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Delivery of Tissue-Targeted Scalpels: Opportunities and Challenges for *in Vivo* CRISPR/Cas-Based Genome Editing

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Abstract

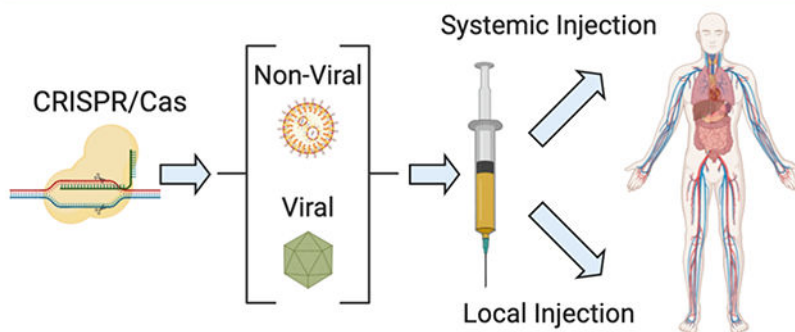
CRISPR/Cas9-based genome editing has quickly emerged as a powerful breakthrough technology for use in diverse settings across biomedical research and therapeutic development. Recent efforts toward understanding gene modification methods *in vitro* have led to substantial improvements in *ex vivo* genome editing efficiency. Because disease targets for genomic correction are often localized in specific organs, realization of the full potential of genomic medicines will require delivery of CRISPR/Cas9 systems targeting specific tissues and cells directly *in vivo*. In this Perspective, we focus on progress toward *in vivo* delivery of CRISPR/Cas components. Viral and nonviral delivery systems are both promising for gene editing in diverse tissues *via* local injection and systemic injection. We describe the various viral vectors and synthetic nonviral materials used for *in vivo* gene editing and applications to research and therapeutic models, and summarize opportunities and progress to date for both methods. We also discuss challenges for viral delivery, including overcoming limited packaging capacity, immunogenicity associated with multiple dosing, and the potential for off-target effects, and nonviral delivery, including efforts to increase efficacy and to expand utility of nonviral carriers for use in extrahepatic tissues and cancer. Looking ahead, additional advances in the safety and efficiency of viral and nonviral delivery systems for tissue- and cell-type-specific gene editing will be required to enable broad clinical translation. We provide a summary of current delivery systems used for *in vivo* genome editing, organized with respect to route of administration, and highlight immediate opportunities for biomedical research and applications. Furthermore, we discuss current challenges for *in vivo* delivery of CRISPR/Cas9 systems to guide the development of future therapies.

Graphical Abstract

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The authors declare competing financial interests. D.G.A. is a founder of CRISPR Tx and a consultant with Translate Bio. For a list of entities with which R.L. is involved, compensated, or uncompensated, see www.dropbox.com/s/yc3xqb5s8s94v7x/Rev%20Langer%20COI.pdf?dl=0. D.J.S. is a co-founder of, and consultant to, ReCode Therapeutics.

Delivery Approaches for CRISPR/Cas Gene Editing



Keywords

CRISPR/Cas9; genome editing; viral delivery; non-viral delivery; nanoparticles; gene delivery; gene therapy; ribonucleoproteins (RNPs); homology-directed repair (HDR)

The clustered regularly interspaced short palindromic repeat (CRISPR) -associated protein (CRISPR/Cas) nuclease system has rapidly become a powerful toolbox for genome engineering with broad applications in biological research, including high-throughput screening applications, animal model creation, *in vivo* and *ex vivo* editing, and disease therapeutics.^{1–6} The canonical CRISPR/Cas9 system involves Cas9 protein and guide RNA structures formed by two complementary sequences consisting of the trans-activating tracrRNA and targeting crRNA. To simplify the system, the tracrRNA/crRNA duplex has been engineered into a single chimeric RNA, termed single guide RNA (sgRNA), making utility of gene editing easier for researchers across disciplines.¹ Initially, Cas9 protein and sgRNA must first assemble into ribonucleoproteins (RNPs). After traversing the cell's nuclear membrane and entering into the nucleus, the target sequence of sgRNA may then recognize a highly specific genomic DNA sequence flanked by a protospacer-adjacent motif (PAM). It is at this point that Cas9 protein creates a double-stranded break (DSB) cut in the genomic DNA. Following cleavage, these DSBs can generally be repaired *via* two distinct pathways: 1) non-homologous end joining (NHEJ), which introduces small deletions or insertions (indels) at random around the targeted gene locus; and 2) homology-directed repair (HDR), which incorporates a template strand of DNA into the DSB site, thereby enabling precise genome modification.^{7–11}

A key component of efficient CRISPR/Cas9-mediated genome editing is the development of efficient delivery strategies that can effectively deliver CRISPR/Cas9 systems into targeted cells.^{6, 12–13} To date, multiple approaches have been reported for *in vitro/ex vivo* delivery of CRISPR/Cas9 machinery, including physical methods (microinjection, electroporation),^{14–15} viral delivery (lentiviral vectors, adenoviral vectors, and adeno-associated vectors),^{7, 13} and nonviral delivery (nanoparticle delivery systems).^{6, 12, 16} These methods are able to deliver CRISPR/Cas9 systems into cells efficiently and have been widely used for various applications, including editing mouse zygotes,^{17–18} stem cells,^{19–22} cultured cells,^{23–26} and organoids.^{27–30} Acknowledging that various reviews have been written on CRISPR/Cas

summarizing gene editing approaches,^{6-7, 9-16} in this Perspective, we focus on how these approaches have been utilized to optimize various strategies for *in vivo* editing—ranging from viral to synthetic carriers as well as local to systemic routes of administration—and the advantages and challenges associated with each strategy. Therefore, we summarize current delivery strategies used for *in vivo* genome editing organized with respect to route of administration, and, looking forward, we elucidate current challenges impeding effective *in vivo* genome editing and propose potential avenues worth pursuing that may overcome these obstacles. Through continued development and optimization, CRISPR/Cas systems hold the potential to enable the realization of truly curative therapeutics that combat diseases at the DNA level, addressing the origin of the disorder rather than simply treating the symptoms.

Viral Delivery Systems for *in Vivo* Genome Editing

Viral vectors are a promising system employed for *in vivo* delivery of CRISPR/Cas9.¹³ Due to their high cellular uptake and editing efficiency, viral vectors have been widely used for gene therapy and genome editing. At present, the most commonly used viral vectors *in vivo* include lentiviral vectors (LVs), adenoviral vectors (AVs), and adeno-associated viruses (AAVs).³¹⁻³² These viral vectors have been used to deliver CRISPR/Cas9 systems successfully to multiple tissues/organs *via* a variety of administration methods (Figure 1).³²

Local Administration

Researchers have investigated LVs, AVs, and AAVs for the generation of various mouse models *via* local injection of gene-editing machinery directly into tissues. For example, LVs expressing both Cas9/sgAlk and Cas9/sgEml4 were delivered either by intratracheal inoculation or by direct intrapulmonary injection into lungs of adult mice, which successfully generated Eml4-Alk rearrangements and thereby induced tumor formation in mouse lungs for cancer model generation.³³ Lentiviral vectors and AAVs have also been shown to deliver CRISPR systems into lung *via* intratracheal and intranasal inoculation. Similarly, these systems successfully knocked out tumor suppressor genes and were able to generate lung tumor mouse models.³⁴ Furthermore, LVs containing CRISPR/Cas9 systems have been adapted for the generation of multiple other cancer models such as pancreatic cancer³⁵ and invasive lobular breast cancer³⁶ *via* local injections into the pancreas and breast tissue, respectively.

