subsequently, the RNA duplex is unwound, and the passenger strand is degraded.[72] The antisense strand then guides the RISC to the complementary mRNA for subsequent endonucleolytic mRNA cleavage, thereby suppressing protein synthesis.

In the field of biomedicine, siRNAs are a popular tool for downregulating genes of interest. For example, in cancer research, siRNA-targeted

Table 3. RNA therapeutics in clinical development

RNA therapeutics	Target(s)	Disease	Company	Identifier and status
ASOs				
1018 ISS (CpG-OND-1018)	TLR9	non-Hodgkin's lymphoma	Dana-Farber Cancer Institute, Brigham and Women's Hospital, Massachusetts General Hospital, University of Rochester	NCT00251394 (phase II)
Apatorsen (OGX-427)	HSP27	urologic, bladder, prostate, urothelial, and non-small-cell lung cancer	Achieve Life Sciences PRA Health Sciences	NCT00487786, NCT01454089 (phase I/II)
Cenersen (EL625)	TP53	acute myelogenous leukemia and lymphoma	Eleos	NCT00074737 (phase II)
ARRx (AZD5312)	AR	prostate cancer	AstraZeneca	NCT02144051, (phase I/II)
Custirsen (OGX-011)	ApoJ	prostate, breast, and non-small-cell lung cancer	Clinical Trials Group Achieve Life Sciences	NCT00054106, NCT00138658, (phase I/II)
Aptamers				
NOX-A12	CXCL12	pancreatic, colorectal cancer, and multiple myeloma	NOXXON Pharma Merck Sharp & Dohme	NCT01521533, NCT01521533, NCT03168139 (phase I/II)
NOX-E36	CCL2	diabetic nephropathy and renal impairment	NOXXON Pharma	NCT01372124, NCT01547897 (phase I/II)
Zimura	C5	geographic atrophy macular degeneration	IVERIC bio	NCT02686658 (phase II/III)
Pegcetacoplan	C3	transplant-associated thrombotic microangiopathy	Swedish Orphan Biovitrum Apellis Pharmaceuticals	NCT05148299 (phase II)
E10030	PDGF	age-related macular degeneration	National Eye Institute	NCT02859441 (phase II)
siRNAs				
TKM-080301	PLK1	cancer with hepatic metastases, and hepatocellular cancer	National Cancer Institute Arbutus Biopharma Corporation	NCT01437007, NCT02191878 (phase I/II)
AGN211745	VEGFR1	choroidal neovascularization-age-related macular degeneration (CNV-AMD)	Allergan	NCT00363714 (phase I/II)
PF-04523655	VEGFR1	CNV-AMD	Quark Pharmaceuticals	NCT00713518 (phase II)
Atu027	Protein kinase N3	advanced solid tumors and pancreatic cancer	Silence Therapeutics	NCT00938574, NCT01808638 (phase I/II)
Cosdosiran (QPI-1002)	p53	acute kidney injury and delayed graft function	Quark Pharmaceuticals	NCT02610283, NCT02610296 (phase I/I)
TD101	KRTGA	pachyonychia congenita	Huntsman Cancer Institute	NCT00716014 (phase I)
siG12D LODER	KRASG12D	pancreatic cancer	Silenseed	NCT01676259, NCT01188785 (phase I/II)
ARO-HIF2	HIF2A	renal cell carcinoma	Arrowhead Pharmaceuticals	NCT04169711 (phase I)
APN401	CBLB	brain cancer, melanoma, pancreatic cancer, and renal cell cancer	Wake Forest University Health Sciences National Cancer Institute	NCT03087591, NCT02166255 (phase I)
STP705	TGF-β1 and COX-2	basal cell carcinoma, keloid, hypertrophic scar, hepatocellular carcinoma, liver metastases, and cholangiocarcinoma	Sirnaomics	NCT04669808, NCT05421013, NCT04844840, NCT02956317, NCT04676633 (phase I/II)
Cosdosiran (QPI-1007)	Caspase-2	nonarteritic anterior ischemic optic neuropathy	Quark Pharmaceuticals	NCT01965106 (phase III)
Tivanisiran (SYL1001)	TRPV1	dry eye disease	Sylentis	NCT03108664, NCT04819269 (phase III)
Nedosiran (DCR-PHXC)	HAO1	primary hyperoxaluria	Dicerna Pharmaceuticals	NCT04042402 (phase III)

(*Continued on next page*)

Table 3. *Continued*

RNA therapeutics	Target(s)	Disease	Company	Identifier and status
Cemdisiran (ALN-CC5)	C5	immunoglobulin A nephropathy	Alnylam Pharmaceuticals	NCT03841448 (phase II)
Fitusiran (ALN-AT3SC)	antithrombin	hemophilia A or B	Genzyme	NCT03417102 (phase III)
saRNAs				
MTL-CEBPA	CEBPA	liver cancer, solid tumor	MiNA Therapeutics	NCT02716012, NCT04710641, NCT04105335 (phase I/II)
MTL-STING	STING	malignant solid tumor	MiNA Therapeutics	Preclinical trial (Enter phase 1 evaluation in 2023)
miRNAs				
TargomiRs	miR-16	malignant pleural mesothelioma, non-small cell lung cancer	EnGeneIC	NCT02369198 (phase I)
MRG-110	miR-92a	wound healing	miRagen	NCT03603431 (phase I)
RG-125 (AZD4076)	miR-103/107	NASH, T2D with NAFLD	AstraZeneca	NCT02612662, NCT02826525 (phase I)
RGLS4326	miR-17	autosomal dominant polycystic kidney disease	Regulus Therapeutics	NCT04536688 (phase I)
Miravirsen	miR-122	HCV	Roche/Santaris	NCT01200420 (phase II)
CDR132L	miR-132	heart failure, acute myocardial infarction	Cardior Pharmaceuticals	NCT05350969 (phase II)
MRG-201 (Remlarsen)	miR-29	keloids	miRagen Therapeutics	NCT03601052 (phase II)
RG-012 (Lademirsen)	miR-21	Alport syndrome	Sanofi	NCT03373786 (phase II)
MRG-106 (Cobomarsen)	miR-155	cutaneous T cell lymphoma/ mycosis fungoides	Fungoides miRagen	Discontinued
RG-101	miR-122	chronic hepatitis C	Regulus Therapeutics	Discontinued
pSil-miR200c and PMIS miR-200a/c	miR200a	tooth extraction	University of Iowa	Discontinued
MRX34	miR-34a	melanoma; primary liver cancer; hematologic malignancies	Mirna Therapeutics	Discontinued
mRNAs				
CVnCoV	SARS-CoV-2 S antigens	COVID-19	CureVac	NCT04652102 (phase III)
CV7202	RABV-G	rabies	CureVac	NCT03713086 (phase I)
AZD8601	VEGF-A	ischemic heart disease	AstraZeneca	NCT03370887 (phase II)
MRT5005	CFTR	CF	Translate Bio	NCT03375047 (phase I/II)
mRNA-3704	MUT	methylmalonic aciduria	Moderna	NCT03810690 (phase I/II)
BNT111	mutated TAAs (NY-ESO-1, MAGEA3, tyrosinase, and TPTE)	advanced melanoma	BioNTech	NCT02410733 (phase I)
mRNA-1273	SARS-CoV-2 S antigens	COVID-19	ModernaTX	NCT05584202 (phase II)

