



Cite this: *Biomater. Sci.*, 2021, **9**,
6001

Harnessing lipid nanoparticles for efficient CRISPR delivery

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The CRISPR-Cas system has revolutionized the biomedical research field with its simple and flexible genome editing method. In October 2020, Emmanuelle Charpentier and Jennifer A. Doudna were awarded the 2020 Nobel Prize in chemistry in recognition of their outstanding contributions to the discovery of CRISPR-Cas9 genetic scissors, which allow scientists to alter DNA sequences with high precision. Recently, the first phase I clinical trials in cancer patients affirmed the safety and feasibility of *ex vivo* CRISPR-edited T cells. However, specific and effective CRISPR delivery *in vivo* remains challenging due to the multiple extracellular and intracellular barriers. Here, we discuss the recent advances in novel lipid nanomaterials for CRISPR delivery and describe relevant examples of potential therapeutics in cancers, genetic disorders, and infectious diseases.

Received 5th April 2021.

Accepted 25th May 2021

DOI: 10.1039/d1bm00537e

rsc.li/biomaterials-science

1. Introduction

Gene manipulation holds tremendous potential for curing genetic and infectious diseases by modifying DNA or RNA sequences of the living organism genome.¹ Since the discovery of targeted gene disruption and integration in eukaryotic yeast cells and mammalian cells in the 1980s, several genome targeting nucleases have been introduced over the years, including meganucleases, zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs).² While all three nucleases target specific DNA sequences *via* protein-DNA binding, targeting of the meganucleases and ZFNs requires protein engineering and TALENs require complex molecular cloning.^{3–6} The discovery of the clustered regularly interspaced short palindromic repeat (CRISPR)-Cas (CRISPR-associated) system revolutionized the gene-editing field: from extensive protein re-programming to simple guide RNA (gRNA) synthesis. The design flexibility and specificity of CRISPR greatly simplify the targeting process, leading to massive adoption of the CRISPR-Cas system in the biological field.^{7,8}

CRISPR was originally known as the immune defense mechanism for prokaryotes to resist virus invasions.⁹ The CRISPR-Cas immunity is developed by adding a new spacer, which is a small segment of the newly encountered virus genome, to the CRISPR locus. The CRISPR locus is then tran-

scribed and processed into CRISPR RNA (crRNA) containing a single spacer that specifically directs the Cas endonucleases to generate a double-stranded break (DSB) at the predetermined viral genome.¹⁰ The resulting DSB undergoes endogenous DNA repair pathways *via* non-homologous end joining (NHEJ) or homology-directed repair (HDR). Both pathways result in genome editing at the cleavage site: the predominant NHEJ pathway pastes together the two ends of the broken DNA, which often introduces insertion or deletion mutations, while HDR results in precise repair with the help of a donor sequence.¹¹ Therefore, the CRISPR-Cas system can be easily re-programmed to target specific genomes with the right gRNA. Due to the specificity and simplicity of the CRISPR system, it has been highly utilized in *in vitro* experiments for gene editing. However, the application of CRISPR technology for humans both *in vivo* and *ex vivo* remains controversial due to safety concerns related to potential off-target effects. In 2020, the results of the first-in-human clinical trial using the CRISPR engineered T cell to treat late-stage lung cancer showed few off-target genome editing events and no severe treatment-related adverse events, thus supporting its safety and feasibility for clinical use.¹²

Many Cas proteins are currently being investigated for gene editing. The widely used class 2 type II endonuclease Cas9 system can be reprogrammed by engineering a single guide RNA (sgRNA), which is the heterologous recombinant of crRNA and transactivating crRNA (tracrRNA).^{13,14} In recent years, many Cas proteins have been repurposed to assemble with gRNA to form effector complexes, resulting in precise sequence recognition and targeted cleavage. Among them, the DNA-targeting Cas12a and RNA-targeting Cas13a are being extensively studied as they only require one short crRNA for

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target editing.^{15,16} Furthermore, second-generation CRISPR, like base editing and prime editing, uses catalytically deficient Cas9 (dCas9) with functionally distinct DNA binding and encodes the desired edit, without inducing DNA double-stranded breaks.^{17–19} For example, the adenine base editor (ABE) enables point mutations of A-T to G-C more efficiently with fewer off-target editing events compared to Cas9 nuclease and does not require DSB, HDR, or donor DNA templates.¹⁷ With its programmable targeting ability, the versatile dCas9 offers other biological applications beyond gene editing, including gene regulation, epigenetic editing, and chromatin imaging and topology.²

While CRISPR technology has the potential to essentially edit any gene, delivering the CRISPR system into the target cell remains a barrier for CRISPR-based gene editing *in vivo*. For example, the Cas-gRNA ribonucleoprotein (RNP) complex may suffer from instability and poor intracellular viability due to its large size, varying surface charge, and fragile tertiary structures.²⁰ Furthermore, CRISPR-Cas gene editing depends on the complex of Cas nuclease and gRNA in the nucleus of the target cell. Thus, the appropriate dosage of all CRISPR components must be delivered to the target cells within the desired time frame.²¹ Early *in vivo* genome editing used viral vectors to deliver CRISPR genes for gene transduction through self-amplification, extrachromosomal amplification, or even host genome integration. However, viral-based delivery may prolong the presence of the RNP complex and may increase off-target mutations.²² To achieve safe delivery with high efficiency,

novel materials and delivery strategies are emerging in the biomaterial research community.²² Here, we discuss the recent advances in lipid nanomaterial-mediated CRISPR delivery with a focus on therapeutic genome-editing applications.

2. The CRISPR delivery system

CRISPR delivery can be divided into two categories: the CRISPR-Cas format and the delivery vehicle. The CRISPR-Cas system can be provided in the format of a plasmid DNA encoding both the Cas nuclease and gRNA; Cas mRNA and gRNA separately; or a Cas-gRNA ribonucleoprotein (RNP) complex (Fig. 1). The selection of delivery vehicles is largely dependent on the application and format of the CRISPR-Cas system.

2.1. The CRISPR-Cas format

To achieve gene editing, a functional RNP complex must be present in the cell nucleus. Direct RNP delivery has two major advantages: quick onset as it skips the transcription and translation processes, and transient expression that reduces off-target editing and related toxicity. However, the large size of the Cas nuclease and the heterogeneous charge of the RNP complex complicates the passage through both the cell and nuclear membranes.²¹ For example, *Streptococcus pyogenes* Cas9 (spCas9) nuclease is relatively large compared to common delivery cargos, with a molecular weight of around 158.3 kDa.²³ The excess positively charged residues of the Cas9



Fig. 1 Intracellular pathways for plasmid-, RNA-, and RNP-based CRISPR delivery.

nuclease and the negatively charged long phosphate backbone of the guide RNA make it difficult to cross multiple cellular membranes and reduce the stability of the RNP complexes.²⁴ Yet, the CRISPR-Cas system is highly flexible and has been adapted into plasmid- and RNA-based formats, allowing the target cell to produce its own RNP.²⁵

Plasmid-based delivery is currently the primary approach used to deliver CRISPR components under laboratory settings due to its stable nature and low production cost. The nucleus-targeted CRISPR-Cas system can be engineered by encoding gRNA sequences into a CRISPR plasmid or using separate plasmids for each component.¹⁴ Similar to RNP-based delivery, plasmids encoding the Cas-gRNA sequence must cross both the cell and nuclear membranes to exert their therapeutic effects. Once inside the nucleus, the plasmid replicates as part of the host genome and results in sustained expression of the Cas nuclease, which may lead to a higher chance of off-target effects and raises safety concerns.^{26,27}