In addition to tumor modeling, viral vectors have been used to treat models of genetic diseases, such as Duchenne muscular dystrophy (DMD),³⁷⁻³⁹ Alzheimer's disease (AD),⁴⁰ and dominant progressive hearing loss.⁴¹ DMD is a fatal genetic disease caused by mutations in the dystrophin gene that result in the absence of this critical structural protein in muscle. Recently, AAVs were used to deliver CRISPR/Cas9 nucleases coupled with paired sgRNAs flanking mutated DMD exon23 in the *mdx* mouse model of DMD. Ultimately, the treatment was able to restore the DMD reading frame and partially recover muscle functional deficiency following intra-muscular injection.³⁸ By deleting DMD exon 44 and exon 50 in DMD mice *via* AAV vector-mediated single-cut genome editing, the expression of dystrophin was rescued and muscle function was improved.⁴²⁻⁴³ It has been shown that a mutation in amyloid precursor protein (APP), termed *APP^{swe}* (*Swedish*), leads to

abnormally high levels of amyloid- β (A β) protein accumulation in the brain. In the interest of creating a mouse model of Alzheimer's disease (AD) with this mutation, AAV vectors coding for sequences of both Cas9 protein and *APP^{Sw}* specific guide RNAs were co-injected into the hippocampus of adult mice. Combinatorial delivery of these AAVs successfully generated an AD model mice with site-specific indels at the mutated *APP^{Sw}* allele.⁴⁰ Beethoven (*Bth*) mice, a mouse model for dominant progressive hearing loss, is related to a *Bth* point mutation in the *Tmc1* allele. György *et al.* screened 14 Cas9/sgRNA combinations and identified one that selectively and efficiently disrupted the mutant *Tmc1* allele, rather than the wild-type *Tmc1/tmc1* allele, in *Beethoven* (*Bth*) mice.⁴¹ They found that AAV-mediated SaCas9-KKH delivery prevented deafness in *Bth* mice for up to 1 year post inner-ear injections. Overall, viral vectors have been able to induce gene editing in multiple tissues *via* local administration, including heart,^{44–45} lung,^{33,46–47} muscle,^{37–39,42–43} brain,^{40,48} breast,³⁶ pancreas,³⁵ inner ear,⁴¹ and retina.^{49–50}

Systemic Administration

In addition to local administration, researchers have identified multiple AAV serotypes that have the capability to deliver CRISPR/Cas9 complexes into different tissues *via* systemic injection. Adeno-associated virus vectors are able to edit genes in liver tissues to treat diseases such as metabolic liver diseases,^{51–53} hemophilia B,⁵⁴ and targets for control of serum cholesterol levels.⁵⁵ For example, Yin *et al.* combined viral delivery (lipid nanoparticles carrying Cas9 mRNA) and nonviral delivery (AAV8 encoding a sgRNA and a HDR template), for the treatment of a mouse model of hereditary tyrosinemia type I (HT-1) *via* systemic injection. They demonstrated that this treatment successfully corrected a mutation in fumarylacetoacetate hydrolase (FAH) in hepatocytes and rescued disease symptoms, such as weight loss and liver damage.⁵¹ Ran *et al.* packaged SaCas9 and a sgRNA expression cassette into AAV8 and administered them *via* tail vein injection. The viruses then accumulated in the mouse liver and targeted the cholesterol regulatory gene PCSK9. At only 1 week post injection, they observed over 40% indel formation in liver, with similar levels maintained at 4 weeks post injection. Meanwhile, they detected significant reductions in serum PCSK9 (~95%) and total cholesterol levels (~40%) that were sustained throughout the whole study.⁵⁵ Another tissue that has been targeted *via* viral vectors is muscle. Specifically, AAV6,⁵⁶ AAV8,³⁹ and AAV9^{42–43} have been used to deliver CRISPR/Cas9 cassettes *via* systemic injection to restore the expression of dystrophin in muscles, resulting in improvements in muscle function in the *mdx* mouse model of DMD. Additionally, researchers have systematically targeted heart⁵⁷ and brain⁵⁸ using AAV9 vectors through the careful inclusion of tissue-specific promoters.

Challenges of Viral Delivery

Despite these achievements, there are several limitations and drawbacks to using viral vectors. First and foremost, viruses have a limited packaging capacity of approximately 5 kilobases (kb),^{9, 12–13} which severely hinders the size of the sequences that can be packaged into the vector. By using smaller Cas9 orthologues (*e.g.*, StCas9, SaCas9, and cjCas9)⁹ or dual AAV vectors,^{59–60} some researchers have been able to circumvent the packaging issues, in part; however, achieving precise genome editing *via* HDR given these constraints remains a significant hurdle. The second cause for concern is the propensity of viruses to generate a

negative and substantial immune response, which then eliminates the possibility of repeat dosing—an aspect that is critically important in cells with high turnover rates.^{12–13} Therefore, a single injection of viral vectors must yield a high enough permanent incidence of gene modification to reach levels that provide a therapeutic benefit, something that is difficult to accomplish in a single dose. A third point of consideration is that the construction process and resources necessary to create a virus with therapeutic potential—from both a financial as well as an industrial perspective—are complex, expensive, and time consuming. Finally, viruses used to achieve genome editing have been known to induce high frequencies of off-target events.^{6–7, 9–10, 12} The long term and/or continuous expression of CRISPR components—as is the case when using viruses—may significantly increase the risks of off-target events *in vivo* after viral delivery, some of which may indeed be deleterious to the host's genome. Efforts to overcome these drawbacks are underway and may be addressed by new approaches. Due to high potency and tissue specificity, viral vectors are still the most widely used method at present for *in vivo* genome editing. Viral delivery systems for *in vivo* gene editing remain an attractive approach for difficult to target tissues, such as heart and brain. Due to their high editing efficiency, viral vectors have been investigated in several preclinical and clinical studies.³²

Nonviral Delivery Systems for *in Vivo* Genome Editing

With the rapid development of synthetic vectors, nonviral delivery systems have already had a transformative impact on the genome editing field and they have the potential to drive advances further in a variety of widespread applications. Unlike viral vectors, which, as noted previously, have a limited packaging capacity, nonviral delivery systems are quite flexible with respect to loading capacity and can accommodate large nucleic acid and protein cargos such as Cas9 mRNA and RNPs (~160 kDa),^{23–24, 61–63} in addition to sgRNAs^{64–65} and donor DNA.⁶⁶ By encapsulating these cargos inside nanoparticles (NPs) rather than in viruses, immune detection, along with most immunogenicity can be minimized, thus allowing for repeat administration of NPs loaded with gene-editing cargo. Another benefit to using NPs as transfection agents is that when delivering Cas9 mRNA or RNPs, Cas9 expression is transient, and this transient expression of the nuclease greatly reduces the probability of insertional mutagenesis. Therefore, the threat of nuclease-induced off-target events is largely mitigated when compared to that of viral vectors.^{67–69} To date, a variety of synthetic vectors have been developed to deliver CRISPR/Cas9 systems *in vivo*. Here, we will introduce them as classified by method of administration (Figure 2).

Local Administration

Intratumoral Injection

Multiple synthetic vectors have been developed that can successfully deliver CRISPR/Cas9 systems designed to knock out tumor-related genes and to suppress tumor growth following intratumoral injection. As an example, Wang *et al.* developed PEGylated NPs based on α -helical polypeptide PPABLG (P-HNPs) that co-deliver Cas9 expression plasmids and sgRNAs targeting polo-like kinase 1 (Plk1), named P-HNP_{pcas9+sgPlk1}.⁷⁰ Following intratumoral injection of P-HNP_{pcas9+sgPlk1} into HeLa xenograft tumor-bearing mice, the gene

editing efficiency at the *Plk1* locus reached 35%, which resulted in significant tumor suppression (higher than 71%) and improved the survival rate of tumor-bearing mice (60%) (Figure 3A). Wang *et al.* used TAT-peptide-coated gold nanoclusters (GNs) as a core to absorb Cas9 protein/sgPlk1 plasmid (they abbreviate the entire complex as GCP).⁷¹ After absorption, GCP was further encapsulated in a cationic lipid shell to form lipid-coated GCP (LGCP). Intratumoral injection of LGCP effectively inhibited tumor growth in a melanoma mice model by deleting the *Plk1* gene in tumors and significantly down-regulating the Plk1 protein expression (Figure 3B). Most recently, the authors used these TAT-peptide-coated AuNPs (20 nm) to replace GNs for encapsulation of Cas9-sgPlk1 plasmids (CPs) and coated the outside in lipid to form lipid-encapsulated AuNPs-condensed CP (LACP). When irradiated with a 514 nm laser, the temperature generated by the localized surface plasmon resonances of AuNPs not only triggered release of the encapsulated cargo, but also provided a means of performing photothermal cancer therapy (Figure 3C). When injected directly into a tumor and irradiated, the tumor volume decreased to approximately 15% of that of the untreated group, indicating LACP-mediated tumor inhibition under irradiation.⁷² More recently, Pan *et al.* developed a near-infrared (NIR) upconversion-triggered CRISPR-Cas9 system (UCNPs-Cas9@PEI).⁷³ The upconverting NPs (UCNPs) convert NIR light (980 nm) into ultraviolet light, which triggers the cleavage of photosensitive molecules, resulting in on-demand release of CRISPR-Cas9 machinery for gene editing. With NIR light irradiation for 20 min every other day, intratumor injection of UCNPs-Cas9@PEI delayed tumor progression by knocking-out the *Plk1* gene in tumor tissues, with no detectable toxic side effects (Figure 3D).