downregulation of sphingosine-1-phosphate lyase 1 (*S1P lyase*) decreased invasiveness of cancer cells by upregulating E-cadherin,[94] siRNA-mediated silencing of remodeling and spacing factor-1 (*RSF-1*) induced apoptosis and cell-cycle arrest of the cervical cancer cells,[95] and *PD-L1*-specific siRNAs significantly improved the efficacy of immunotherapy against melanoma and colon cancer cells.[96] siRNA-based therapy has also been applied to neurodegenerative diseases. Zhou et al. developed a glycosylated "triple-interaction" stabilized polymeric siRNA nanomedicine (Gal-NP@siRNA) to target the β-site amyloid precursor protein (APP) cleavage enzyme 1 (*BACE1*) in the APP/PS1 transgenic mouse model of Alzheimer's disease. Gal-NP@siRNA efficiently penetrated the blood-brain barrier through glycemia-controlled GLUT1-mediated transport, thereby inducing siRNA-mediated downregulation of *BACE1* expression to alter related signaling pathways without significant side effects.[97]

To date, the FDA has approved six siRNA agents (Table 2): patisiran (Onpattro), givosiran (Givlaari), lumasiran (Oxlumo), inclisiran (Leqvio), vutrisiran (Amvuttra), and nedosiran (Rivfloza). Patisiran was the first siRNA agent that received FDA approval in 2018. Patisiran targets mutated transthyretin (*TTR*) mRNA and significantly reduces *TTR* deposition caused by hATTR amyloidosis in patients with polyneuropathy.[93] A lipid nanoparticle formulation was used to shield and stabilize the novel siRNA (ALN-18328) in patisiran from nuclease

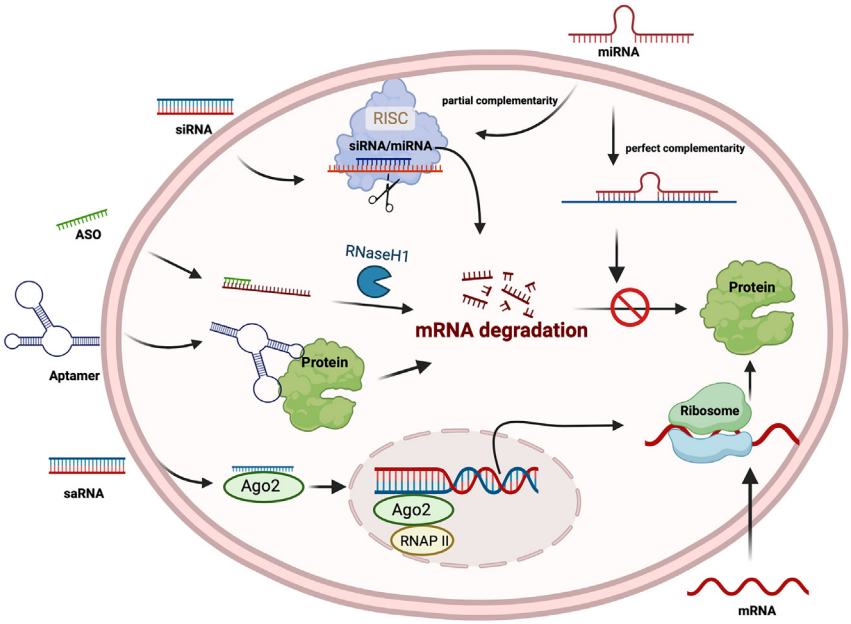

Figure 3. Schematic illustrating different classes of RNA therapeutics

ASO, antisense oligonucleotide; RNA, ribonucleic acid; siRNA, small interfering RNA; saRNA, small activating RNA; miRNA, microRNA; mRNA, messenger RNA; RISC, RNA-induced silencing complex; Ago2, Argonaute 2; RNase H, ribonuclease H; RNAP II, RNA polymerase II. Graph was created with BioRender (https://app.biorender.com).

degradation.[98] In 2019, the FDA approved givosiran for the treatment of acute hepatic porphyria (AHP), which is caused by defective heme biosynthesis resulting in a toxic buildup of porphobilinogen (PBG) and delta-aminolevulinic acid (ALA).[59] Givosiran targets the mRNA of ALA synthase 1 (*ALAS1*) in the liver and reduces the serum levels of neurotoxic intermediates such as aminolevulinic acid and porphobilinogen.[99] Lumasiran was approved by the FDA in 2020 for the treatment of primary hyperoxaluria type 1 (PH1) in pediatrics and adults by decreasing the levels of oxalate in the urine and blood. Lumasiran targets hydroxyacid oxidase 1 (*HAO1*) mRNA and reduces the levels of the glycolate oxidase. This reduces the levels of glyoxylate, a substrate for the synthesis of oxalate.[100] Inclisiran injection was approved by the FDA in 2021. It acts by targeting the proprotein convertase subtilsin/kexin type 9 (*PCSK9*) mRNAs to reduce the levels of LDL. *PCSK9* is a key player in lipid metabolism and participates in the regulation of cholesterol levels. Inclisiran injection was approved by the FDA for use in combination with statin therapy for the treatment of heterozygous familial hypercholesterolemia (HeFH) and clinical atherosclerotic cardiovascular disease (ASCVD).[101] Furthermore, vutrisiran was approved in June 2022 by the FDA for the treatment of polyneuropathy caused by hATTR amyloidosis in adults through degradation of the variant and wild-type *TTR* mRNAs.[102] In September 2023, the FDA approved nedosiran, an siRNA drug, to treat primary hyperoxaluria 1 (PH1) once monthly by inhibiting the expression of *LDH* enzyme.[103] Givosiran, lumasiran, inclisiran, vutrisiran, and nedosiran are all composed of 2′-O-Me, 2′-F, and PS chemical modifications that enhance stability and reduce immunogenicity.[104] These five drugs are also conjugated to the GalNAc ligand, which was developed to overcome the biological barriers and facilitate targeted delivery.[105] When the bound siRNAs are internalized into hepatocytes through ASGPR-mediated endocytosis, GalNAc siRNAs are rapidly cleaved from the target moiety within acidic endosomes. Subsequently, these siRNAs silence the activity of the targeted protein in the liver.[106,107] Except as mentioned above, several siRNA-based therapies are currently being evaluated in clinical trials and are shown in Table 3.