The third option is to use mRNAs encoding the Cas protein sequence, which can be translated by ribosomes in the cytoplasm and then joined together with gRNA to form RNP.²⁸ Similar to RNP-based delivery, the transient nature of mRNA greatly reduces the off-target effect by limiting the number of Cas nucleases in the cell.²⁹ Furthermore, both mRNA and RNP delivery methods offer no risk of integrating the host genome with exogenous DNA. However, the complex manufacturing process and preservation of the biological activities of RNA and RNP restrict the widespread application of these delivery methods.²⁸ Recent progress has been made towards an engineered CRISPR-Cas system by either redesigning crRNA or modifying the Cas nuclease-encoded mRNA.^{30,31} crRNA can be engineered through structural alteration in the stem region and chemical modification in the linkage, ribose, and nitrogenous base. For example, 2'-O-methyl-3'-phosphonoacetate (MS) or 2'-O-methyl-3'-thioPACE (MSP) modification at specific sites in the crRNA significantly reduces the off-target effect and enhances the genome editing efficiency for both RNA- and RNP-based deliveries.^{32,33} Chemical modification of mRNA modulates the stability, expression, and biological activities of mRNAs in the cell.³⁴ Pseudouridine- (Ψ -), 5-methoxyuridine- (5moU-) and methylpseudouridine-modified ($me^1\Psi$ -) mRNAs have been reported to increase the mRNA half-life, improve protein expression, and reduce immunogenicity.³⁵⁻³⁷ Optimized chemical modifications of CRISPR-Cas mRNA and gRNA lead to further development of CRISPR-associated biological applications *in vivo*.

2.2. Current CRISPR delivery platforms

Three common approaches are used for the CRISPR-Cas system delivery: physical delivery, viral vector, and non-viral vector.³⁸ The common physical delivery is accompanied by cell membrane disruption: microinjection delivers a controlled dosage of the cargo to the intended cellular sites using a micron-level needle, while electroporation and hydrodynamic delivery manipulate external forces to disrupt the cellular membrane.²¹ Viral vectors, including adeno-associated virus

(AAV), adenovirus, and lentivirus-based vectors, are commonly used for CRISPR delivery due to their long-term editing efficacy and high versatility for *in vitro*, *ex vivo*, and *in vivo* applications.³⁹⁻⁴¹ However, virus-based vectors may provoke off-target effects that can trigger mutagenesis or carcinogenesis which questions the safety of repeated administration.⁴² Alternatively, a wide variety of non-viral vectors using organic or inorganic materials have been reported over the last decade as an emerging field of research. Common non-viral vectors include lipid- and polymer-based nanoparticles, DNA nanoclews, cell-penetrating peptides (CPPs), and inorganic nanoparticles.^{38,43} This article focuses on the recent advances in lipid-based nanoparticles (LNPs) for CRISPR delivery (Fig. 2).

LNPs are colloidal lipophilic systems composed of multiple components, like phospholipids, cholesterol, and polyethylene glycol (PEG).^{44,45} PEGylation cloaks the LNPs from the body's reticuloendothelial system and increases their colloidal stability, resulting in prolonged circulation time.⁴⁶ LNPs can be produced through various methods, such as the thin-film hydration method, the active loading method, or the recently developed microfluidic technology.^{47,48} LNPs have unique features that are beneficial for CRISPR delivery, including great biocompatibility, high loading efficiency, good stability, ease of modification and large-scale production.⁴⁹ However, concerns have been raised over the biodistribution and toxicity of LNPs, as well as their tendency to trigger immune responses *in vivo*.⁵⁰

To overcome the aforementioned concerns, several approaches were developed by modifying the lipid chemical structure and its pharmacological properties.⁵¹⁻⁵⁶ Zhang *et al.* developed and screened a library of functionalized N^1, N^3, N^5 -tris(2-aminoethyl)benzene-1,3,5-tricarboxamide derivatives (FTT) to achieve efficient liver delivery. Among them, FTT5-mediated delivery of mRNA encoding adenine base editors (ABEs) and sgRNA targeting *PCSK9* showed high genome editing efficiency in the mouse liver at a low dose of 0.125 mg kg⁻¹.⁵⁷ Cell-specific LNP delivery reduces toxicity and off-target effects by binding to the desired cell *via* targeting ligands, such as proteins, antibodies, aptamers, small molecules, and carbohydrates.⁵⁸ Targeted delivery can also be achieved through the engineering of multifunctional LNPs, which utilizes the physicochemical properties and lipid composition of LNPs to achieve different biodistributions.^{59,60} Sun *et al.* developed a pH-sensitive multifunctional amino-lipid, (1-aminoethyl)iminobis[*N*-(oleoylcysteinyl-1-aminoethyl)propionamide] (ECO), for plasmid- and RNA-based CRISPR delivery as ECO-derived lipids efficiently self-assemble with nucleic acids. Nucleic acids can form an electrostatic complex with the protonatable amino headgroup in ECO, conjugate with cysteinyl residues *via* disulfide bond cross-linking, and be further condensed by the hydrophobic lipid tails. The ECO lipid carriers induced minimal hemolysis at neutral pH, whereas the hemolytic activity increased significantly in an acidic environment.⁵⁹ Additionally, changes in lipid compositions and structures can also achieve different LNP biodistributions. For example, incorporation of additional cationic



Fig. 2 An illustration of the types of lipid-based nanoparticles (LNPs) for CRISPR delivery.

or anionic lipids can lead to selective organ targeting (SORT) in the lungs, spleen and liver following intravenous administration *in vivo*.⁶¹ More recently, Liu *et al.* synthesized a library of multi-tailed ionizable phospholipids (iPhos) for mRNA-based CRISPR delivery. By tinkering with the chemical structures of iPhos, they were able to alter *in vivo* editing efficiencies and enable organ selectivity.⁶²

Complex delivery systems that combine lipid materials with other carriers provide an attractive avenue to improve the specificity and efficiency of CRISPR delivery. One approach is to deliver each CRISPR component separately by taking advantage of different platforms. For example, in a mouse model of human hereditary tyrosinemia, mice were treated with LNP-encapsulated Cas9 mRNA to allow transient nuclease expression while AAV-encapsulated U6-sgRNA cassette and HDR repair template prolonged the expression of sgRNA. This combination corrected more than 6% fumarylacetoacetate hydrolase (*Fah*)-splicing mutation in the mouse liver.⁶³ Another approach is to fuse or coat the carrier with lipid materials to maximize the beneficial properties and specificity of both carriers. Hybrid nanoparticles that fuse exosomes and liposomes significantly increase an exosome's efficiency in encapsulating large CRISPR plasmids while maintaining their ability to cross stringent biological barriers.⁶⁴ Moreover, applying a positively charged lipid bilayer to colloiddally stable stellate mesoporous silica nanoparticles (MSN) results in rapid degradation into non-toxic silicic acid under physiological conditions, which makes it possible to accommodate CRISPR components for a degradation-mediated delivery.⁶⁵

3. Applications of LNP-mediated CRISPR delivery

3.1. Cancers

Cancers are caused by certain changes in the genome that affect normal cellular functions, especially proliferation and differentiation. CRISPR provides a novel anti-tumor strategy by targeting essential genes for survival or by correcting the genome errors in tumor cells. LNPs greatly facilitate the accumulation of the CRISPR system at the tumor site through multiple mechanisms, such as the enhanced permeability and retention (EPR) effect, surface modification with a targeting ligand, and pH/H₂O₂-responsive nanoparticles. This section focuses on LNP-based CRISPR therapy *via* direct tumor targeting and cancer immunotherapy (Table 1).