Intracranial Injection

In addition to viral delivery, there is great interest in developing nonviral vectors capable of editing genes in the brain for new disease therapies. Staahl *et al.* directly engineered Cas9 RNP complexes with multiple nuclear localization signals that enabled neuronal editing in the brain *via* intracranial injection.⁷⁴ Park *et al.* constructed Cas9 nanocomplexes by mixing amphiphilic R7L10 peptide and Cas9/sgRNA RNPs to edit genes in post-mitotic neurons of the adult brain (Figure 4A).⁷⁵ The Cas9 nanocomplex targeting beta-secretase 1 (*Bace1*) significantly reduced expression of amyloid beta (A β) peptides and suppressed A β -associated phenotypes and cognitive deficits in both five familial Alzheimer's disease (5XFAD) and amyloid precursor protein (APP) knock-in Alzheimer's disease mouse models.⁷⁵ Beyond neuronal editing, the recently developed CRISPR-Gold was also able to induce gene editing in non-neuronal cells, including astrocytes and microglia, *via* delivery of Cas9 and Cpf1 RNPs. Upon intracranial injection, CRISPR-Gold inhibited 40–50% of *Grm5* in the striatum and prompted behavioral improvements in a mouse model of fragile X syndrome (Figure 4B).⁷⁶

Intramuscular Injection

There are more than 800 monogenic disorders that can cause dysfunction of skeletal muscles. Among them, DMD is the most severe and lethal monogenic disorder, resulting from mutations at the gene locus encoding dystrophin, a protein that maintains integrity of striated muscles.⁷⁷ The development of genome-editing technology provides a possible means of permanently removing or repairing genetic mutations related to DMD, thereby

restoring the expression of dystrophin protein and recovering muscle functions. For this purpose, CRISPR-Gold has been used to edit genes in muscles.⁷⁸ Intramuscular injection of CRISPR-Gold into Ai9 mice resulted in deletion of a stop sequence in genomic DNA, which then induced expression of tdTomato, a fluorescent protein. Moreover, CRISPR-Gold produced correction of a point mutation in the dystrophin gene in the *mdx* model of DMD by using intramuscularly injected Cas9 RNPs along with donor DNA templates. Using this technique, researchers were able to achieve a local point mutation correction rate of 5.4% in *mdx* mice, leading to reduced muscle fibrosis and an increase in muscle function (Figure 5A). In addition, Chen *et al.* utilized *in-situ* polymerization to create nanocapsules (NCs) that are capable of encapsulating RNPs. Local injection of these RNP-loaded NCs into the tibialis anterior muscle of tdTomato mice efficiently induced the expression of tdTomato *via* gene editing (Figure 5B).⁷⁹ Recently, Wei *et al.* reported a methodology that allows engineering of modified LNPs to deliver RNPs into muscle and other tissues.⁸⁰ Optimized LNPs were able to deliver RNPs to restore 4.2% of dystrophin protein in Ex44 DMD mice.

Intra-Inner Ear Injection

Nonviral delivery approaches have been used to treat genetic hearing loss disorders through local injection into the inner ear. Zuris *et al.* used the commercial cationic liposomal reagents Lipofectamine 2000 and RNAiMax to deliver Cas9 protein and EGFP sgRNA and then locally injected them into the cochlea of P2 Atoh1-GFP mice, where all hair cells express GFP.⁸¹ After 10 days, loss of GFP fluorescence in $20 \pm 3\%$ outer hair cells was observed in the Lipofectamine 2000 treatment group (Figure 6A). Given this high potential to treat genetic diseases of the inner ear, Zuris *et al.* then evaluated the ability of their system to treat autosomal-dominant hearing loss in a mouse model of a human genetic disease (*Tmc1^{Bth/+}* mice). After injection, they were able to knock out the mutated *Tmc1^{Bth}* allele using Cas9-guideRNA-lipid complexes. This treatment effectively promoted hair cell survival and reduced progressive hearing loss in *Tmc1^{Bth/+}* mice (Figure 6B).⁸²

Intraocular Injection

Nonviral delivery systems have also been assessed for delivery of CRISPR/Cas9 cargo into the eye *via* local injection. For example, Yang *et al.* functionalized nanodiamonds with mCherry protein and conjugated two linear DNA constructs onto the mCherry proteins (named cND-mC-C/C9).⁸³ One of these DNA constructs encoded Cas9 protein and a GFP reporter protein, and the other encoded sgRNA and an HDR template targeting the *Rsl* gene mutation, which is associated with X-linked retinoschisis (XLRs). Intravitreal injection of cND-mC-C/C9 NPs into mouse eye results in the presentation of pathological features of XLRs and, as such, may be used for the creation of an XLRs mouse model. Similarly, Chen *et al.* functionalized RNP-encapsulating nanocapsules (NCs) with a targeting ligand ATRA for retinal pigment epithelium (termed NC-ATRA), and found that subretinal injection of NC-ATRA into the eyes of transgenic Ai14 tdTom reporter mice induced higher gene editing efficiency than negative control groups.⁷⁹

Transdermal Administration

Skin is also a targetable tissue for local treatment. Ryu *et al.* encapsulated RNP complexes into nanosized liposomes and conjugated them onto the surface of microbubbles.⁸⁴ They

then used this delivery system to edit the mouse *SRD5A2* locus (*mSRD5A2*) for mouse androgenic alopecia therapy. They depilated the dorsal backs of C57BL/6 mice after an adaption, and topically applied testosterone daily for 7 weeks to maintain telogen retention as an androgenic alopecia model. Once activated by ultrasound, sonoporation generated by microbubble cavitation facilitated RNPs release and delivery into dermal papilla cells in hair follicles and resulted in *mSRD5A2* gene knockout, leading to recovery of hair growth.

Systemic Administration

Targeting Liver

The liver is an attractive target for nonviral vector delivery because most NPs accumulate in the liver following systemic administration. To date, multiple lipid-based NPs have been identified for hepatocyte-specific delivery in liver *via* systemic injection.^{51, 64–65, 85–88} For example, Miller *et al.* developed zwitterionic amino lipid (ZAL) nanoparticles NPs (ZNPs) for delivery of Cas9 mRNA and sgLoxP targeting the stop cassette of a tdTomato reporter mouse model.⁶⁴ Systemic injection of ZNPs enabled deletion of the stop cassette and induced tdTomato fluorescence in the liver, kidneys, and lungs of tdTomato mice (Figure 7A). Jiang *et al.* developed TT3 lipid-like nanoparticles (LLNs) that can efficiently deliver Cas9 mRNA and guide RNA to the liver, thereby disrupting *HBVDNA* and the *PCSK9* gene for liver-related disease treatments.⁶⁵ To increase gene editing efficiency *in vivo* further, end-modified sgRNA and fully modified sgRNA were synthesized. Upon single intravenous injection of biodegradable lipid nanoparticles (LNPs; LNP-INT01) co-delivering Cas9 mRNA and highly modified sgRNA targeting mouse transthyretin (*Ttr*) gene to CD-1 mice, ~70% of gene editing at *Ttr* locus and >97% knockdown of TTR serum protein were detected for at least 12 months (Figure 7B).⁸⁵ Also, a single IV injection of CKK-E12 LNPs loaded with Cas9 mRNA and chemically modified sgRNA into mouse tail vein induced > 80% gene editing of the *PCSK9* gene in the liver and resulted in an undetectable level of PCSK9 protein in the serum (Figure 7C).⁸⁷ In addition to gene knock-out, researchers have also used LNPs for gene correction in the liver. Yin *et al.* used C12-200 formulated LNPs to encapsulate Cas9 mRNA and AAV vectors loaded with a cassette encoding a sgRNA and a donor template.⁵¹ The combined delivery of these components successfully repaired a *Fah* mutation in the *Fah^{mut/mut}* mouse model of hepatorenal tyrosinemia type 1 (HT1) (0.81% of gene correction at *Fah* locus) and rescued the mice from weight loss and liver damage (Figure 7D).