saRNA

The clinical research on siRNA has led to the use of small RNAs to inhibit the transcription of disease-related genes. However, RNA therapeutics are not yet available for specifically rescuing the transcription of silenced functional genes in specific diseases. RNA activation involves specific upregulation of the target gene expression using small RNA oligos, which are called saRNAs. Multiple studies have shown that saRNAs activate a wide variety of genes in several mammalian species.[108–110] The saRNAs are double-stranded noncoding RNAs that are 21 nucleotides long with a two-nucleotide overhang at the 3′ ends.[111] The saRNAs form a complex consisting of guide RNA, heterogeneous nuclear ribonucleoproteins (hnRNPs), and Ago2 in the cytoplasm. Subsequently, this complex translocates into the nucleus, binds to the corresponding DNA sequence, facilitates assembly of an RNA-induced transcriptional activation (RITA) complex, and recruits RNA polymerase II to initiate transcription and productive elongation of the target mRNA.[112,113] Thus, saRNAs expose the target gene promoter and facilitate the binding of RNA polymerase II (RNAPII) at the transcription start site and assembly of the transcription pre-initiation complex.[112] In some cases, saRNAs alter the target mRNAs by binding to the promoter-associated transcripts or long noncoding RNAs instead of the complementary DNA sequences.[114]

MTL-CCAAT/enhancer binding protein alpha (CEBPA) is a first-in-class saRNA-based therapy that has recently progressed in the clinical trials. MTL-CEBPA uses liposomal nanoparticles called SMARTICLES (which are composed of different mixtures of 1-palmi- toyl-2-oleoyl-glycero-3-phosphocholine (POPC), DOTAP, DMGSucc, and cholesterol) to encapsulate a modified 2 'O-Me-conjugated saRNA (21-mer), which activates transcription of the tumor suppressor *CEBPA* gene for the treatment of hepatocellular carcinoma (HCC).[111,115] In the phase I and Ib study (OUTREACH-2; NCT02716012), MTL-CEBPA was evaluated as a monotherapy or in combination with sorafenib in 51 HCC patients with cirrhosis that resulted from non-alcoholic steatohepatitis or liver metastases. This

clinical trial is estimated to be completed in January 2023. The preliminary results from this clinical trial show that MTL-CEBPA mediated RNA activation and reduced immune suppression biomarkers in HCC patients; moreover, MTL-CEBPA in combination with sorafenib showed significant tumor suppression and a good safety profile.[116] Therefore, in January 2022, MiNA Therapeutics announced the global phase II clinical trial (OUTREACH-2; NCT04710641) in advanced HCC patients for MTL-CEBPA in combination with sorafenib.[117] Furthermore, MTL-CEBPA in combination with pembrolizumab (PD-L1 inhibitor) is undergoing phase 1 clinical trial (NCT04105335) in patients with advanced solid tumors.

Recently, MiNA Therapeutics announced the proof-of-mechanism data for MTL- Stimulator of interferon genes (STING) as a second drug candidate for RNA therapeutics. *STING* is a master regulatory protein for the identification of cancer cells by the immune system. The downregulation of *STING* is a key immune evasion mechanism in the cancer patients and is also the main mechanism for inactivating the cGAS/cGAMP/STING pathway in the innate immune response.[118] MTL-STING is initially being developed as a combination treatment for solid tumor malignancies and is expected to enter phase I evaluation in 2023.[119]

miRNAs

miRNAs were first identified in *Caenorhabditis elegans* and represent an abundant class of noncoding single-stranded RNAs that are 22–61 nucleotides in length.[120] The miRNA coding genes are initially transcribed as primary miRNA transcripts (pri-miRNAs) and are subsequently processed into shorter precursor miRNAs (pre-miRNAs) by the RNase III enzyme, Drosha.[72,120] Some pre-miRNAs are directly excised from the introns of protein-coding genes.[121] The pre-miRNAs are then exported from the nucleus into the cytoplasm by Exportin-5 and converted to double-stranded miRNA molecules by the cytoplasmic endoribonuclease, Dicer. Subsequently, miRNAs are loaded into miRNA-induced silencing complexes (miRISCs) and repress protein translation through selective base pairing between the single-stranded guide sequence of the miRNA and the target mRNA.[122,123] miRNAs have been the focus of several clinical studies for the treatment of human diseases such as cancers, viral infections, and inflammatory diseases.[124–126] Furthermore, miRNA mimics or miRNA antagonists (anti-miRs) have been proposed as potential agents for the restoration of miRNA expression levels.[59,127]

Currently, the FDA has not approved any miRNA or anti-miR drugs. However, several studies are ongoing regarding the use of miRNA and anti-miR therapeutics. So far, 12 miRNA drugs have been evaluated in clinical trials. Among these, four are in phase I trials, three are in phase II trials, and five have been terminated (Table 3). Currently, none of the potential miRNA therapeutics are in phase III trials, and more than 40% of the tested miRNA drugs have been terminated in clinical trials, which indicates significant obstacles for the miRNA therapeutics. Due to the off-target effects, the miRNAs show a flexible complementary ratio with the target sequence (range: 20%–90%), and none of the miRNAs are 100% complementary. Therefore, most of the miRNAs target 30–1,000 genes, through mRNA degradation or translation blockage, thus leading to gene-silencing effects.[128] In comparison, the siRNA mechanism requires 100% target sequence specificity. siRNA drugs downregulate target genes via mRNA cleavage.[128] Therefore, siRNAs show significantly higher specificity than the miRNAs.[129] However, even though the flexible complementary miRNAs allow the regulation of multiple target genes, the off-target effects are one of the major challenges of miRNA therapeutics.

MesomiR-1 is a phase1 study (NCT02369198) aimed at delivering miR-16 mimics packaged in EDV nanocells targeted with EGFR antibodies (TargomiRs) for the treatment of malignant pleural mesothelioma and non-small cell lung cancer (NSCLC). The phase 1 trial of TargomiRs (NCT02369198) concluded successfully and is expected to continue to phase II despite some complications.[130] RG-125 (AZD4076) is a GalNAc-conjugated anti-miR targeting miR-103/107 for the treatment of non-alcoholic steatohepatitis (NASH) in patients with type 2 diabetes or pre-diabetes through reduction of fasting glucose and insulin levels.[131] MRG-110 has completed phase I clinical trials to test the safety and efficacy of the miR-92a inhibitor in healthy subjects (NCT03603431). MRG-110 shows potential for treating impaired wound healing in conditions such as diabetes because of the pro-angiogenic effects of miR-92a inhibition.[59,132] CDR132L is a noncoding RNA inhibitor that targets miR-132, a central regulator of the pathological cardiac remodeling process in patients with cardiac diseases such as heart failure. Since miR-132 levels are elevated in the cardiac tissues of patients with heart failure, it is hypothesized that the inhibition of miR-132 by CDR132L would potentially restore normal cardiac muscle function by correcting the aberrant signaling pathways.[133] MRG-201 (Remlarsen) is a synthetic miRNA agonist of miR-29 for reversing fibrosis because miR29 decreases synthesis of collagen and other related proteins involved in fibrosis.[134]

mRNAs

The mRNAs are excellent candidates for the treatment of diseases with a known genetic component because of their high translation efficiency and immunostimulatory properties.[135] However, mRNA drugs can be associated with toxicity because mRNA expression at nontarget sites results in unwanted protein expression,[136] the off-target effects of mRNA accumulation in the liver and spleen requiring a higher dose of mRNA treatment,[137] and instability related to the relatively long mRNA sequence.[136] These concerns have hampered the use of mRNAs for *in vivo* clinical applications. Furthermore, the field of mRNA therapeutics has received a major boost due to the development of sophisticated regulatory systems for mRNA expression and the increased stability of mRNAs through chemical modifications, as well as the advancement of delivery platforms.[138]