3.1.1. Direct tumor targeting. Direct tumor targeting directs the LNPs specifically to tumor cells, followed by the release of the CRISPR-Cas system that targets essential genes for survival and growth. The most straightforward strategy for locating tumor cells is to target tumor-specific extracellular proteins or other membrane-associated compositions. For example, R8-dGR is a modified cell-penetrating peptide that binds to the integrin $\alpha_v\beta_3$ and neuropilin-1 receptors, which are often overexpressed in various cancers.⁷⁰ Li *et al.* encapsulated paclitaxel and CRISPR plasmids encoding Cas9 nuclease and sgRNA targeting hypoxia-inducible factors-1 α (HIF-1 α), which is a transcriptional modulator of cellular responses to adapt to the oxygen-deficient tumor microenvironment, in R8-dGR-modified cationic liposomes (R8-dGR-lip). Systemic

Table 1 Examples of lipid-based CRISPR-Cas delivery systems for cancer treatments

		Delivery vehicle	Cell line	Cargo	Editing target	Model system	Ref.
Direct tumor targeting	Targeting genome	Cationic lipid-encapsulated TAT peptide-modified gold nanoparticles (LACP)	A375	Plasmid	<i>Plk-1</i>	<i>In situ</i> injection in xenograft mice model of human melanoma	66
		Ionizable amino lipid nanoparticle	GBM 005	mRNA	<i>Plk-1</i>	Intracerebral injection of LNPs into GBM 005 bearing mice	67
		Ionizable amino lipid nanoparticle with EGFR-targeting ligand	Human OV8	mRNA	<i>Plk-1</i>	Intraperitoneal injections of EGFR-targeted LNPs into ovarian mice model	67
	Targeting tumor cell	Poly(ethylene glycol)- <i>b</i> -poly(lactic acid- <i>co</i> -glycolic acid) (PEG-PLGA)-based cationic lipid-assisted polymeric nanoparticles (CLANs)	K562	Plasmid	<i>BCR-ABL</i> fusion	Intravenous injection of CLANs into chronic myeloid leukemia (CML) mice model	68
		Phenylboronic acid (PBA) derived lipid nanoparticles (PBA-BADP LNPs)	HeLa	mRNA	<i>HPV18E6</i>	HeLa cell culture	69
Cancer immunotherapy		R8-dGR modified cationic liposome (R8-dGR-lip)	BxPC-3	Plasmid	Hypoxia-inducible factor-1 α (<i>HIF-1α</i>)	Intravenous injections of R8-dGR-lip into pancreatic cancer xenograft and metastasis mouse model	70
		Virus-like nanoparticles (VLN) co-delivering CRISPR-Cas9 system with small molecule drugs	B16F10	RNP	<i>PD-L1</i>	Intravenous injection into B16F10 melanoma xenograft mouse model	71

administration of R8-dGR-lip encapsulating Cas9/*sgHIF-1 α* plasmids markedly enhanced the cellular uptake and downregulated HIF-1 α expression in pancreatic BxPC-3 tumor cells.⁷⁰ In addition, they showed that the HIF-1 α blockade could work synergistically with paclitaxel in inhibiting tumor growth and suppressing pancreatic cancer metastasis.⁷⁰ In another study, phenylboronic acid (PBA)-derived LNPs significantly enhanced the cellular uptake of Cas9 mRNA/sgRNA in cancer cells overexpressing sialic acid (SA) through surface PBA/SA interactions.⁶⁹ PBA LNP-mediated Cas9 mRNA/*sgHPV18E6* delivery knocks out the *HPV18E6* gene, which functions as an oncogene by inducing p53 degradation, thus effectively inhibiting HeLa cervical cancer cell growth and reducing cell viability by 50% *in vitro*.⁶⁹

Targeting an oncogene is also a commonly used strategy to limit toxicity within the tumor. Most chronic myeloid leukemia (CML) is caused by a chromosomal translocation that fuses the breakpoint cluster region (*BCR*) gene with the Abelson murine leukemia viral oncogene homolog (*ABL*) gene, which leads to the overproduction of tyrosine kinase in abnormal blood cells.⁷² Liu *et al.* designed a lipid-assisted polymeric CRISPR delivery system to specifically target the overhangs of *BCR-ABL* fusion without interfering with the *BCR* and *ABL* genes in the normal cells. Intravenous injection of the Cas9/*sgBCR-ABL* plasmid encapsulated in PEG-PLGA-based LNPs significantly improved CML symptoms with minimal off-target effects and increased the overall survival rate of CML mice.⁶⁸ Also, the mitotic protein kinase Polo-like kinase 1 (*Plk-1*) is often overexpressed in tumor cells; thus targeting the *Plk-1* gene demonstrates great anti-tumor potency.⁷³ Wang *et al.* condensed the Cas9/*sgPlk-1* plasmid (CP) into nucleus-targeting TAT peptide-modified gold nanoparticles (AuNPs). The resulting AuNPs/CP were further coated with lipids (DOTAP, DOPE, cholesterol, and PEG) to maintain high stability of the inner core and

facilitate tumor cell internalization. After entering the tumor cell, AuNPs are subjected to laser-triggered photothermal treatments and release the Cas9/*sgPlk-1* plasmid in the cytosol, thereby enabling efficient *Plk-1* knockout in melanoma tumor cells and inhibiting tumor growth.⁶⁶ Most recently, Rosenblum *et al.* demonstrated efficient orthotopic glioblastoma inhibition with a single intracerebral injection of amino-ionizable LNPs encapsulating Cas9 mRNA and sgRNAs targeting the *Plk-1* gene (*sgPlk-1*-cLNPs).⁶⁷ To reach disseminated ovarian tumors, they further engineered the *sgPlk-1*-cLNPs with EGFR-targeting antibodies. Intraperitoneal injections of EGFR-targeted *sgPlk-1*-cLNPs selectively delivered into disseminated ovarian tumors, which resulted in ~80% of gene editing *in vivo*, inhibited tumor growth and increased the overall survival rate by 80%.⁶⁷ Although significant progress has been made in utilizing the CRISPR system to directly modify cancer cells, the mutagenic and evolving nature of cancer cells may become resistant to such strategies. Thus, additional efforts are needed to ensure lasting, safe, and efficient editing to combat cancer.

3.1.2. Cancer immunotherapy. Most cancers can be recognized and attacked by the body's immune system, but the immune suppressive nature of the tumor microenvironment restricts the endogenous anti-tumor activities. Delivery of the CRISPR system has broad prospects in cancer immunotherapy by engineering therapeutic immune cells and the tumor microenvironment.^{74,75} Immune checkpoint blockade disrupts the negative immune regulatory signals and reactivates the immune response against tumors. Immune checkpoint inhibitors toward programmed cell death 1 (PD-1) and cytotoxic T lymphocyte antigen 4 (CTLA-4) on T cells have shown early success in the clinic.⁷⁶ Recently, the first-in-human clinical trial reported that CRISPR-Cas9 knocked out PD-1 in T cells with limited off-target effects, and extended the median survi-

val time in non-small cell lung cancer patients.¹² Besides editing T cells, attenuated programmed death-ligand 1 (PD-L1) expression on tumor cells can also disturb the PD-1/PD-L1 pathway.⁷⁷ Liu *et al.* designed a virus-like nanoparticle (VLN) to regulate multiple cancer-associated pathways by delivering the CRISPR-Cas system with small molecule drugs. After loading Cas9/sgPD-L1 RNP and axitinib in mesoporous silica nanoparticles (MSN), a lipid layer composed of DOTAP, DOPC, and PEG₂₀₀₀-DSPE was formed on the surface to extend the circulation time and protect RNP from enzymatic degradation. CRISPR-mediated PD-L1 knockout in tumor cells reversed the “cold” tumor immunity and enhanced the anti-tumor efficacy of axitinib.⁷¹

The aforementioned clinical and preclinical studies demonstrate the potential therapeutic applications of lipid nanoparticles in overcoming CRISPR delivery limitations and developing long-lasting gene editing efficiency in cancer treatments. However, the high recurrence rate and the emergence of drug resistance in most types of cancer have prompted the continuous development of new treatment modalities.