Targeting Lungs and Spleen

As mentioned above, most systemic therapeutic efforts for gene editing using nonviral carriers have focused on targets in the liver. Delivering gene-editing components to extrahepatic tissues remains challenging. Solving this challenge represents a significant area of research for future expansion of gene editing because many important targets for gene correction are in tissues such as the lungs, spleen, kidney, bone marrow, and others. In the past few years, there have been reports of delivering siRNA and mRNA to extrahepatic tissues.^{64, 89–99} Although the mechanisms of targeting are not fully elucidated, these works provide a foundation upon which to develop tissue-targeted genomic medicines. For example, Sago *et al.* developed a high-throughput *in vivo* screening method, fast

identification of nanoparticle delivery (FIND), to identify LNPs that can functionally deliver mRNA to different cell types.⁹⁹ With this method, they identified two LNPs (7C2 and 7C3) that can deliver mRNA to endothelial cells, especially endothelial cells in the spleen. After IV injecting LSL-Tom mice twice with 7C3 encapsulating Cas9 mRNA:modified sgRNA (esgRNA), they observed gene editing in splenic endothelial cells (~20%) as efficiently as hepatocytes. As NPs developed for other drugs (*e.g.*, siRNA) are reengineered for delivery of CRISPR/Cas, it is envisioned that more extrahepatic carriers will be discovered, especially as the mechanisms of targeting become clearer. *In vivo* screening methodologies, such as DNA barcoding, will play an important role in these efforts.

As an alternative to high-throughput screening, Cheng *et al.* recently developed a generalizable methodology, selective organ targeting (SORT), which enables researchers to reengineer LNPs for tissue-specific mRNA delivery targeting liver, lungs, and spleen.¹⁰⁰ The approach involves the addition of SORT molecules into established NPs (including DLNP (Dendrimer-based lipid nanoparticles), Stable nucleic acid lipid particles (SNALPS), and Lipid-like nanoparticles (LLNPs)) to redirect delivery to extrahepatic tissues (Figure 8A). Liver-, lung-, and spleen-targeted SORT LNPs co-delivering Cas9 mRNA/sgTom selectively activated strong td-Tomato expression in liver, lung, and spleen, respectively, in tdTomato mice (Figure 8A). Lung-targeted SORT LNPs transfected 65% of all endothelial cells and 40% of all epithelial cells in the lungs of tdTomato mice following a single injection of 0.3 mg/kg Cre mRNA, suggesting potential for systemic correction of diseases that affect the lung epithelium. This approach also enabled the therapeutic editing of targets such as PCSK9, where SORT LNPs induced 60% editing at the *PCSK9* locus and 100% PCSK9 reduction in both liver tissue and serum *via* co-delivery of Cas9 mRNA/sgPCSK9 (Figure 8A). In parallel, it was realized that 5-component SORT LNPs containing permanently cationic lipids (positively charged at neutral pH) could mediate self-assembly using neutral buffer solutions of RNPs to preserve protein structure and stability for effective genome editing.⁸⁰ The developed 5A2-DOT-X LNPs could mediate tissue-specific, multiplexed editing of multiple genes following intravenous injection in mice. In addition to therapeutic applications, liver and lung tropic LNPs were used to create cancer models *in situ* through knockout of multiple tumor suppressor genes or by induction of programmed chromosomal rearrangement.⁸⁰ With further development, the SORT strategy¹⁰⁰ may enable editing in additional tissues in the future, thereby expanding the development of gene editing/correction therapeutics in difficult-to-access cells and organs.

Targeting Tumors

Nonviral delivery systems have been widely explored for antitumor therapy. With the development of genome-editing technology, knocking out tumor-related genes using CRISPR/Cas9 systems becomes a promising strategy to inhibit tumor proliferation. Chen *et al.* constructed iRGD conjugated liposome-templated hydrogel nanoparticles (LHNPs) as a targeted CRISPR/Cas9 delivery system with the ability to encapsulate Cas9 protein and mini circle DNA encoding sgRNA.¹⁰¹ Systemic treatment with iRGD-LHNPs targeting *Plk1* gene suppressed tumor proliferation in mice bearing U87 flank tumors. Furthermore, they encapsulated Lexiscan, a small drug known to improve blood-brain barrier permeability, into iRGD-LHNPs. Intravenous administration of these engineered LHNPs increased survival

rates for mice bearing intracranial U87 tumors (Figure 8B). Recently, Liu *et al.* designed a tumor-environment-responsive copolymer (PLys₁₀₀-CA-mPEG₇₇) that was able to form nanostructures by mixing with Cas9 ribonucleoproteins, named nanoRNP.¹⁰² Upon intravenous injection, nanoRNP carrying two sgRNA sequences targeting *STAT3* and *RUNX1* gene loci effectively inhibited tumor growth in a heterogeneous tumor model (Figure 8C).

Targeting Macrophages

Nonviral vectors deliver CRISPR/Cas9 systems into macrophages *via* systemic administration. Wang *et al.* constructed Cas9 expression plasmids driven by a macrophage-specific promoter (human CD68 promoter).¹⁰³ Cationic lipid-assisted PEG-*b*-PLGA nanoparticles (CLANs) encapsulating these plasmids were shown to express Cas9 protein in macrophages and monocytes *in vivo*. By adding a guide RNA sequence targeting *Ntn1* into these plasmids, CLANs encapsulating these plasmids efficiently accomplished three things: 1) knock-out of the *Ntn1* gene, specifically in the macrophages and monocytes *in vivo* after intravenous injection; 2) inhibition of netrin-1 expression in macrophages; and 3) improvement of type 2 diabetes symptoms in the mouse model (Figure 8D). In addition to CRISPR/Cas9 plasmids, the authors also screened and found an optimized CLAN that co-delivered Cas9 mRNA and sgRNA into macrophages *in vivo* for the treatment of inflammatory diseases.¹⁰⁴ Lee *et al.* reported the discovery of AuNP-based nanocomposites that were generated through the co-engineering of AuNPs and Cas9 proteins including a 20-glutamic acid tag (Cas9 E20). Systemic injection of this complex into mice led to 8% and 4% of gene editing at the *PTEN* locus in the macrophages of the liver and spleen.¹⁰⁵

Challenges for Nonviral Delivery

Efficiency

Of paramount importance will be the ability of nonviral vectors to achieve gene editing at levels that are high enough to result in therapeutic benefits to patients in the clinic. Great progress has been made for high editing efficiency in the liver, and this efficiency will next need to be achieved in other tissues and cell types. As discussed previously, nonviral delivery methods enable multiple doses to be administered sequentially, which inherently increases the total cumulative editing levels that are achievable. Advantages of synthetic carriers, such as minimized immunogenicity, a reduction in the number of off-target effects, and the ability to administer repeat doses, may overcome some issues related to viral delivery. An additional concern is the fact that many genetic diseases are not able to be treated effectively *via* knockout of specific genes and/or gene sequences. Rather, many of these genetic diseases require correction of a mutated genetic sequence or knock-in of a specific gene. Currently, the frequency of HDR, which would enable these types of corrections, is much lower than that of indels. Therefore, the elucidation of methods that increase the efficiency of HDR over NHEJ is of exceptional importance and yet also challenging, as the exact mechanisms underlying these processes are, at this time, not well understood. Some recent efforts have been focused on increasing HDR. For example, covalently linking a DNA repair template to Cas9/sgRNA RNPs has been reported to improve HDR efficiency.^{106–107} Additionally, Cpf1 (or Cas12a) with staggered cutting was

demonstrated to prefer HDR more than traditional Cas9 protein, which generates blunt-end cutting.²⁰ Also, a fusion nickase named hRad51-Cas9(D10A) was able to mediate HDR without generating double-stranded DNA breaks, which again favors a higher HDR:indel ratio and low off-target events.¹⁰⁸ Thus, developments in Cas protein design may further aid advancement of HDR correction in combination with improved synthetic carriers.

Specificity

Developing nonviral vectors that can preferentially deliver CRISPR components into targeted tissues and targeted cell types will expand the potential targets of genome-editing therapies. Currently, LNPs targeting hepatocytes in the liver *via* systemic delivery are the most advanced,¹⁰⁹ but a number of studies have described LNP delivery to nonhepatic tissues such as the endothelium^{80, 99–100, 110} and immune cells.^{80, 100, 110} One obstacle to broad application of nonviral vector-based gene editing is the lack of nanovectors that can target specific tissues or cell types for precise gene modification. That being said, the SORT technology described above is a promising advance that may overcome some of these problems due to its predictable nature and tissue selectivity.¹⁰⁰

Off-Target

In addition to high on-target efficiency and specificity, the risk of off-target effects, which may potentially knockout other genes and lead to fatal diseases, should also be seriously considered. Many efforts have been made to minimize off-target events. Several bioinformatic online tools have been developed to help design sgRNAs with an explanation of all possible off-target sites, so that sgRNA sequences can be selected with minimized numbers of off-target sites for *in vivo* gene editing. The choice of Cas9 protein and sgRNAs is also essential, as high-fidelity Cas9 proteins were identified to reduce off-target mutation *in vivo*.¹¹¹ Chemically or structurally modified sgRNAs also demonstrate a reduction in off-target cleavage compared to unmodified sgRNAs.¹¹² Off-target edits may also be related to the delivery strategies of the CRISPR/Cas9 system. Viral delivery and plasmid delivery show significantly higher off-target effects than do mRNA delivery and RNP delivery.¹¹³ Even though they are targeting the correct gene loci, gene cutting at unwanted tissues or cells (*i.e.*, low specificity) may also be considered off-target effects. Therefore, developing nonviral vectors that can target specific tissues will largely reduce off-target events.