The mRNA therapeutics are used in protein replacement therapy, including delivery of VEGF-A to sites of myocardial infarction,[139] production of vaccines for infectious diseases,[135] and the *in vivo* production of programmed cell death protein 1 (PD-1) human mAbs.[140,141] Furthermore, during the recent onset of the worldwide

COVID-19 pandemic, clinical trials of mRNA-based vaccines against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) progressed rapidly and resulted in the approval of two mRNA vaccines for emergency use by the World Health Organization (WHO).[72] The first mRNA vaccine approved by the FDA was BNT162b2 (Comirnaty), which was developed by Pfizer-BioNTech and consisted of the coding sequence for the full-length membrane-anchored spike (S) glycoprotein of SARS-CoV-2 with two minor mutations (K986P and V987P) to increase conformational stability. The second FDA-approved mRNA vaccine was mRNA-1273 (Spikevax), which was developed by Moderna and contained the coding sequence for the S glycoprotein of SARS-CoV-2, which was stabilized with two proline substitutions (K986P and V987P) and an intact S1–S2 cleavage site.[142] (Table 2). Both vaccines were encapsulated in lipid nanoparticles, including 1-methyl-pseudouridine, to escape the innate immune-sensing mechanisms and increase translational capacity and mRNA stability.[59,138] Furthermore, two additional mRNA vaccine candidates are currently in clinical trials (Table 3). CVnCoV is a COVID-19 vaccine candidate that is produced by CureVac and is currently in phase III clinical trials. CVnCoV is a chemically unmodified mRNA that encodes the full-length S glycoprotein of SARS-CoV-2.[143] CV7202 is another mRNA vaccine candidate in phase I clinical trials for rabies prevention. CV7202 is composed of the rabies virus glycoprotein mRNA to induce a rabies-neutralizing antibody response.[144]

There are several mRNA drug candidates in clinical trials for various human diseases. AZD8601 is a candidate VEGF-A drug manufactured by AstraZeneca for ischemic heart disease and is currently being evaluated in phase II clinical trials.[145] MRT5005 is a candidate drug for producing the cystic fibrosis transmembrane conductance regulator protein (CFTR). MRT5005 is manufactured by Translate Bio and is currently in phase I/II clinical trials for the potential treatment of cystic fibrosis (CF) lung disease.[146] Moderna is currently conducting phase I/II clinical trials for mRNA-3704, which is an mRNA therapeutic for restoring function of the mitochondrial enzyme methylmalonic-CoA mutase (MUT) in rare cases of methylmalonic acidemia.[62] FixVac (BNT111) is an intravenously administered liposomal RNA (RNA-LPX) vaccine that targets four highly prevalent tumor-associated antigens in melanoma, including New York esophageal squamous cell carcinoma 1 (NY-ESO-1), melanoma-associated antigen A3 (MAGE-A3), tyrosinase, and transmembrane phosphatase with tensin homology (TPTE). This drug is currently in phase I dose escalation clinical trials for the treatment of advanced melanoma and is manufactured by BioNTech.[147]

Challenges for the RNA therapeutics

Research in the last two decades has established that RNA-based therapeutics is a promising area of drug development in various human diseases, especially those that are undruggable. However, several factors have limited their clinical use, including issues regarding RNA stability, penetration efficiency, endosomal escape, immunogenetic problems, and off-target effects. The main obstacles are discussed in this section.

RNA stability

In vivo stability of the RNA molecules is an important criterion for RNA-based therapeutics. The naked RNA oligonucleotides have a very short half-life of 6 min in the plasma and are rapidly cleared by the RNases in the systemic circulation after intravenous injections.[148] Furthermore, there are other biological barriers that prevent naked oligonucleotides from reaching the target cells and tissues. For example, glomerular filtration in the kidney eliminates small molecules that are <50 kDa. These small molecules cannot cross the cell membrane and return into circulation. Therefore, it is estimated that the renal system eliminates intravenously injected naked oligonucleotides into urine within 1 h.[149] The liver also contributes to the clearance of oligonucleotides from the human body. In the reticuloendothelial system (RES), circulating naked oligonucleotides are eliminated from the liver by the Kupffer cells and from the spleen by the macrophages.[150] Therefore, prolonged *in vivo* stability of the RNA therapeutics in circulation requires specialized or modified delivery systems, which are discussed in later sections.

Penetration efficiency and endosomal escape

Another critical factor for the efficiency of RNA drugs is the ability to penetrate the cell membrane. The lipid bilayer prevents the passage of large amounts of negatively charged molecules, including RNA.[59,151] Furthermore, the intracellular route of nonviral oligonucleotide delivery occurs via endocytosis. RNA molecules in the endosomal vesicles are targeted to the lysosomes for degradation.[152] Therefore, endosomal escape is a significant challenge for the efficient delivery of RNA-based therapeutics. Effective strategies are required for the oligonucleotides to escape the endosomes and lysosomal degradation. Currently, this has been achieved by using lipid and dendrimer nanoparticles, as well as ligand conjugation, which results in the release of the loaded therapeutic cargo into the cytoplasm.[153]

Immunogenicity

The endogenous immune system considers the injected small RNA molecules as pathogens. Therefore, injection of foreign RNA stimulates the innate immune response and results in undesirable adverse effects and poor therapeutic effects because of drug elimination.[62] Systemic administration of the RNA duplexes stimulates excessive production of inflammatory cytokines and type I interferons (IFNs) through the Toll-like receptors 7/8 (TLR7/8) and represents a key challenge for RNA therapy.[154] The immune response also depends on the length and the nature of the injected RNAs. Longer dsRNAs induce a stronger immune response with high inflammatory cytokine levels.[155] The immune effects can be partially overcome through chemical modifications of the RNA molecules, which are discussed in detail later.

Off-target effects

RNA therapeutics can cause off-target effects and compromise specificity and safety. These off-target effects occur when small

RNA molecules are partially complementary to one or more cellular mRNAs besides the target gene or mRNA.[72] The imperfectly matched oligonucleotide binds non-specifically to these nontarget mRNAs and induces silencing through miRNAs binding to their 3′-UTR sequences.[156] Additionally, due to the lack of effective delivery system, RNA drugs could not reach the on-target cells, tissues, and organs, which also lead to off-target effects, thereby resulting in toxicity and reducing the efficacy of the RNA therapeutics.[157]

Low exposure

Low exposure to oligonucleotides is another major challenge in the clinical practice of RNA-based therapeutics.[148] Poor enzymatic stability is one of the reasons for this low exposure, as the phosphodiester bond of oligonucleotides is extremely susceptible to RNases and phosphatases.[106] Once the RNA molecule enters the circulation, it is rapidly degraded into segments by endonucleases or exonuclease enzymes, preventing the accumulation of entire RNA molecules *in vivo*.[106] On the other hand, because the RNA molecule is relatively small, has a short half-life, and lacks the ability to strongly bind to plasma proteins, it is easily cleared during glomerular filtration in the kidneys and eliminated into urine,[158] which also leads to low exposure to RNA drugs.