3.2. Genetic disorders

CRISPR technology also holds clinical potential for curing many genetic disorders besides cancers, as a change in a single nucleotide may reverse the disease-causing situation. Recently, two patients were treated for sickle-cell anemia and β -thalassemia by deleting the *BCL11A* gene in the stem cell using CRISPR-Cas9 technology. Initial results showed long-term allelic editing in hematopoietic stem cells, increased fetal hemoglobin expression, and elimination of vaso-occlusive episodes.⁷⁸ Gene-editing procedures have the potential to overcome the barriers related to traditional treatment such as stem cell or bone marrow transplant, namely donor availability and compatibility. Treatments for most genetic disorders include correcting the disease-associated mutation and inactivating deleterious protein expression (Table 2).

Correcting the disease-associated mutation is the most straightforward treatment for genetic disorders. For example, an out-of-frame deletion mutation in the Duchenne muscular dystrophy (*DMD*) gene alters the structure of the dystrophin protein, which is primarily located in the skeletal and heart muscles for movement. *DMD* is a severe and fatal monogenic disease as patients suffer from breathing complications and cardiomyopathy.⁸⁷ CRISPR technology provides possibilities to permanently remove the disease-causing mutation, thereby restoring dystrophin expression and rescuing muscle functions.^{79,88,89} For this purpose, sgRNA has been designed to restore the reading frame by deletion of *DMD* exons. Incorporation of 10% (molar ratio) permanent cationic lipid DOTAP into the 5A2-SC8 ionizable LNPs formulation (5A2-DOT-10 LNPs) promotes the encapsulation of Cas9/sg*DMD* RNP in a neutral buffer. Mice with *DMD* exon 44 deletion, which disrupts the dystrophin reading frame through splicing of exons 43 and 45, were intramuscularly injected with 5A2-DOT-10 LNPs encapsulating Cas9/sg*DMD* RNP to allow splicing between exons 43 and 46. The treated mice showed a restored

dystrophin reading frame as well as increased *DMD* protein expression by 4.2%.⁷⁹ More recently, CRISPR-editing was applied to treat hereditary deafness by disrupting the transmembrane channel-like gene family 1 (*Tmc1*), which is the major deafness-associated allele in the Beethoven (*Bth*) mouse model. Cas9/sg*Tmc1*^{Bth} RNPs were mixed with cationic lipids to form nanocomplexes and intracochlearly injected into neonatal *Tmc1*^{Bth/+} mice, which induced up to 10% of *Tmc1*^{Bth} allele disruption *in vivo* and significantly reduced progressive hearing loss.⁸⁰

Inactivating deleterious protein expression has also been investigated. Hypercholesterolemia has been widely investigated in the last decade due to the discovery of proprotein convertase subtilisin/kexin type 9 (PCSK9). Disrupting the function of PCSK9 helps maintain low cholesterol levels and reduces the risk of atherosclerotic cardiovascular disease.⁹⁰ *In vivo* CRISPR editing presents a possible approach for the therapeutic antagonism of PCSK9.^{79,82,91} Zhang *et al.* developed galactose-modified lipid encapsulated gold nanoclusters complexed with Cas9/sgPCSK9 RNPs to target the asialoglycoprotein receptor (ASGPR) on hepatocytes. Compared with PEG-coated nanoclusters, the galactose-lipid layer significantly increased RNP delivery to the liver by eight-fold *in vivo* and resulted in ~30% cholesterol reduction.⁸¹ Besides RNP, Cas9 mRNA/sgPCSK9 delivered by BAMEA-O16B bioreducible LNPs was shown to accumulate in the mouse liver after systemic administration and reduce serum PCSK9 levels down to 20% of the untreated group. The ionizable head in BAMEA-O16B facilitated RNA encapsulation, while glutathione (GSH)-induced disulfide bond degradation triggers the release of the RNAs intracellularly.⁸² The optimized cationic lipid-assisted PEG-*b*-PLGA nanoparticle (CLAN) has also been used to treat multiple genetic disorders. CLAN represents a safe, effective, and controllable platform to protect nucleic acids, overcome delivery barriers, and increase the encapsulation efficiency.⁹² For example, the CLAN targeting B-cell activating factor receptor (*BAFFR*) genome (CLAN_{Cas9/sgBAFFR}) alleviates rheumatoid arthritis by downregulating the number of B cells.⁸⁵ In a separate study, CLAN_{pM330/sgNtn1} inhibited macrophage netrin-1 expression in Type 2 Diabetes (T2D) mice using the Cas9/sg*Ntn1* plasmid driven by the macrophage-specific CD68 promoter, which subsequently improved glucose tolerance and insulin sensitivity.⁸³

Although in its infancy, CRISPR-based genome editing holds tremendous potential for tackling a number of genetic disorders that are unattainable by traditional therapies. With an efficient and safe delivery method, the accessible and affordable CRISPR-based therapy will undoubtedly be a game-changer in clinical applications.

3.3. Infectious diseases

The sequence-specific targeting capability of CRISPR-Cas allows it to easily recognize and eliminate foreign genetic elements. The CRISPR-Cas machinery can be easily repurposed into an offense against specific viruses or bacteria by directing the sgRNA to essential chromosomal genes. The major form of

Table 2 Examples of lipid-based CRISPR-Cas delivery systems for genetic diseases

	Disease	Delivery vehicle	Cargo	Editing target	Model system	Ref.
Correcting the disease-associated mutation	Duchenne Muscular Dystrophy (DMD)	5A2-DOT-10 lipid nanoparticles	RNP	Dystrophin gene	Intramuscular injection into <i>DMD</i> exon 44 deletion mice	79
	Hearing loss	Cas9 : guide RNA : lipid complexes	RNP	<i>Tmc1^{Bth}</i> allele	In cochlea injection into neonatal <i>Tmc1^{Bth/+}</i> mice	80
Inactivating deleterious protein expression	Hypercholesterolemia	Galactose-modified lipid-coated gold nanoclusters (Gal-LGCP)	RNP	Serine protease proprotein convertase subtilisin/kexin type 9 (<i>PCSK9</i>)	Intravenous injection into C57BL/6 mice	81
		5A2-DOT-5 lipid nanoparticles	RNP	Serine protease proprotein convertase subtilisin/kexin type 9 (<i>PCSK9</i>)	Intravenous injection into C57BL/6 mice	79
		BAMEA-O16B bio-reducible lipid nanoparticles	mRNA	Serine protease proprotein convertase subtilisin/kexin type 9 (<i>PCSK9</i>)	Intravenous injection into C57BL/6 mice	82
	Type 2 Diabetes Mellitus (T2DM)	Functionalized TT derivatives (TT3 and FTT5) lipid nanoparticles	mRNA	Serine protease proprotein convertase subtilisin/kexin type 9 (<i>PCSK9</i>)	Intravenous injection into BALB/c mice	57
		Cationic lipid-assisted PEG- <i>b</i> -PLGA nanoparticles (CLAN)	Plasmid	Netrin-1 gene (<i>Ntn1</i>)	Intravenous injection into T2D mice	83
		Lecithin-based liposomal nanocarrier particle (NL)	RNP	Dipeptidyl peptidase-4 gene (<i>DPP-4</i>)	Intravenous injection into type 2 diabetes mellitus (T2DM) <i>db/db</i> mice	84
Rheumatoid Arthritis (RA)	Cationic lipid-assisted nanoparticle (CLAN) system	Plasmid	B-cell activating factor receptor gene (<i>BAFFR</i>)	Intravenous injection into DBA/1 mice	85	
Transthyretin amyloidosis (ATTR)	Biodegradable, ionizable lipid nanoparticles (LNP-INT01)	mRNA	Transthyretin (<i>Ttr</i>)	Lateral tail vein injection in CD-1 mice	86	