Immunogenicity

Components of CRISPR/Cas9 systems (Cas9 proteins, Cas9 mRNAs, or sgRNAs) delivered by nonviral vectors may trigger host immune responses. Anti-Cas9 antibodies have been reported to exist in human populations.¹¹⁴ RNA cargos delivered may also activate innate immune responses *in vivo*.^{115–116} In addition, some nonviral vectors may also be recognized by host immune systems. The activation of the immune system may not only significantly decrease editing efficiency *in vivo* but may also result in severe safety concerns in patients. Encapsulating CRISPR components inside nanovectors partially shields their recognition by the host immune system and largely reduces the generation of immune responses in comparison to viral systems. In sum, the potential of nonviral vectors to trigger an immune response should be carefully considered when designing nonviral CRISPR delivery systems.

Conclusions and Prospects

The CRISPR/Cas9 platform is an unprecedented technological leap in genome editing that is quickly reaching all aspects of biomedical research and therapeutic discovery. In the short time since CRISPR/Cas was first reported, numerous biotechnology companies have been founded to develop the therapeutic potential of this revolutionary technology further. Large pharma has also become involved, positioning CRISPR to affect many therapeutic modalities and areas. Currently, most of the ongoing clinical trials employing CRISPR/Cas-mediated gene editing are relating to chimeric antigen receptor T cells (CAR-T) therapies, which are generated using *ex vivo* editing approaches (Table 1). These approaches may largely avoid current challenges associated with *in vivo* techniques (*e.g.*, specificity, off-targets, and immunogenicity), but *ex vivo* approaches are still challenging and could induce innate negative immune responses. For the clinical translation of direct *in vivo* genome editing, challenges remain ahead. Encouragingly, many pharmaceutical companies are developing gene therapies employing CRISPR/Cas9 systems for genetic disease treatments. Vertex Pharmaceuticals recently acquired Exonics Therapeutics, a company focusing on DMD therapies using *in vivo* CRISPR/Cas9 technology to repair exon mutations and restore dystrophin expression. CRISPR Therapeutics and Editas Medicine are also developing DMD therapies *via in vivo* CRISPR-based gene editing. Likewise, Vertex and Editas are also actively developing CRISPR/Cas9-based gene therapies for cystic fibrosis (CF) treatments. In conjunction with DMD and CF, local delivery of CRISPR/Cas9 systems targeting other genetic diseases such as Leber congenital amaurosis 10 and alpha-I antitrypsin deficiency, are also under development, according to these companies' pipelines and clinical trial registries. Going forward, developing delivery strategies that combine high tissue specificity, high editing efficiency, and minimized toxicity are critical to accelerate the clinical translation of *in vivo* CRISPR/Cas9 based therapies. Given the rapid progress of CRISPR/Cas9 therapeutics in just a few short years, there is optimism that advances in viral and nonviral delivery approaches will someday yield the creation of safe and effective cell-specific therapies to treat human disease.

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Routes of Administration for Viral Delivery

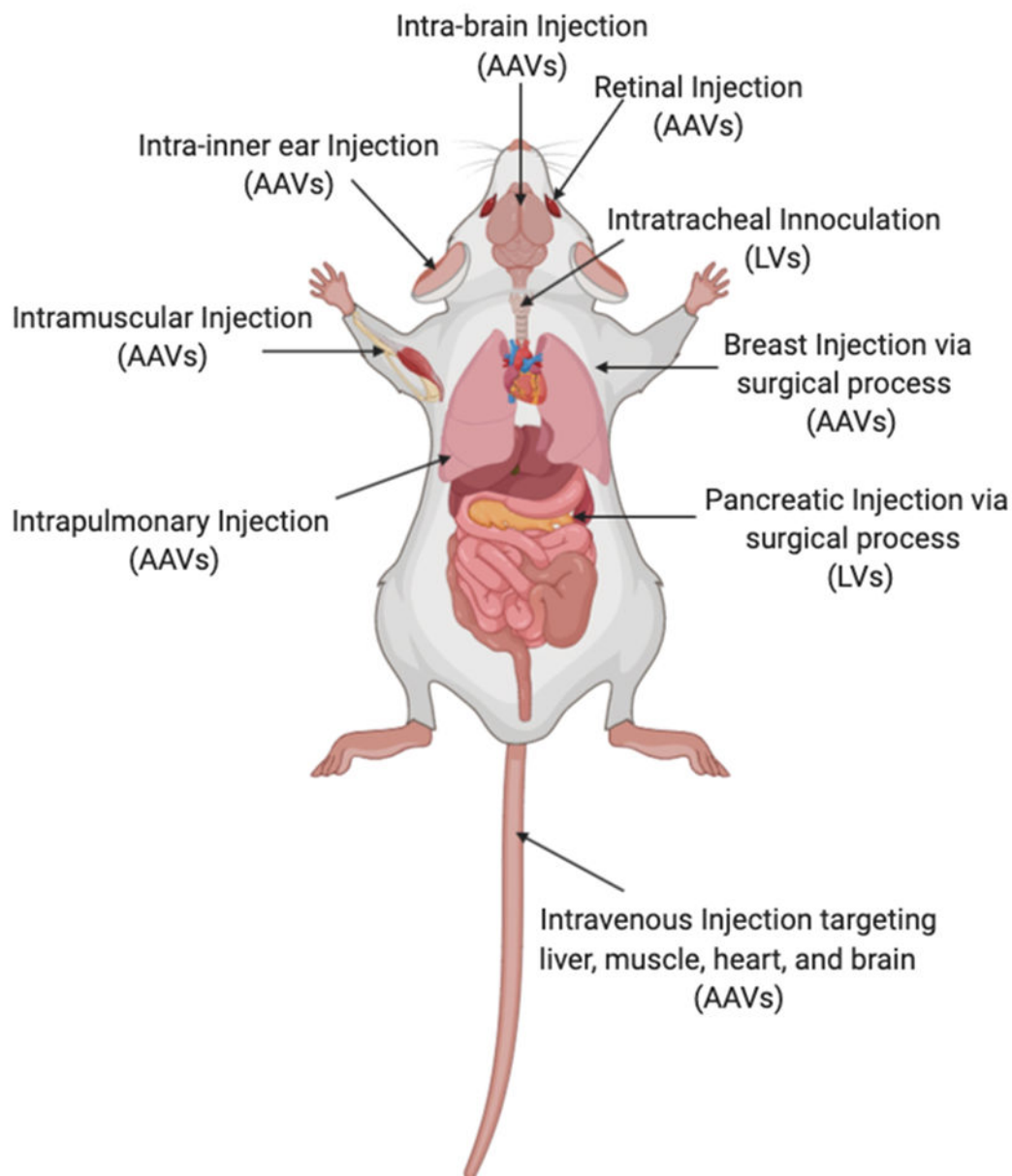


Figure 1. Routes of administration for viral-based delivery systems, including lentivirus (LV) and adeno-associated viruses (AAV), pertaining to *in vivo* genome editing.