CHEMICAL MODIFICATIONS OF RNA-BASED DRUGS

Efficient outcomes of the systemic administration of RNA therapeutics depend on the ability to overcome the physiological challenges mentioned above. Therefore, an optimal RNA drug should also be biocompatible, biodegradable, and nonimmunogenic.[159] Chemical modification of RNA molecules can enhance their stability and protect against the potential immunogenic barrier. Modified nucleotide bases can increase stability, binding affinity, and specificity of the potential RNA drugs to the target sequence.[106,160] The most common chemical modifications of the nucleotide bases are 5′-methylcytosine (5′-mC) and 5-propynyl pyrimidine.[161] However, these modifications are associated with steric hindrance, which affects the function of RNA drugs by interfering with the interaction between siRNAs and the RISC.[162]

The deoxyribose sugar moiety in the DNA and the ribose sugar moiety in the RNA can be modified to increase the stability of the oligos against nuclease degradation and reduce the adverse immune responses. For example, 2′ modified nucleosides of short RNA, such as 2′-O-methyl (2′- OMe) or 2′-fluoro-2′-deoxy (2′-F) nucleoside, have a prolonged half-life because of increased resistance to endonucleases.[163,164] These modifications are incorporated by connecting the 2′-O to the C4′ with a methylene linkage and have been used effectively in the construction of siRNAs, gapmers, splice-switching, and antagomirs.[161] Modification of the phosphorothioate (PS) backbone can also improve the stability of the RNA therapeutics by suppressing exonuclease-mediated degradation and clearance. However, this modification can decrease binding affinity with the target RNA and induce non-specific protein binding. To overcome this problem, PS chemistry is often combined with the base and/or sugar modifications, such as the PMOs, which are short single-stranded DNA analogs that consist of a backbone of morpholine rings connected by the phosphorodiamidate linkages.[161,165] The FDA has recently approved two PMO-based ASO drugs, eteplirsen and golodirsen, to resolve the skipping of DMD exons 51 and 53. Both drugs bind to the dystrophin pre-mRNA, alter the exon splicing of the RNA, and enhance the production of full-length dystrophin. The progression of DMD can be prevented or suppressed by increasing the quantity of the full-length dystrophin protein through these modified RNA therapeutics. The uncharged nature of the PMO also protects them against biological degradation.[166,167] Another method is the covalent attachment of the hepatocyte asialoglycoprotein receptor-binding N-acetylgalactosamine (GalNAc) to the ASOs, siRNAs, miRNAs, and aptamers to achieve gene silencing.[168,169] PS modifications can improve the target-binding affinity of the aptamers. Several strategies have been employed to improve the stability of the *in vitro*-transcribed (IVT) mRNAs, with greater focus on the structural modification of the nucleic acid. It is well known that the length of the poly(A) tail, incorporation of 5′ and 3′ UTR,[136] and inclusion of the regulatory sequences are essential in determining the intracellular stability of the mRNAs.[164]

In vivo delivery of RNA-based drugs

Currently, there is an urgent requirement for innovative delivery systems to improve the *in vivo* efficacy of RNA therapeutics. In recent years, nanoparticle-based (NP) delivery systems have been developed to provide effective delivery of the loaded RNA cargo with improved protection. NP delivery systems also reduce drug-associated toxicity by minimizing drug accumulation in the off-target tissues and organs.[170] Furthermore, recent efforts have been directed toward developing NP systems with stimuli-responsive drug release, including those triggered by light, redox reactions, temperature, or pH.[171–173]

Lipid-based nanoparticles

Liposomes were the earliest lipid-based nano-delivery systems that were used for drug delivery. Hence, they are the most thoroughly studied and successfully developed nano-delivery systems. The advantages of liposomes for the delivery of genes or chemical drugs include the ease of preparation, low cost, and low toxicity. Liposomes are formed by the dispersion of polar lipids such as phospholipids (which contain polar head groups and nonpolar tails) in an aqueous phase. Surface-modified liposome-based particles are advantageous in delivering a variety of therapeutics. Doxil[174] is a liposome-encapsulated formulation of doxorubicin for the treatment of cancer with reduced cardiac toxicity. This breakthrough demonstrated the efficacy of nanoparticle-based drug delivery with altered biodistribution of the drug and increased safety.[175] Lipid-based nanoparticles (LNPs) approved by the FDA contain variations of the following four basic components: (1) cationic or ionizable lipids for electrostatic interactions with the RNA, (2) cholesterol for improving cell entry, (3) helper lipids such as phospholipids, and (4) polyethylene glycol (PEG) for improving stability and circulation time by preventing serum protein binding.[176] Scientists have investigated the structure of lipid-based

nucleic acid delivery systems and demonstrated that the lipid structure alters the interaction of the LNPs with cells, protects them from degradation, and prolongs their time of circulation. Onpattro (Patisiran) is an FDA-approved siRNA drug (2018, Alnylam Pharmaceuticals) that is encapsulated inside four lipid components, namely DSPC, cholesterol, DLin-MC3-DMA, and PEG2000-C-DMG.[98]

Recently, Alnylam, Moderna, and Pfizer/BioNTech/Acuitas developed LNPs for delivering mRNAs with four components: (1) cationic or ionizable lipids such as DLin-MC3-DMA (Alnylam), SM-102 (Moderna), or ALC-0315 (Pfizer/BioNTech/Acuitas); (2) cholesterol; (3) PEG-lipids such as PEG-2000-C-DMG (Alnylam), PEG-2000-DMG (Moderna), or ALC-0159 (Pfizer/BioNTech/Acuitas); and (4) DSPC.[177] Although most preclinical studies have shown that the structure of the cationic or ionizable lipid is the most important criterion for drug delivery, the other three components also influence drug delivery.[178] For example, an LNP-based siRNA delivery to the pulmonary and cardiovascular endothelial cells in mice was re-targeted to deliver siRNAs to the bone marrow by altering the cholesterol and PEG-lipid components.[179] The interaction of PEG and lipid components of the LNPs with the aqueous component in blood determined the interaction of LNPs with the cells.[180] Luo et al. incorporated the leukocyte membrane proteins into the surface of the liposomes to generate leukosomes, which could localize to the sites of inflammation, reduce the expression levels of pro-inflammatory genes (interleukin [IL]-6, IL-1b, and tumor necrosis factor [TNF]-α), and increase the expression levels of the anti-inflammatory genes (IL-10 and transforming growth factor [TGF]-β).[181] This is a promising strategy to generate novel mRNA therapeutics that selectively target sites of inflammation, but further investigations are necessary. To further enhance the capability of drug delivery at targeted sites, many ligands conjugating with lipid-based nanoparticles have also been employed, including small compounds, carbohydrates, peptides, proteins, and antibodies.[182,183] For example, Sato et al. used vitamin A-conjugated liposomes to deliver siRNA against heat-shock protein 47 (*HSP47*) for reversing human liver cirrhosis.[184] Another study has developed human epidermal growth factor (hEGF) or anti-HER2 Affibody as targeting moieties on the surface of liposomes exhibit better antitumor outcomes.[185]