the viral genome in hepatitis B virus (HBV)-infected hepatocytes is the HBV covalently closed circular DNA (*cccDNA*). Using optimized N^1, N^3, N^5 -tris(2-aminoethyl)benzene-1,3,5-tricarboxamide-3 (TT3) derived lipid-like nanoparticles (LLNs), Jiang *et al.* demonstrated robust Cas9 protein expression in mouse liver 6 h post tail vein injection of Cas9 mRNA encapsulated in TT3 LLNs. TT3 LLNs efficiently diminished HBV protein production by targeting the HBV *cccDNA* in the established HBV mouse model.⁹¹ CRISPR-Cas based gene-editing has also been applied to disrupt essential entry co-receptors of the human immunodeficiency virus (HIV), like chemokine receptor type 5 (CCR5) and C-X-C chemokine receptor type 4 (CXCR4) for the host-targeting antiviral approach.^{88,93} In 2019, a global pandemic erupted due to the rise of a sudden yet highly disruptive virus, the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). To combat this pandemic, Abbott and co-workers developed an RNA-guided RNA-targeting Cas13d PAC-MAN (prophylactic antiviral CRISPR in human cells) system that recognizes and degrades highly conserved RNA sequences from SARS-Cov2 in respiratory epithelial cells. While PAC-MAN identified six crRNAs that can target more than 90% of sequenced coronaviruses, an effective and safe delivery method is needed.⁹⁴ It has been reported that Cas13d and its cognate crRNA can be delivered *in vivo* using polymer or lipid nanoparticles.^{23,95,96}

Antimicrobial-resistant (AMR) bacteria is a worldwide public health challenge that threatens the effective prevention and treatment of bacterial infections. Various LNPs have been developed to encapsulate antibacterial peptides or small molecule drugs for systemic or topical antimicrobial treatments.^{97–99} CRISPR-based antibacterials represent a novel and programmable platform to attack disease-causing bacteria by designing gRNAs to target essential genes for pathogen virulence or survival.¹⁰⁰ CLANs encapsulating Cas9 mRNA/sgRNA targeting *NLRP3* (CLAN_{mCas9/sgNLRP3}) inhibited NLRP3 inflammasome activation in macrophages, and subsequently mitigated lipopolysaccharide (LPS)-induced septic shock.¹⁰¹ Besides lipid-based delivery of the CRISPR system, polymer-based nanoparticles have been developed for antimicrobial treatment.^{102,103} Recently, covalent binding of Cas9 nuclease to cationic polymer was utilized for delivery into methicillin-resistant *Staphylococcus aureus* (MRSA). Subsequent complexation of the nanoparticle with sgRNAs targeting *mecA*, a major gene in methicillin resistance, significantly reduced the MRSA growth rate in the presence of oxacillin by 1/5th of the control group.¹⁰⁴

Overall, CRISPR technology demonstrates great potential for treating numerous infectious diseases. Additional research needs to be conducted to be better prepared for future pandemics and the rise of antibiotic-resistant bacteria.

4. Conclusion and perspective

The CRISPR platform represents an unprecedented leap in the field of novel gene-editing technologies and treatments for various diseases, with simplified target design and higher editing efficiency compared to previous genome-editing strategies. Here, we summarized the recent progress in lipid nanoparticle-assisted CRISPR delivery in the treatment of cancers, genetic disorders, and infectious diseases. Despite the great potential for the translation of CRISPR into clinical applications in the near future, off-target editing remains a major concern. Newly evolved CRISPR systems, such as base editors, can introduce point mutations more efficiently and have fewer off-target editing events. Therefore, improving delivery specificity is needed to accommodate these emerging technologies for safe and effective delivery *in vivo*. The versatile lipid-based nanoparticles can be modified for specific-cell/tissue targeting. For example, LNPs not only accumulate in the tumor tissue through the EPR effect, but can also target *via* appropriate surface modification with a targeting ligand or pH/hydrogen peroxide-responsive lipids. Furthermore, LNPs are capable of delivering all forms of CRISPR, including plasmids, mRNAs, and RNPs, circumventing immune system surveillance and avoiding blood protease degradation. In spite of LNPs' outstanding performance in delivering the CRISPR system, more research needs to be conducted regarding the manufacturing complications and *in vivo* biodistribution, toxicity, and immunogenicity. Given the fact that the CRISPR system is a powerful and promising tool for gene editing, we envision that the discovery of novel effective delivery platforms is of great importance for bringing advanced CRISPR therapeutics to the clinic.

Conflicts of interest

Y. D. is a scientific advisory board member of Oncorus Inc and serves as a consultant to Rubius Therapeutics. The authors have no competing interest to declare.

Acknowledgements

Y. D. acknowledges the support from the NIH through the National Heart, Lung, and Blood Institute (R01HL136652), as well as the start-up fund from the College of Pharmacy at The Ohio State University. The authors acknowledge that figures were created using biorender.com.