Non-Viral Delivery Systems for Gene Editing

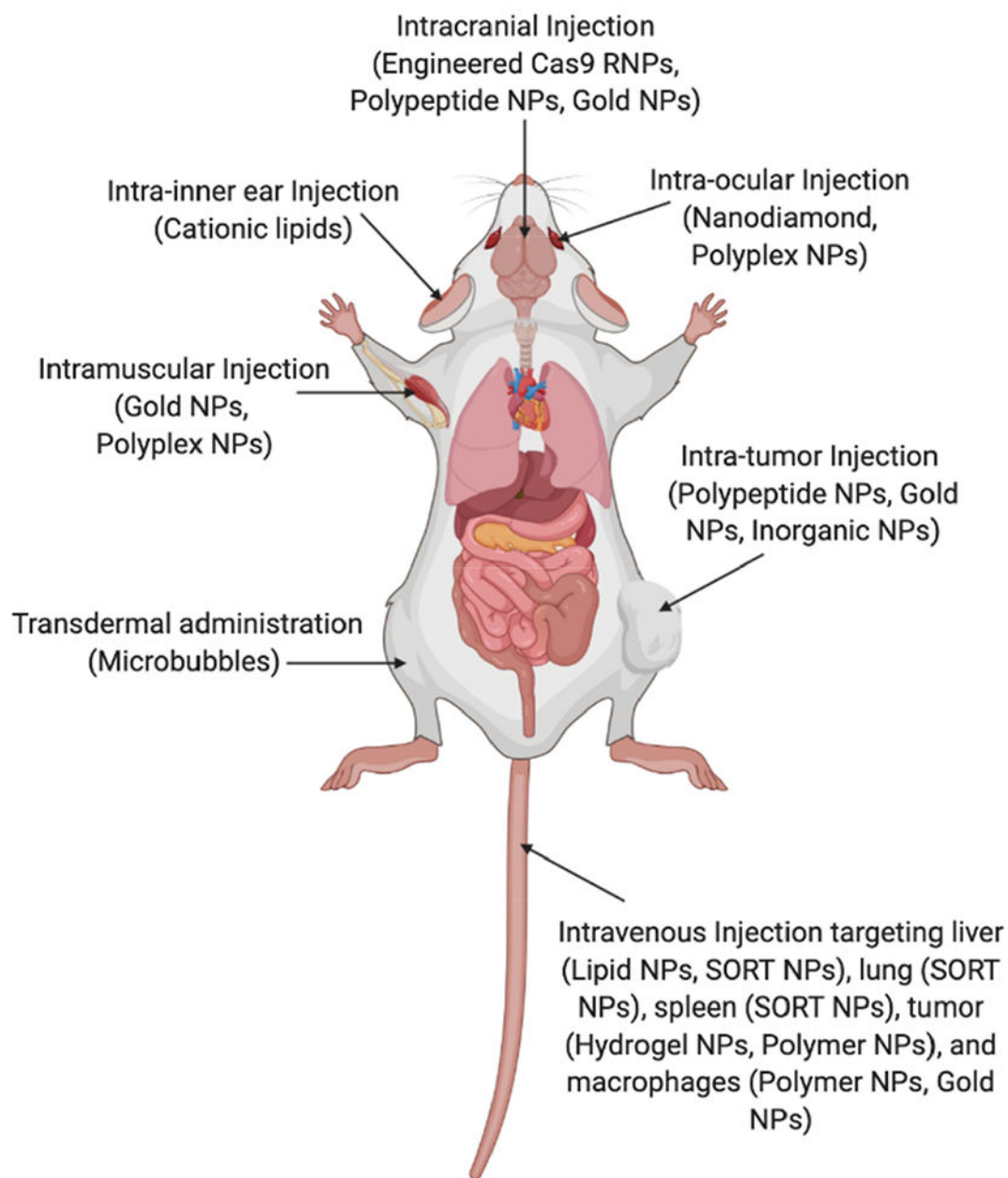


Figure 2. Different routes of nonviral-based delivery systems for *in vivo* genome editing. Nanoparticles, NPs; ribonucleoproteins, RNPs; selective organ targeting, SORT.

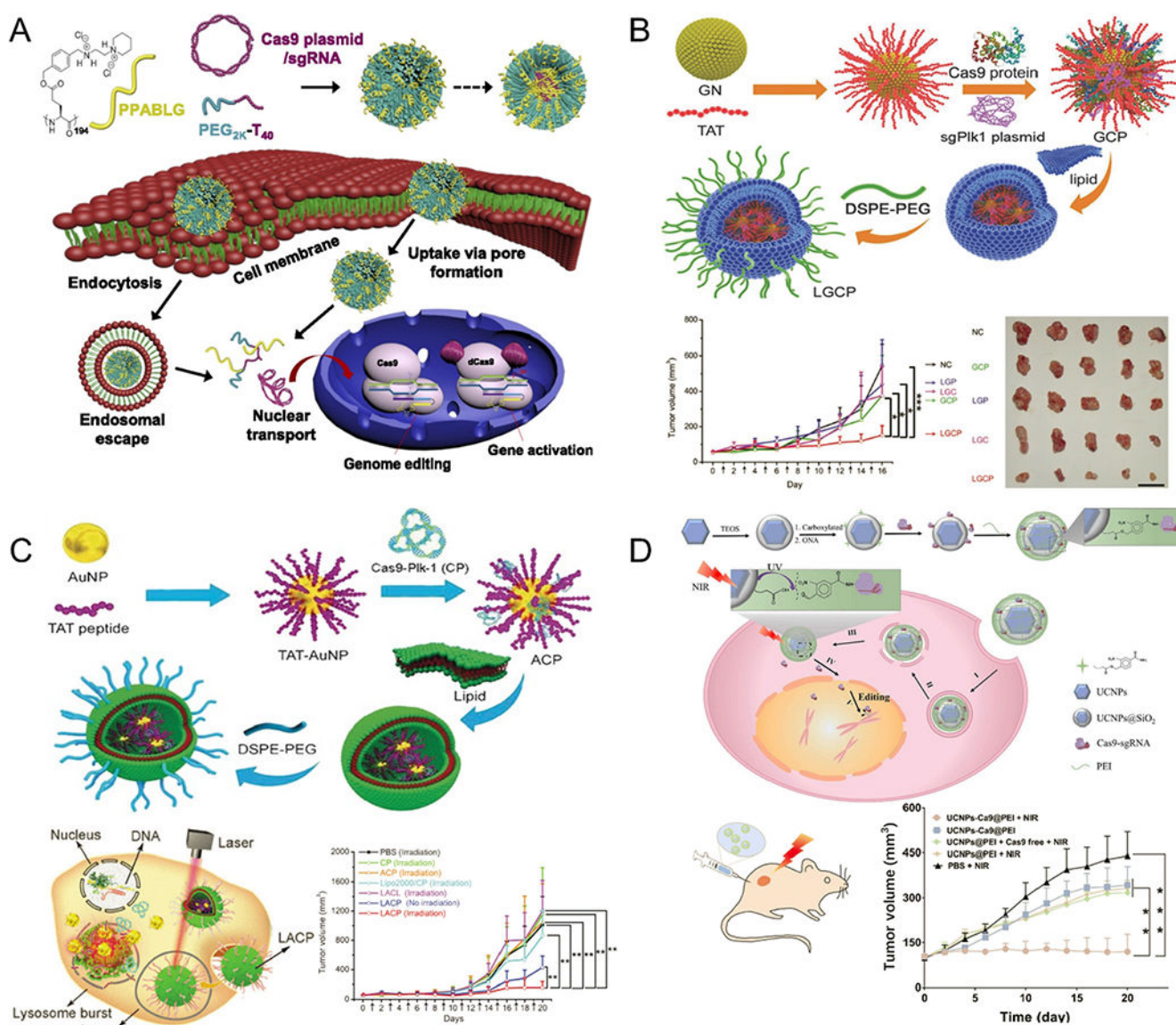


Figure 3. Nonviral, intratumor injection delivery systems for *in vivo* genome editing. (A) Scheme of formation of PEGylated nanoparticles based on α -helical polypeptide PPABLG (P-HNPs) and their intracellular delivery of Cas9 expression plasmid/single guide RNA for genome editing or gene activation. Reproduced with permission from ref 70. Copyright 2018 National Academy of Sciences. (B) Schematic illustration of synthesis process of nanoformulations (polyethylene glycol-lipid/Gold nanoclusters/Cas9 protein/sgPlk1 plasmid, LGCP). LGCP delivering Cas9 protein/sgPlk1 plasmid successfully inhibited tumor growth by knocking-out the Plk1 gene. Reproduced with permission from ref 71. Copyright 2017 John Wiley & Sons, Inc. (C) Schematic representation of synthesis process for lipid-encapsulated AuNPs-condensed Cas9-sgPlk1 plasmids (LACPs) and laser-enhanced knock-outs of targeted gene by LACPs, resulting in tumor inhibition in mouse melanoma models. Reproduced with permission from ref 72. Copyright 2018 John Wiley &