Exosomes

Exosomes are nanosized extracellular vesicles with a diameter of 30–150 nm that are composed of DNA, RNA, lipids, cytosolic metabolites, and cell surface proteins.[186,187] Since exosomes originate in cellular secretion, they possess a natural ability in intercellular cargo delivery.[187,188] Once targeted to recipient cells, exosomes can transfer their genetic materials and molecules by endocytosis, receptor interaction, or cell membrane fusion, thus regulating cellular functions.[187,189] Similar to a lipid-based nanoparticle, exosomes have a special lipid bilayer structure with an aqueous core and a lipophilic shell.[189] Exosomes have high stability and longer circulation time *in vivo*, efficient cellular uptake, lower immunogenicity, and lower toxicity.[189] Therefore, the unique structure and physicochemical characteristics make the exosome a highly efficient and promising natural carrier for RNA delivery.[189] Li et al. applied arrowtail RNA nanoparticles to ginger-derived exosome-like nanovesicles (GDENs) with folic acid (FA) ligands on the surface for siRNA delivery, this system successfully inhibited tumor growth through intravenous administration.[190] Another recent study has established exosomes for RNA loading by constructing a fusion protein, CD9-HuR, which successfully delivers miR-155 to recipient cells.[191] Furthermore, the exosome can be applied to mRNA delivery as well. Tsai et al. demonstrated that exosomes can efficiently deliver mRNAs encoding immunogenic forms of the SARS-CoV-2 Spike and nucleocapsid proteins *in vitro* and *in vivo*, further supporting the development of this system as a novel vaccine and therapeutic approach.[192]

Polymer-based nanoparticles

Natural and artificial polymers can be used to construct nanoparticles. The polymer-based nanoparticles show high structural stability, drug encapsulation efficiency, cellular uptake, drug release rate, and ease of modification for the multifunctional and targeted delivery of RNA therapeutics.[193] The polymer properties, including charge, degradability, and molecular weight, can be varied to alter the RNA delivery into the cells.[177]

Polyethyleneimine and poly(L-lysine)

Cationic polymers with amine groups such as polyethyleneimine (PEI) and poly(L-lysine) (PLL) can complex with RNAs through electrostatic interactions and can be used to deliver drugs into cells.[177,194] However, chemical modifications of PEI and PLL are required to enhance their *in vivo* efficacy and tolerability. For example, nanoparticles generated with PEG-grafted PEI and cyclodextrin-PEI conjugates have been used for the *in vivo* delivery of mRNAs.[195,196]

Poly(beta-amino ester)s

Poly(beta-amino ester)s (PBAEs) are another class of cationic polymers that are synthesized by conjugating amine monomers to the diacrylates. PBAEs with cationic amines and ester bonds show better biodegradation and cytocompatibility compared to PEI and PLL.[197] PBAEs have been used for the intranasal delivery of mRNAs and for the siRNA delivery in a mouse orthotopic glioblastoma tumor model.[198] Furthermore, studies on lipid-polymer hybrids have shown that the serum stability and delivery are enhanced by the addition of lipids to PBAE.[199]

Dendrimers

Dendrimers are highly branched polymeric molecules with a 3D structure that is composed of multiple perfect branches radially emanating from a central core and includes multiple functional groups on the surface.[170] Organic and inorganic dendrimer-based nanoparticles have been widely studied and exhibit great potential in the targeted delivery of cargo, including drugs or chemicals, to specific cells.[170] The dendrimer structure has also been modified for protecting the nucleic acids from enzymatic degradation and improving endosomal escape. There are many distinct types of dendrimers that have been used for drug delivery. Here, we focus on the functions and

applications of poly(amidoamine) (PAMAM), poly(propylene imine) (PPI), and peptide dendrimers.

PAMAM dendrimers

PAMAM dendrimer is the most thoroughly studied dendrimer type with a 3D spherical structure.[200] PAMAM dendrimer consists of ammonia or ethylenediamine (EDA) core and multiple extended branch points. However, advances in synthetic chemistry have resulted in triethanolamine being frequently used as the core because it offers better loading of monomer molecules.[170] PAMAM dendrimers regulate drug release through a combination of complexation or conjugation. Complexation refers to the loading of drugs into the internal voids of the dendrimers or the gaps surrounded by multiple dendrimers through electrostatic interactions, hydrogen bonding, or hydrophobic interactions. Conjugation involves covalent binding of the drug molecules to the surface of the dendrimer through chemical bonds.[201] Modifications on the surface of the PAMAM dendrimers could improve drug targeting and reduce immunotoxicity. Lai et al. reported that the PAMAM-amide-DOX conjugate for cancer treatment showed slower drug release and reduced cytotoxicity compared to the free drug control at pH = 4.5.[201] Luong et al. reported the use of Fe_3O_4 nanoparticles decorated with folic acid-PAMAM for delivering an anticancer drug, 3,4-difluorobenzylidene-curcumin, in the treatment of ovarian and cervical cancers.[202] Amreddy et al. reported the use of FA and PEI-conjugated PAMAM nanocarriers loaded with *cis*-diamine platinum drugs and siRNAs for lung cancer treatment.[203]

Amphiphilic dendrimers (ADs) are a new type of PAMAM-based carriers with an affinity for the cell membrane lipids and with improved ability to bind to DNA in the cationic dendrimers.[204] Since the structure of the ADs is more flexible, the amino terminal and carbon chain of ADs can be easily modified. Therefore, modified ADs are widely used in the field of biomedicine. In the nucleic acid delivery process, the positively charged amino terminal of the ADs interacts with the negatively charged nucleic acid molecules through electrostatic interactions.[205] Yu et al. demonstrated that ADs synthesized with different alkyl tail lengths efficiently delivered siRNAs and produced significant gene-silencing effect.[204]

PPI dendrimers

PPI dendrimers were first synthesized by Buhleier and Vogtle.[206] PPI dendrimers are synthesized using a divergent approach that involved a two-reaction sequence consisting of repeated Michael addition reaction and amidation from the core to the propylenimine branching unites.[207] Typically, 1,4-butane diamine is used as the core of PPI dendrimers and complexing with the nucleic acid molecules reduces the cytotoxicity of the PPI dendrimers.[208] In order to improve the ability of drug targeting, the numerous groups on the surface of PPI can also be modified.[209] PPI is also used in the construction of nucleic acid biosensors.[210]