References

- 1 D. B. T. Cox, R. J. Platt and F. Zhang, *Nat. Med.*, 2015, **21**, 121–131.
- 2 M. Adli, *Nat. Commun.*, 2018, **9**, 1911.
- 3 A. Thierry and B. Dujon, *Nucleic Acids Res.*, 1992, **20**, 5625–5631.
- 4 Y. G. Kim, J. Cha and S. Chandrasegaran, *Proc. Natl. Acad. Sci. U. S. A.*, 1996, **93**, 1156–1160.
- 5 M. Bibikova, M. Golic, K. G. Golic and D. Carroll, *Genetics*, 2002, **161**, 1169–1175.
- 6 M. Christian, T. Cermak, E. L. Doyle, C. Schmidt, F. Zhang, A. Hummel, A. J. Bogdanove and D. F. Voytas, *Genetics*, 2010, **186**, 757–761.
- 7 S. H. Sternberg and J. A. Doudna, *Mol. Cell*, 2015, **58**, 568–574.
- 8 G. Gasiunas, R. Barrangou, P. Horvath and V. Siksnys, *Proc. Natl. Acad. Sci. U. S. A.*, 2012, **109**, E2579–E2586.
- 9 E. V. Koonin, K. S. Makarova and F. Zhang, *Curr. Opin. Microbiol.*, 2017, **37**, 67–78.
- 10 L. A. Marraffini, *Nature*, 2015, **526**, 55–61.
- 11 K. S. Pawelczak, N. S. Gavande, P. S. VanderVere-Carozza and J. J. Turchi, *ACS Chem. Biol.*, 2018, **13**, 389–396.
- 12 Y. Lu, J. Xue, T. Deng, X. Zhou, K. Yu, L. Deng, M. Huang, X. Yi, M. Liang, Y. Wang, H. Shen, R. Tong, W. Wang, L. Li, J. Song, J. Li, X. Su, Z. Ding, Y. Gong, J. Zhu, Y. Wang, B. Zou, Y. Zhang, Y. Li, L. Zhou, Y. Liu, M. Yu, Y. Wang, X. Zhang, L. Yin, X. Xia, Y. Zeng, Q. Zhou, B. Ying, C. Chen, Y. Wei, W. Li and T. Mok, *Nat. Med.*, 2020, **26**, 732–740.
- 13 M. Jinek, K. Chylinski, I. Fonfara, M. Hauer, J. A. Doudna and E. Charpentier, *Science*, 2012, **337**, 816–821.
- 14 L. Cong, F. A. Ran, D. Cox, S. Lin, R. Barretto, N. Habib, P. D. Hsu, X. Wu, W. Jiang, L. A. Marraffini and F. Zhang, *Science*, 2013, **339**, 819–823.
- 15 B. Paul and G. Montoya, *Biomed. J.*, 2020, **43**, 8–17.
- 16 G. J. Knott and J. A. Doudna, *Science*, 2018, **361**, 866–869.
- 17 N. M. Gaudelli, A. C. Komor, H. A. Rees, M. S. Packer, A. H. Badran, D. I. Bryson and D. R. Liu, *Nature*, 2017, **551**, 464–471.
- 18 A. V. Anzalone, P. B. Randolph, J. R. Davis, A. A. Sousa, L. W. Koblan, J. M. Levy, P. J. Chen, C. Wilson, G. A. Newby, A. Raguram and D. R. Liu, *Nature*, 2019, **576**, 149–157.
- 19 J. E. Dahlman, O. O. Abudayyeh, J. Joung, J. S. Gootenberg, F. Zhang and S. Konermann, *Nat. Biotechnol.*, 2015, **33**, 1159–1161.
- 20 Z. Gu, A. Biswas, M. Zhao and Y. Tang, *Chem. Soc. Rev.*, 2011, **40**, 3638–3655.
- 21 Z. Glass, M. Lee, Y. Li and Q. Xu, *Trends Biotechnol.*, 2018, **36**, 173–185.
- 22 S. Tong, B. Moyo, C. M. Lee, K. Leong and G. Bao, *Nat. Rev. Mater.*, 2019, **4**, 726–737.
- 23 C.-F. Xu, G.-J. Chen, Y.-L. Luo, Y. Zhang, G. Zhao, Z.-D. Lu, A. Czarna, Z. Gu and J. Wang, *Adv. Drug Delivery Rev.*, 2019, **168**, 3–29.
- 24 D. N. Nguyen, T. L. Roth, P. J. Li, P. A. Chen, R. Apathy, M. R. Mamedov, L. T. Vo, V. R. Tobin, D. Goodman, E. Shifrut, J. A. Bluestone, J. M. Puck, F. C. Szoka and A. Marson, *Nat. Biotechnol.*, 2020, **38**, 44–49.
- 25 L. Li, S. Hu and X. Chen, *Biomaterials*, 2018, **171**, 207–218.