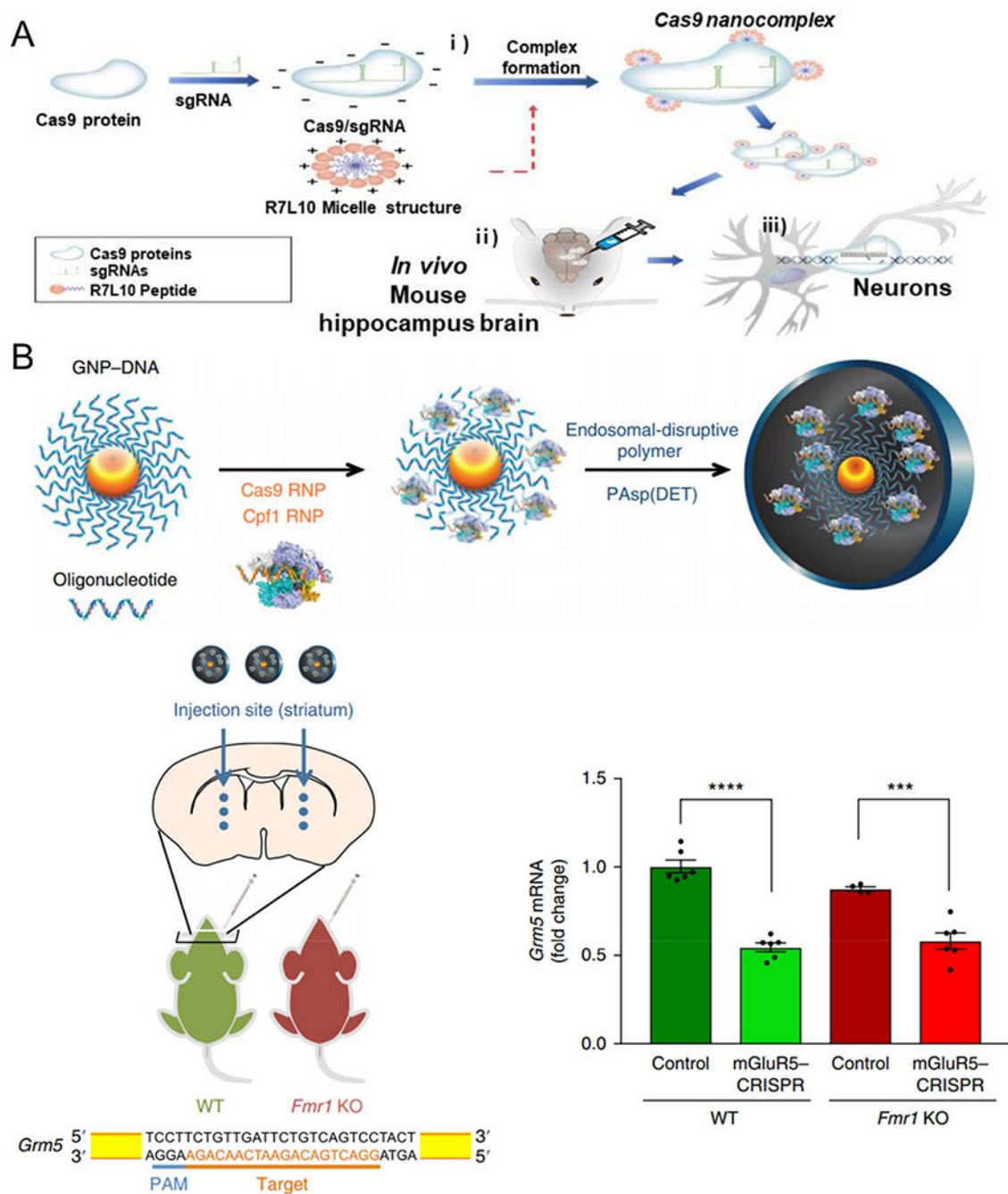
Sons, Inc. (D) Design of upconverting nanoparticles (UCNPs)-based CRISPR-Cas9 delivery system for near-infrared (NIR) light-controlled gene editing. When exposed to NIR light, UCNPs-Cas9@PEI platform successfully inhibited tumor proliferation. Reproduced with permission from ref 73. Copyright 2019 American Association for the Advancement of Science.

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ref 76. Copyright 2018 Springer Nature. Single-guide RNA, sgRNA; ribonucleoprotein, RNP.

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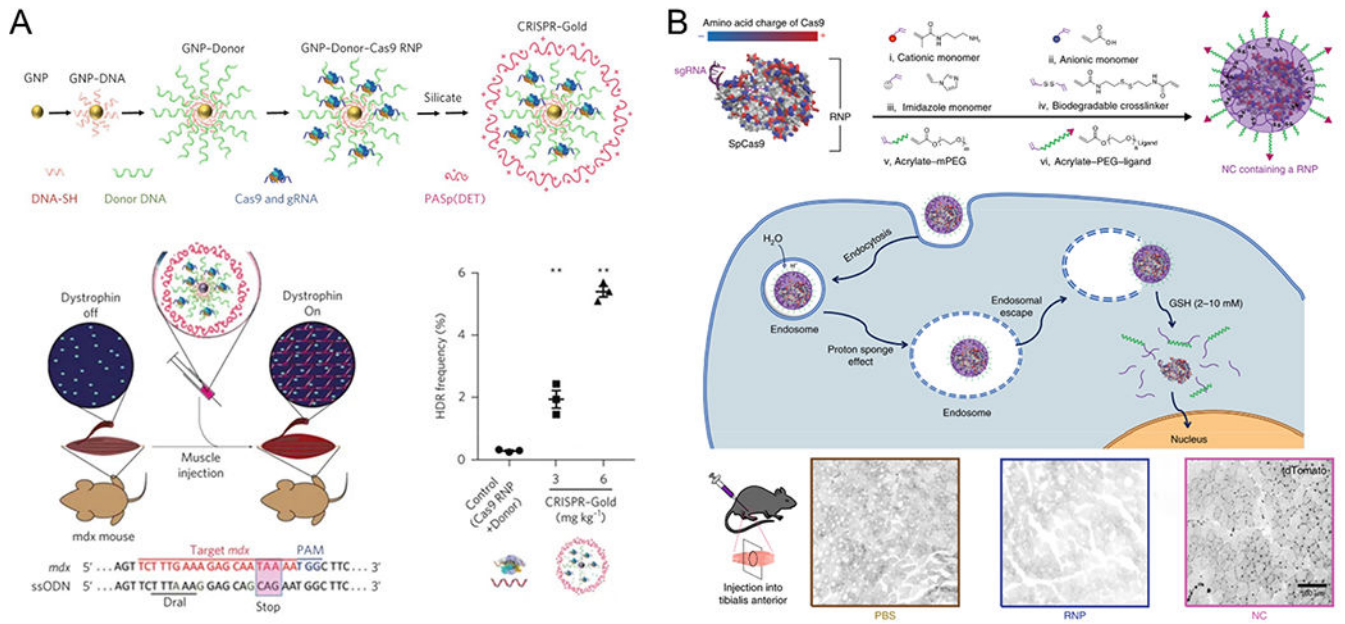


Figure 5.

Nonviral delivery systems for *in vivo* genome editing via intramuscular injection. **(A)** Schematic representation of CRISPR–Gold synthesis. Intramuscular injection of CRISPR–Gold in *mdx* mice promoted homology-directed repair efficacy in the dystrophin gene, enhanced dystrophin protein expression, and reduced muscle fibrosis. Reproduced with permission from ref 78. Copyright 2017 Springer Nature. **(B)** A schematic illustration of the *in situ* free-radical polymerization of biodegradable nanocapsules (NCs) for the delivery of the Cas9 ribonucleoprotein (RNP) complex and its internalization and the subcellular release of the RNP. Local injections of NCs into the tibialis anterior muscle induced robust gene editing and strong tdTomato signals were observed. Reproduced with permission from ref 79. Copyright 2019 Springer Nature.