Peptide dendrimers

Peptide dendrimers are radial or wedge-like branched macromolecules that consist of a peptidyl branching core and/or covalently attached surface functional units.[211] Peptide dendrimers are widely used as biomedical diagnostic reagents, protein mimetics, anticancer and antiviral agents, vaccines, as well as drug and gene delivery vehicles.[212] Zhang et al. generated a mPEGylated peptide dendrimer-doxorubicin conjugate-based nanoparticle using an enzyme-responsive tetrapeptide linker sequence (Gly-Phe-Leu-Gly) as a drug delivery carrier and showed efficient inhibition of breast cancer growth in a mouse model.[213]

Cell-penetrating peptide

Cell-penetrating peptides (CPPs), consisting of 5–30 amino acid residues,[214] are another effective tool for the delivery of membrane-impermeable nucleic acids *in vitro* and *in vivo*.[215] Due to its cationic or amphipathic nature, it can transport nucleic acid into the cytoplasm via electrostatic interactions, resulting in the self-assembly of peptides with nucleic acids and the formation of nanosized complexes.[169,214] CPP delivery facilitates by direct cell membrane penetration or improves cellular uptake.[216] For example, Yang et al. successfully synthesized graphene oxide (GO)/PEI/PEG/CPP/siRNA targeting the Rictor system, which significantly suppressed tumorigenicity in triple-negative breast cancer (TNBC) cells.[217] Another study has described Lp-PPRP, which contains a cationic polymer and a palmitic acid-modified CPP (R8-PA) for a novel ASO delivery tool in tumor therapy.[218] Dastpeyman et al. found that a CPP, HA2-ApoE, used in combination with Nusinersen, an FDA-approved ASO drug for the treatment of SMA, exhibits efficient endosomal escape and cytoplasmic delivery capabilities and significantly improved blood-brain-barrier permeability and central-nervous-system activity of transgenic mouse models.[219]

Silica nanoparticles

Silica nanoparticles have gained considerable attention in drug-delivery applications because of good biocompatibility, ease of synthesis, and surface modifications.[220] There are three main types of silica nanoparticles, namely solid, nonporous, and mesoporous. The amorphous particle size, stability, porosity, and surface of the silica nanoparticles depend on the synthesis parameters.[221,222] Mesoporous silica nanoparticles consist of many empty pores or compartments like a honeycomb. Therefore, the surface of mesoporous silica nanoparticles can be modified by positively charged moieties to transport substantial amounts of negatively charged RNA. Silica nanoparticles are suitable for the controlled release of therapeutic agents because of their large surface area, pore volume, and high stability.[222,223] However, particle aggregation causes safety issues. Lee et al. developed a positively charged structure with a large pore to load anionic siRNAs against *Bcl-2* and a negatively charged structure with a small pore to load the anticancer drug doxorubicin; these dual-pore hybrid silica nanoparticles were generated to deliver a combination of genetic and chemotherapeutic drugs simultaneously and their efficacy was demonstrated in the HeLa cells *in vitro*.[224]

Carbon and gold nanomaterials

Gold nanoparticles, quantum dots, nanographene oxide, and carbon nanotubes are synthesized nanostructures that can harbor RNA

molecules, protect them from degradation, and deliver them to the targeted site.[62] Jayasekara et al. demonstrated the potential of AuNPs-AR agonist/antagonist conjugates as GPCR therapeutic targets and were associated with biological properties similar to their monomeric counterparts.[225] Xue et al. developed a novel siRNA delivery nanosystem with encapsulated siRNA and aptamer-incorporated core/shell (CS) gold nanoparticle (siRNA/Ap-CS).[226] This system exhibited high serum stability and long circulation time, and it significantly inhibited the growth of malignant tumor.[226] He et al. showed that siRNA conjugated with quantum dots (QDs) can be used to target genes by overcoming the cellular bilayer and deliver the QD-siRNA complex into cells.[227]

ADMINISTRATION ROUTES FOR RNA-BASED DRUGS

Due to the different physicochemical properties of RNA, the routes and sites of administration have a significant impact on the clinical efficacy and biodistribution to the RNA drugs.[128,228] Currently, there are two main clinical administration routes for RNA therapy: (1) local administered by intramuscular, intrathecal, intravitreal, or intradermal injections, etc.; or (2) systemic administration by intravenous, subcutaneous, or intraperitoneal injections.[228,229]

Local administration

RNA-based drugs treated by local administration normally exhibit therapeutic effect at the specific sites and could reduce the problems in terms of pharmacokinetic and RNA stability.[228] Intrathecal injection of RNA agents has been explored for the central nervous system (CNS) [230]; for example, Nusinersen is used for the treatment of SMA by intrathecal injection every 4–6 months.[231] Local administration has also become the preferred method of administration for ophthalmic treatment due to the complexity of the ocular surface.[228] For example, fomivirsen and pegaptanib are used to treat ocular diseases via direct intravitreal injections.[231] Furthermore, naked mRNA has been used to heal various skin diseases and to improve wound healing in the skin by subcutaneous or intradermal injection.[232] Local administration can also be used for siRNA drugs with liposome carriers.[106] For example, intratracheal administration of siRNA targeting *Plekhf1* with liposome carrier could effectively inhibit Plekhf1 expression levels in the lungs, and this strategy can be used to protect against pulmonary fibrosis disease.[233]

However, the effectiveness of local administration is limited by the low bioavailability of RNA therapeutics and the increased absorption time.[231] Therefore, systemic administration is the preferred route of administration for the treatment of many diseases, especially cancer, because it allows the drugs to reach both local and metastatic sites.[234]

Systemic administration

Systemic RNA delivery is more advanced and complicated compared to local administration.[228] Typically, a carrier is required to systematically deliver RNA drugs to prolong the half-life of the drugs. Currently, 50% of siRNA clinical trials have been conducted via systemic delivery, and all these siRNA-based therapies involve nanoparticle delivery systems.[234,235] siRNA-lipid nanoparticles are easily selectively concentrated in the liver due to the high apolipoprotein E (ApoE) content and resulting in significant hepatic specificity drug responses.[228] For example, Patisiran is used to inhibit hepatocyte-derived transthyretin by intravenous infusions every 3 weeks.[235] Melamed et al. demonstrated that intraperitoneal administration of mRNA delivered by lipid nanoparticles containing cationic helper lipids facilitate protein expression in pancreatic islets.[228,229] Furthermore, givosiran, lumasiran, inclisiran, and vutrisiran represent approved GalNAc-siRNA conjugate drugs, which are administered subcutaneously for acute liver porphyria, primary hyperoxaluria type 1, ASCVD, and transthyretin-mediated amyloidosis, respectively.[104] However, the development of naked RNA drug for systemic administration is hampered by RNA stability and rapid renal clearance considerations.[115] The only exception is QPI-1002, a naked siRNA targeting *p53* to treat acute kidney injury,[106] in which intravenous administration of QPI-1002 is used to target tubule cells with high renal siRNA concentration.[104]

APPLICATION OF THE RNA THERAPEUTIC PLATFORM IN TARGETING GPCRs

GPCRs are the most intensively studied therapeutic targets for a plethora of human diseases. However, it is difficult to develop drugs targeted to specific GPCRs because of their complex structure, presence of multiple subtypes, and common activation mechanisms between related GPCRs. RNA therapeutics has opened a new avenue of developing specific drugs targeting GPCRs for various human diseases.