- 26 P. Mali, L. Yang, K. M. Esvelt, J. Aach, M. Guell, J. E. DiCarlo, J. E. Norville and G. M. Church, *Science*, 2013, **339**, 823–826.
- 27 M. Jinek, A. East, A. Cheng, S. Lin, E. Ma and J. Doudna, *eLife*, 2013, **2**, e00471.
- 28 P. S. Kowalski, A. Rudra, L. Miao and D. G. Anderson, *Mol. Ther.*, 2019, **27**, 710–728.
- 29 J. Eoh and L. Gu, *Biomater. Sci.*, 2019, **7**, 1240–1261.
- 30 B. Li, C. Zeng and Y. Dong, *Nat. Protoc.*, 2018, **13**, 899–914.
- 31 T. Jiang, J. M. Henderson, K. Coote, Y. Cheng, H. C. Valley, X.-O. Zhang, Q. Wang, L. H. Rhym, Y. Cao, G. A. Newby, H. Bihler, M. Mense, Z. Weng, D. G. Anderson, A. P. McCaffrey, D. R. Liu and W. Xue, *Nat. Commun.*, 2020, **11**, 1979.
- 32 A. Hendel, R. O. Bak, J. T. Clark, A. B. Kennedy, D. E. Ryan, S. Roy, I. Steinfeld, B. D. Lunstad, R. J. Kaiser, A. B. Wilkens, R. Bacchetta, A. Tsalenko, D. Dellinger, L. Bruhn and M. H. Porteus, *Nat. Biotechnol.*, 2015, **33**, 985–989.
- 33 D. E. Ryan, D. Taussig, I. Steinfeld, S. M. Phadnis, B. D. Lunstad, M. Singh, X. Vuong, K. D. Okochi, R. McCaffrey, M. Olesiak, S. Roy, C. W. Yung, B. Curry, J. R. Sampson, L. Bruhn and D. J. Dellinger, *Nucleic Acids Res.*, 2018, **46**, 792–803.
- 34 J. Song and C. Yi, *ACS Chem. Biol.*, 2017, **12**, 316–325.
- 35 B. Li, X. Luo and Y. Dong, *Bioconjugate Chem.*, 2016, **27**, 849–853.
- 36 B. Li, W. Zhao, X. Luo, X. Zhang, C. Li, C. Zeng and Y. Dong, *Nat. Biomed. Eng.*, 2017, **1**, 1–10.
- 37 E. Robinson, K. D. MacDonald, K. Slaughter, M. McKinney, S. Patel, C. Sun and G. Sahay, *Mol. Ther.*, 2018, **26**, 2034–2046.
- 38 C. A. Lino, J. C. Harper, J. P. Carney and J. A. Timlin, *Drug Delivery*, 2018, **25**, 1234–1257.
- 39 K. Lundstrom, *Diseases*, 2018, **6**, 42.
- 40 H. C. Verdera, K. Kuranda and F. Mingozzi, *Mol. Ther.*, 2020, **28**, 723–746.
- 41 C. Li and R. J. Samulski, *Nat. Rev. Genet.*, 2020, **21**, 255–272.
- 42 J.-C. Nault, S. Datta, S. Imbeaud, A. Franconi, M. Mallet, G. Couchy, E. Letouzé, C. Pilati, B. Verret, J.-F. Blanc, C. Balabaud, J. Calderaro, A. Laurent, M. Letexier, P. Bioulac-Sage, F. Calvo and J. Zucman-Rossi, *Nat. Genet.*, 2015, **47**, 1187–1193.
- 43 Y.-X. Lin, Y. Wang, S. Blake, M. Yu, L. Mei, H. Wang and J. Shi, *Theranostics*, 2020, **10**, 281–299.
- 44 C. Desfrancois, R. Auzély and I. Texier, *Pharmaceuticals*, 2018, **11**, 118.
- 45 S. Patel, N. Ashwanikumar, E. Robinson, Y. Xia, C. Mihai, J. P. Griffith, S. Hou, A. A. Esposito, T. Ketova, K. Welsher, J. L. Joyal, Ö. Almarsson and G. Sahay, *Nat. Commun.*, 2020, **11**, 983.
- 46 J. V. Jokerst, T. Lobovkina, R. N. Zare and S. S. Gambhir, *Nanomedicine*, 2011, **6**, 715–728.
- 47 C. Chan, S. Du, Y. Dong and X. Cheng, *Curr. Top. Med. Chem.*, 2021, **21**, 92–114.
- 48 J. A. Kulkarni, D. Witzigmann, J. Leung, R. van der Meel, J. Zaifman, M. M. Darjuan, H. M. Grisch-Chan, B. Thöny, Y. Y. C. Tam and P. R. Cullis, *Nanoscale*, 2019, **11**, 9023–9031.
- 49 H. Yin, R. L. Kanasty, A. A. Eltoukhy, A. J. Vegas, J. R. Dorkin and D. G. Anderson, *Nat. Rev. Genet.*, 2014, **15**, 541–555.
- 50 K. A. Whitehead, R. Langer and D. G. Anderson, *Nat. Rev. Drug Discovery*, 2009, **8**, 129–138.
- 51 M.-S. Martina, V. Nicolas, C. Wilhelm, C. Ménager, G. Barratt and S. Lesieur, *Biomaterials*, 2007, **28**, 4143–4153.
- 52 E. S. Hosseini, M. Nikkhah and S. Hosseinkhani, *Int. J. Nanomed.*, 2019, **14**, 4353–4366.
- 53 K. A. Hajj, R. L. Ball, S. B. Deluty, S. R. Singh, D. Strelkova, C. M. Knapp and K. A. Whitehead, *Small*, 2019, **15**, e1805097.
- 54 J. B. Miller, S. Zhang, P. Kos, H. Xiong, K. Zhou, S. S. Perelman, H. Zhu and D. J. Siegwart, *Angew. Chem., Int. Ed.*, 2017, **56**, 1059–1063.
- 55 Y. Li, J. Bolinger, Y. Yu, Z. Glass, N. Shi, L. Yang, M. Wang and Q. Xu, *Biomater. Sci.*, 2019, **7**, 596–606.
- 56 K. A. Hajj, J. R. Melamed, N. Chaudhary, N. G. Lamson, R. L. Ball, S. S. Yerneni and K. A. Whitehead, *Nano Lett.*, 2020, **20**, 5167–5175.
- 57 X. Zhang, W. Zhao, G. N. Nguyen, C. Zhang, C. Zeng, J. Yan, S. Du, X. Hou, W. Li, J. Jiang, B. Deng, D. W. McComb, R. Dorkin, A. Shah, L. Barrera, F. Gregoire, M. Singh, D. Chen, D. E. Sabatino and Y. Dong, *Sci. Adv.*, 2020, **6**, eabc2315.
- 58 M. J. Mitchell, M. M. Billingsley, R. M. Haley, M. E. Wechsler, N. A. Peppas and R. Langer, *Nat. Rev. Drug Discovery*, 2021, **20**, 101–124.
- 59 D. Sun, Z. Sun, H. Jiang, A. M. Vaidya, R. Xin, N. R. Ayat, A. L. Schilb, P. L. Qiao, Z. Han, A. Naderi and Z.-R. Lu, *Bioconjugate Chem.*, 2019, **30**, 667–678.
- 60 N. Kong, W. Tao, X. Ling, J. Wang, Y. Xiao, S. Shi, X. Ji, A. Shajii, S. T. Gan, N. Y. Kim, D. G. Duda, T. Xie, O. C. Farokhzad and J. Shi, *Sci. Transl. Med.*, 2019, **11**, eaaw1565.
- 61 Q. Cheng, T. Wei, L. Farbiak, L. T. Johnson, S. A. Dilliard and D. J. Siegwart, *Nat. Nanotechnol.*, 2020, **15**, 313–320.
- 62 S. Liu, Q. Cheng, T. Wei, X. Yu, L. T. Johnson, L. Farbiak and D. J. Siegwart, *Nat. Mater.*, 2021, **20**, 701–710.
- 63 H. Yin, C.-Q. Song, J. R. Dorkin, L. J. Zhu, Y. Li, Q. Wu, A. Park, J. Yang, S. Suresh, A. Bizhanova, A. Gupta, M. F. Bolukbasi, S. Walsh, R. L. Bogorad, G. Gao, Z. Weng, Y. Dong, V. Koteliansky, S. A. Wolfe, R. Langer, W. Xue and D. G. Anderson, *Nat. Biotechnol.*, 2016, **34**, 328–333.
- 64 Y. Lin, J. Wu, W. Gu, Y. Huang, Z. Tong, L. Huang and J. Tan, *Adv. Sci.*, 2018, **5**, 1700611.
- 65 A. Nouredine, A. Maestas-Olguin, E. A. Saada, A. E. LaBauve, J. O. Agola, K. E. Baty, T. Howard, J. K. Sabo, C. R. S. Espinoza, J. A. Doudna,

- J. S. Schoeniger, K. S. Butler, O. A. Negrete, C. J. Brinker and R. E. Serda, *Acta Biomater.*, 2020, **114**, 358–368.
- 66 P. Wang, L. Zhang, W. Zheng, L. Cong, Z. Guo, Y. Xie, L. Wang, R. Tang, Q. Feng, Y. Hamada, K. Gonda, Z. Hu, X. Wu and X. Jiang, *Angew. Chem., Int. Ed.*, 2018, **57**, 1491–1496.
- 67 D. Rosenblum, A. Gutkin, R. Kedmi, S. Ramishetti, N. Veiga, A. M. Jacobi, M. S. Schubert, D. Friedmann-Morvinski, Z. R. Cohen, M. A. Behlke, J. Lieberman and D. Peer, *Sci. Adv.*, 2020, **6**, eabc9450.
- 68 Y. Liu, G. Zhao, C.-F. Xu, Y.-L. Luo, Z.-D. Lu and J. Wang, *Biomater. Sci.*, 2018, **6**, 1592–1603.
- 69 Q. Tang, J. Liu, Y. Jiang, M. Zhang, L. Mao and M. Wang, *ACS Appl. Mater. Interfaces*, 2019, **11**, 46585–46590.
- 70 M. Li, H. Xie, Y. Liu, C. Xia, X. Cun, Y. Long, X. Chen, M. Deng, R. Guo, Z. Zhang and Q. He, *J. Controlled Release*, 2019, **304**, 204–215.
- 71 Q. Liu, C. Wang, Y. Zheng, Y. Zhao, Y. Wang, J. Hao, X. Zhao, K. Yi, L. Shi, C. Kang and Y. Liu, *Biomaterials*, 2020, **258**, 120275.
- 72 B. J. Druker, S. Tamura, E. Buchdunger, S. Ohno, G. M. Segal, S. Fanning, J. Zimmermann and N. B. Lydon, *Nat. Med.*, 1996, **2**, 561–566.
- 73 Z. Liu, Q. Sun and X. Wang, *Transl. Oncol.*, 2016, **10**, 22–32.
- 74 L. O. Afolabi, A. O. Adeshakin, M. M. Sani, J. Bi and X. Wan, *Immunology*, 2019, **158**, 63–69.
- 75 W. Song, S. N. Musetti and L. Huang, *Biomaterials*, 2017, **148**, 16–30.
- 76 P.-W. Huang and J. W.-C. Chang, *Biomed. J.*, 2019, **42**, 299–306.
- 77 X.-Y. He, X.-H. Ren, Y. Peng, J.-P. Zhang, S.-L. Ai, B.-Y. Liu, C. Xu and S.-X. Cheng, *Adv. Mater.*, 2020, **32**, e2000208.
- 78 H. Frangoul, D. Altshuler, M. D. Cappellini, Y.-S. Chen, J. Domm, B. K. Eustace, J. Foell, J. de la Fuente, S. Grupp, R. Handgretinger, T. W. Ho, A. Kattamis, A. Kernysky, J. Lekstrom-Himes, A. M. Li, F. Locatelli, M. Y. Mapara, M. de Montalembert, D. Rondelli, A. Sharma, S. Sheth, S. Soni, M. H. Steinberg, D. Wall, A. Yen and S. Corbacioglu, *N. Engl. J. Med.*, 2021, **384**, 252–260.
- 79 T. Wei, Q. Cheng, Y.-L. Min, E. N. Olson and D. J. Siegwart, *Nat. Commun.*, 2020, **11**, 3232.
- 80 X. Gao, Y. Tao, V. Lamas, M. Huang, W.-H. Yeh, B. Pan, Y.-J. Hu, J. H. Hu, D. B. Thompson, Y. Shu, Y. Li, H. Wang, S. Yang, Q. Xu, D. B. Polley, M. C. Liberman, W.-J. Kong, J. R. Holt, Z.-Y. Chen and D. R. Liu, *Nature*, 2018, **553**, 217–221.
- 81 L. Zhang, L. Wang, Y. Xie, P. Wang, S. Deng, A. Qin, J. Zhang, X. Yu, W. Zheng and X. Jiang, *Angew. Chem., Int. Ed.*, 2019, **58**, 12404–12408.
- 82 J. Liu, J. Chang, Y. Jiang, X. Meng, T. Sun, L. Mao, Q. Xu and M. Wang, *Adv. Mater.*, 2019, **31**, e1902575.
- 83 Y.-L. Luo, C.-F. Xu, H.-J. Li, Z.-T. Cao, J. Liu, J.-L. Wang, X.-J. Du, X.-Z. Yang, Z. Gu and J. Wang, *ACS Nano*, 2018, **12**, 994–1005.
- 84 E. Y. Cho, J.-Y. Ryu, H. A. R. Lee, S. H. Hong, H. S. Park, K. S. Hong, S.-G. Park, H. P. Kim and T.-J. Yoon, *J. Nanobiotechnol.*, 2019, **17**, 19.
- 85 M. Li, Y.-N. Fan, Z.-Y. Chen, Y.-L. Luo, Y.-C. Wang, Z.-X. Lian, C.-F. Xu and J. Wang, *Nano Res.*, 2018, **11**, 6270–6282.
- 86 J. D. Finn, A. R. Smith, M. C. Patel, L. Shaw, M. R. Youniss, J. van Heteren, T. Dirstine, C. Ciullo, R. Lescarbeau, J. Seitzer, R. R. Shah, A. Shah, D. Ling, J. Growe, M. Pink, E. Rohde, K. M. Wood, W. E. Salomon, W. F. Harrington, C. Dombrowski, W. R. Strapps, Y. Chang and D. V. Morrissey, *Cell Rep.*, 2018, **22**, 2227–2235.
- 87 Y.-L. Min, R. Bassel-Duby and E. N. Olson, *Annu. Rev. Med.*, 2019, **70**, 239–255.
- 88 K. Lee, M. Conboy, H. M. Park, F. Jiang, H. J. Kim, M. A. Dewitt, V. A. Mackley, K. Chang, A. Rao, C. Skinner, T. Shobha, M. Mehdipour, H. Liu, W. Huang, F. Lan, N. L. Bray, S. Li, J. E. Corn, K. Kataoka, J. A. Doudna, I. Conboy and N. Murthy, *Nat. Biomed. Eng.*, 2017, **1**, 889–901.
- 89 M. R. Emami, C. S. Young, Y. Ji, X. Liu, E. Mokhonova, A. D. Pyle, H. Meng and M. J. Spencer, *Adv. Ther.*, 2019, **2**, 1900061.
- 90 M. D. Shapiro, H. Tavori and S. Fazio, *Circ. Res.*, 2018, **122**, 1420–1438.
- 91 C. Jiang, M. Mei, B. Li, X. Zhu, W. Zu, Y. Tian, Q. Wang, Y. Guo, Y. Dong and X. Tan, *Cell Res.*, 2017, **27**, 440–443.
- 92 C.-F. Xu, S. Iqbal, S. Shen, Y.-L. Luo, X. Yang and J. Wang, *Small*, 2019, **15**, 1900055.
- 93 Z. Liu, S. Chen, X. Jin, Q. Wang, K. Yang, C. Li, Q. Xiao, P. Hou, S. Liu, S. Wu, W. Hou, Y. Xiong, C. Kong, X. Zhao, L. Wu, C. Li, G. Sun and D. Guo, *Cell Biosci.*, 2017, **7**, 47.
- 94 T. R. Abbott, G. Dhamdhare, Y. Liu, X. Lin, L. Goudy, L. Zeng, A. Chemparathy, S. Chmura, N. S. Heaton, R. Debs, T. Pande, D. Endy, M. F. La Russa, D. B. Lewis and L. S. Qi, *Cell*, 2020, **181**, 865–876.
- 95 C. D. Sago, M. P. Lokugamage, K. Paunovska, D. A. Vanover, C. M. Monaco, N. N. Shah, M. G. Castro, S. E. Anderson, T. G. Rudoltz, G. N. Lando, P. M. Tiwari, J. L. Kirschman, N. Willett, Y. C. Jang, P. J. Santangelo, A. V. Bryksin and J. E. Dahlman, *Proc. Natl. Acad. Sci. U. S. A.*, 2018, **115**, E9944–E9952.
- 96 C. J. McKinlay, J. R. Vargas, T. R. Blake, J. W. Hardy, M. Kanada, C. H. Contag, P. A. Wender and R. M. Waymouth, *Proc. Natl. Acad. Sci. U. S. A.*, 2017, **114**, E448–E456.
- 97 L. M. Arévalo, C. J. Yarce, J. Oñate-Garzón and C. H. Salamanca, *Pharmaceuticals*, 2019, **12**, 1.
- 98 W. Gao, D. Vecchio, J. Li, J. Zhu, Q. Zhang, V. Fu, J. Li, S. Thamphiwatana, D. Lu and L. Zhang, *ACS Nano*, 2014, **8**, 2900–2907.
- 99 X. Hou, X. Zhang, W. Zhao, C. Zeng, B. Deng, D. W. McComb, S. Du, C. Zhang, W. Li and Y. Dong, *Nat. Nanotechnol.*, 2020, **15**, 41–46.
- 100 A. C. Greene, *Trends Biotechnol.*, 2018, **36**, 127–130.

- 101 C. Xu, Z. Lu, Y. Luo, Y. Liu, Z. Cao, S. Shen, H. Li, J. Liu, K. Chen, Z. Chen, X. Yang, Z. Gu and J. Wang, *Nat. Commun.*, 2018, **9**, 4092.
- 102 R. Verma, R. Sahu, D. D. Singh and T. E. Egbo, *Semin. Cell Dev. Biol.*, 2019, **96**, 44–52.
- 103 Q. Li, P. Zhao, L. Li, H. Zhao, L. Shi and P. Tian, *Antimicrob. Agents Chemother.*, 2020, **64**, e01789–e01719.
- 104 Y. K. Kang, K. Kwon, J. S. Ryu, H. N. Lee, C. Park and H. J. Chung, *Bioconjugate Chem.*, 2017, **28**, 957–967.