The targeting of GPCRs is mainly focused on either directly modulating GPCR expression level or the downstream signaling molecules. The most widely used strategy for targeting GPCRs is siRNA therapy. Candidate oncogenes are highly expressed in tumors and play critical roles in tumor growth, metastasis, angiogenesis, and drug resistance. Cobalt (III) oxide nanoparticles (Co_3O_4NPs) were used to deliver *β-arrestin1* siRNAs into the 1321N1 cells; MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay showed that Co_3O_4NPs were non-toxic in this cell line.[236] G protein-coupled receptor 1 (OGR1 or GPR68) mediates contraction of the airway smooth muscle signaling in response to pH changes and contributes to asthma pathology; therefore, siRNAs against *OGR1* represent valuable therapeutics in obstructive lung diseases.[237,238] Ku et al. reported that *Gpr27* was a positive regulator of insulin production by using an MIN6-based siRNA screening system with four independent siRNAs targeting mouse-selected GPCR-related genes that were transfected into a reporter cell line.[239] This suggested that siRNA-based targeting of a specific GPCR can be used to modulate insulin production and represents a new avenue for treating diabetes.

Currently, there is only one report on the activation of GPCRs using saRNA. Xiong et al. reported that saRNAs delivered using an amphiphilic dendrimer vector significantly increased the expression of the MAS receptor (*MAS1*), a GPCR, and inhibited tumorigenesis of multiple cancers by suppressing the classical angiotensin II pathway.[240] This study was the first to use saRNAs to modulate GPCR signaling

in cancer therapy and presented a new methodology for effectively targeting GPCRs.

Chemokine receptor 4 coupled with the G protein (CXCR4) is specifically associated with cancer metastasis and HIV-1 infection. The CXCR4-CXCL12 axis plays a key role in orchestrating the recruitment of immune and stromal cells within the tumor microenvironment (TME), thereby influencing tumor cell growth and progression.[241] Therefore, many studies have focused on using RNA therapeutics to target the *CXCR4-CXCL12* axis. For example, Dong et al. showed that COL1A1-014 sponges miR-1273 h-5p and increases the levels of the CXCL12 and CXCR4 proteins, thereby promoting the proliferation and metastasis of gastric cancer cells.[242,243] Furthermore, overexpression of lncNORAD reduces the growth of NSCLC cells by suppressing the expression levels of CXCR4 and CXCL12.[244] Moreover, overexpression of miR-193a-5p reduces colorectal cancer (CRC) proliferation by suppressing CXCR4 expression levels. Therefore, miR-193a-5pin combination with 5-fluorouracil (5-FU) and oxaliplatin is postulated to be an effective therapy for CRC.[243,245] LncRNA FEZF1 antisense RNA 1 (FEZF1-AS1) induces proliferation of osteosarcoma cells and their resistance to apoptosis by inhibiting miR-144, which directly targets CXCR4.[243,246] NOX-A12 is a PEGylated 45-nucleotide L-form RNA aptamer (L-RNA aptamer; Spiegelmer is the trade name) and a new RNA drug under phase 2 clinical trial. It is designed to selectively target CXCL-12 with high affinity. It has shown efficacy in preventing the binding of CXCL-12 to its receptors, CXCR4/7, thereby inhibiting angiogenesis and metastasis and improving other anticancer therapies.[83,247]

Noncoding RNAs (ncRNAs) have been widely used to modulate GPCRs. CD97 is a pro-metastatic GPCR and a direct target of miR-126, and it has been shown to promote breast cancer cell invasion, migration, and angiogenesis.[248] MiR-138-5p enhanced gefitinib sensitivity of the NSCLC cells by regulating GPR124/ADGRA2.[249] EPI-2010 is a respirable ASO (RASON) that is used to target the adenosine A1 receptor and inhibit asthma. Low doses of RASONs can be administered to target respiratory tissues and have been shown to be safe and long acting.[250] Many RNA aptamers have been shown to bind and stabilize the β_2-adrenoceptor (β_2AR), which is a non-peptide ligand GPCR with inactive or ligand-specific conformation.[251] Aptamers function like neutralizing antibodies and block the interactions between ligands and their receptors. Anti-PD-L1 aptamers reduce tumor growth and improve immune surveillance by blocking the PD-1/PD-L1 signaling axis.[41]

OUTLOOK AND FUTURE PERSPECTIVES

RNA molecules are highly versatile therapeutics and have great potential for a wide range of medical conditions. The success of COVID-19 mRNA vaccines also demonstrated the immense potential of RNA-based technologies in the field of infectious disease prevention and in RNA therapeutics for a wide range of applications. More importantly, RNA drugs offer a promising solution for targeting "untagged GPCR," particularly orphan receptors. However, the challenge lies in our limited understanding of these orphan receptors. Hence, a concerted effort is needed to elucidate the physiological functions of these receptors before translating the RNA drug approach into therapeutic applications.

Precision targeting of specific GPCRs within subfamilies poses difficulties due to structural similarities among multiple subtypes. Classic small-molecule drugs raise concerns about cross-reactivity, as seen in chemokine and β-adrenergic receptor families. RNA drugs therefore present an excellent alternative, although achieving broad inhibition of multiple targets may prove challenging. However, controlling common downstream signaling molecules, if feasible, could overcome this obstacle.

The success of oliceridine, the first GPCR-biased agonist, highlights the potential to reduce side effects by preferentially activating the G protein pathway over the β-arrestin pathway GPCR.[252,253] This achievement poses a challenge for RNA drugs, mainly because most approaches involve regulating receptor expression and translation. But recent research on using the A62 agonist aptamer in activating insulin receptor[7] suggests the potential of using aptamer for selectively activating receptor-biased signaling of GPCRs.

RNA drugs, based on the delivery systems, could provide organ- or tissue-specific drug effects, crucial for cancer therapy. In addition, nanoparticle delivery systems enable RNA drugs to synergize with other therapeutic approaches. Combining multiple cell-killing strategies, such as chemotherapy and immunotherapy,[254] not only enhances drug responses but also mitigates potential drug resistance in cancer cells.

In addition, since genetic variations affect an individual's response to drugs, combining pharmacogenomics analysis and RNA therapeutics may provide innovative and personalized treatments to maximize efficacy and minimize potential adverse effects. In the future, by addressing patient-specific variability, RNA therapeutics could lead to more informed, precise, and tailored treatments that ultimately could optimize outcomes and improve quality of life for patients.

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AUTHOR CONTRIBUTIONS

Conceptualization/design, W.Y. and L.L.; original draft preparing and writing, W.Y.; preparation of tables and figures, W.Y.; manuscript reviewing, editing, and supervision, X.S. and L.L. All authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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