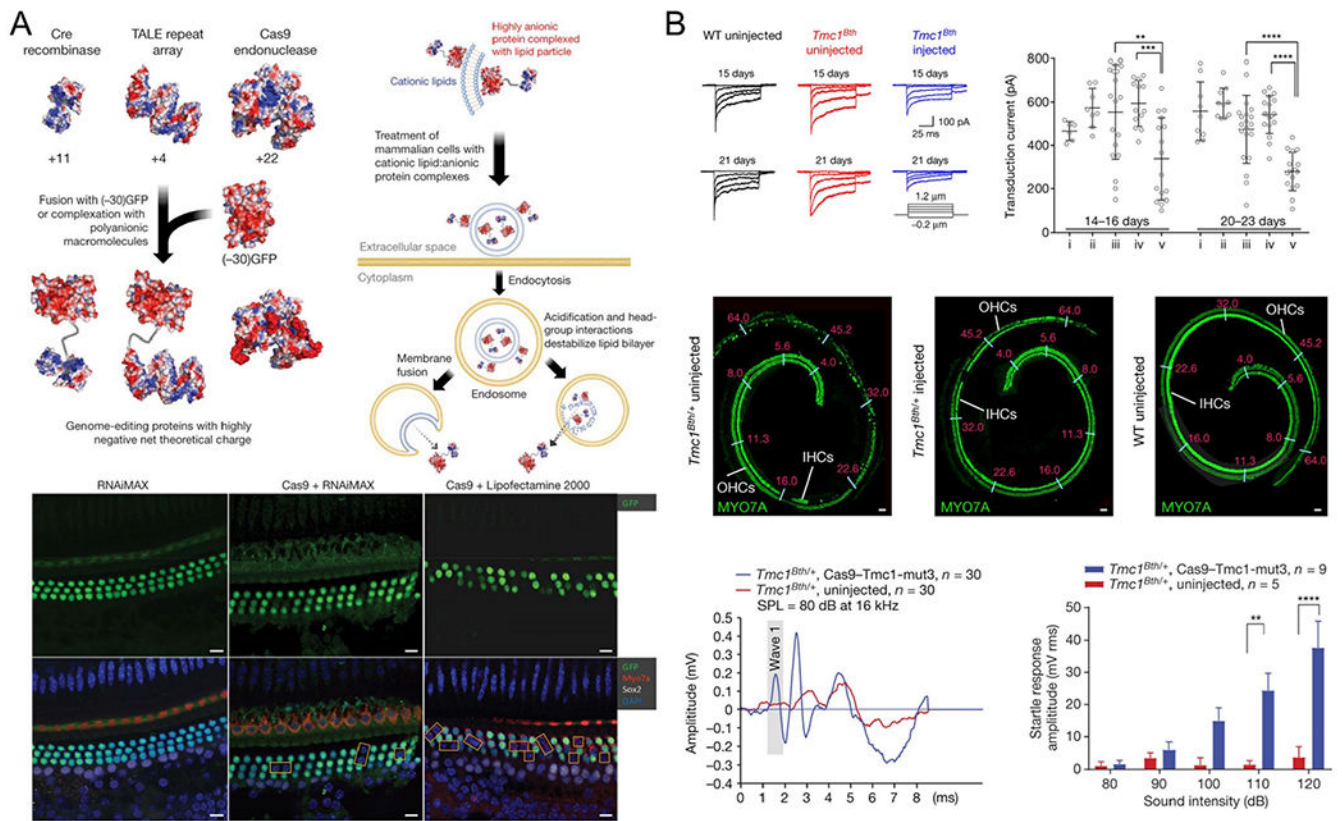


Figure 6. Nonviral delivery systems for *in vivo* genome editing *via* intra-inner ear injection. **(A)** Cationic lipids mediated delivery of proteins into mammalian cells through engineering proteins by fusion or noncovalent complexation with polyanionic macromolecules. Local injection of Cas9/sgRNA complexes delivered by RNAiMAX or Lipofectamine 2000 into the scala media successfully resulted in loss of green fluorescent protein (GFP) expression in hair cells. Reproduced with permission from ref 81. Copyright 2015 Springer Nature. **(B)** Scala media injection of Cas9/Tmc1-mut3 complexed with Lipofectamine 2000 (Cas9-Tmc1-mut3-lipid) promoted hair cell survival and reduced hearing loss in *Tmc1*^{Bth/+} mice. Reproduced with permission from ref 82. Copyright 2018 Springer Nature.

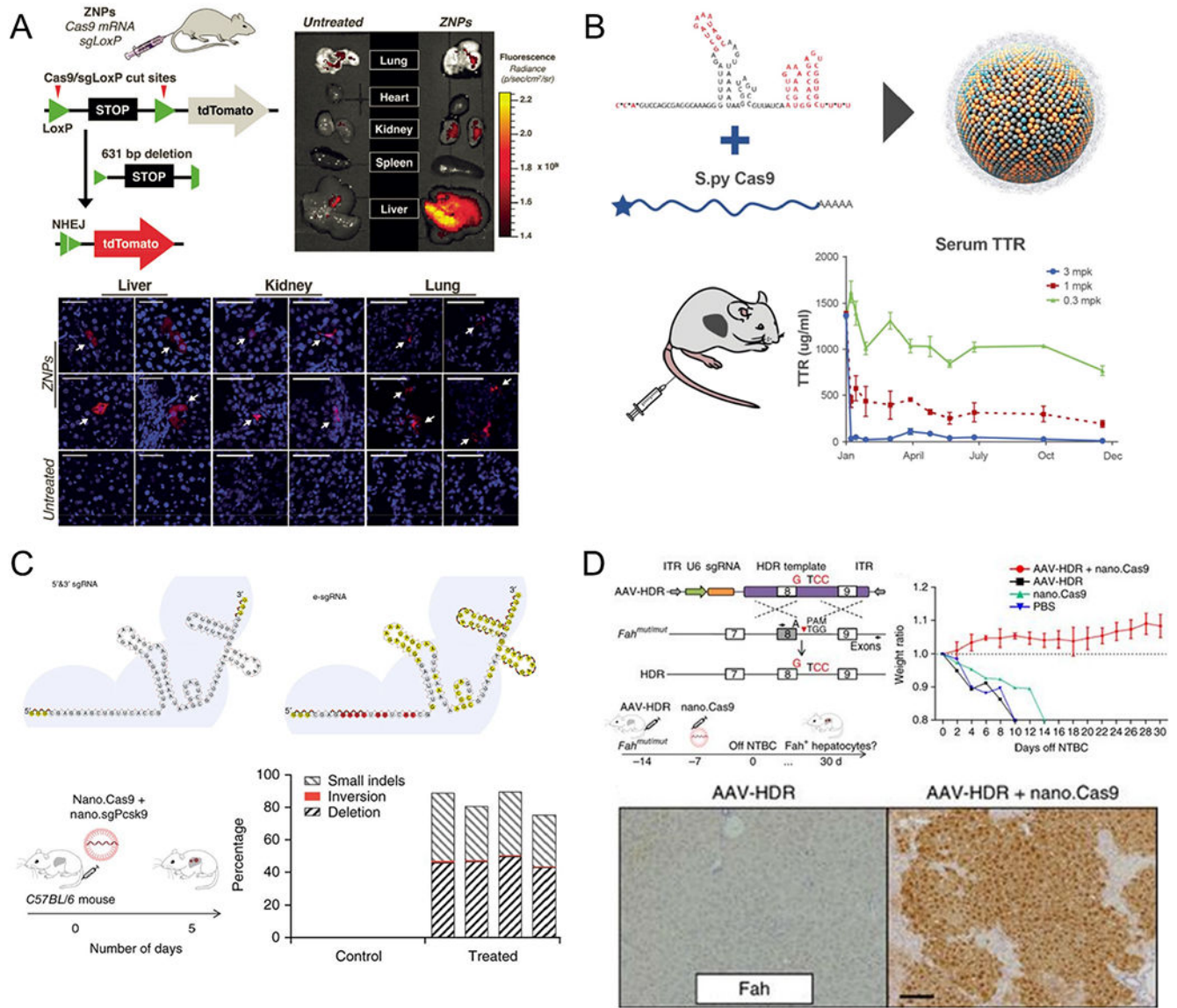


Figure 7. Nonviral delivery systems for liver-targeted genome editing *via* systemic administration. **(A)** zwitterionic amino lipid nanoparticles (ZNPs) co-delivering Cas9 mRNA and sgLoxP successfully deleted the stop cassette and activated expression of tdTomato protein mainly in the liver of tdTomato mouse model. Reproduced with permission from ref 64. Copyright 2017 John Wiley & Sons, Inc. **(B)** Single IV administration of a biodegradable lipid nanoparticle (LNP)-based delivery system (LNP-INT01) achieved and maintained ~70% of gene editing at *Ttr* locus and >97% knockdown of serum TTR levels for at least 12 months. Reproduced with permission from ref 85. Copyright 2018 Elsevier. **(C)** Structure illustration of the conventional 5' and 3' end modification (5' and 3' sgRNA) and the new e-sgRNA design. *In vivo* CKK-E12 LNP-based delivery of two e-sgRNAs targeting *Pcsk9* and Cas9 mRNA mice induced > 80% gene editing of *PCSK9* gene in the mouse liver. Reproduced with permission from ref 87. Copyright 2017 Springer Nature. **(D)** Combination of LNP-

mediated delivery of Cas9 mRNA with adeno-associated viruses (AAVs) encoding a sgRNA and a repair template corrected the causative Fah-splicing mutation, generated Fah-positive hepatocytes, and rescued disease symptoms (including weight loss and liver damage) in type I tyrosinemia mice. Reproduced with permission from ref 51. Copyright 2016 Springer Nature.

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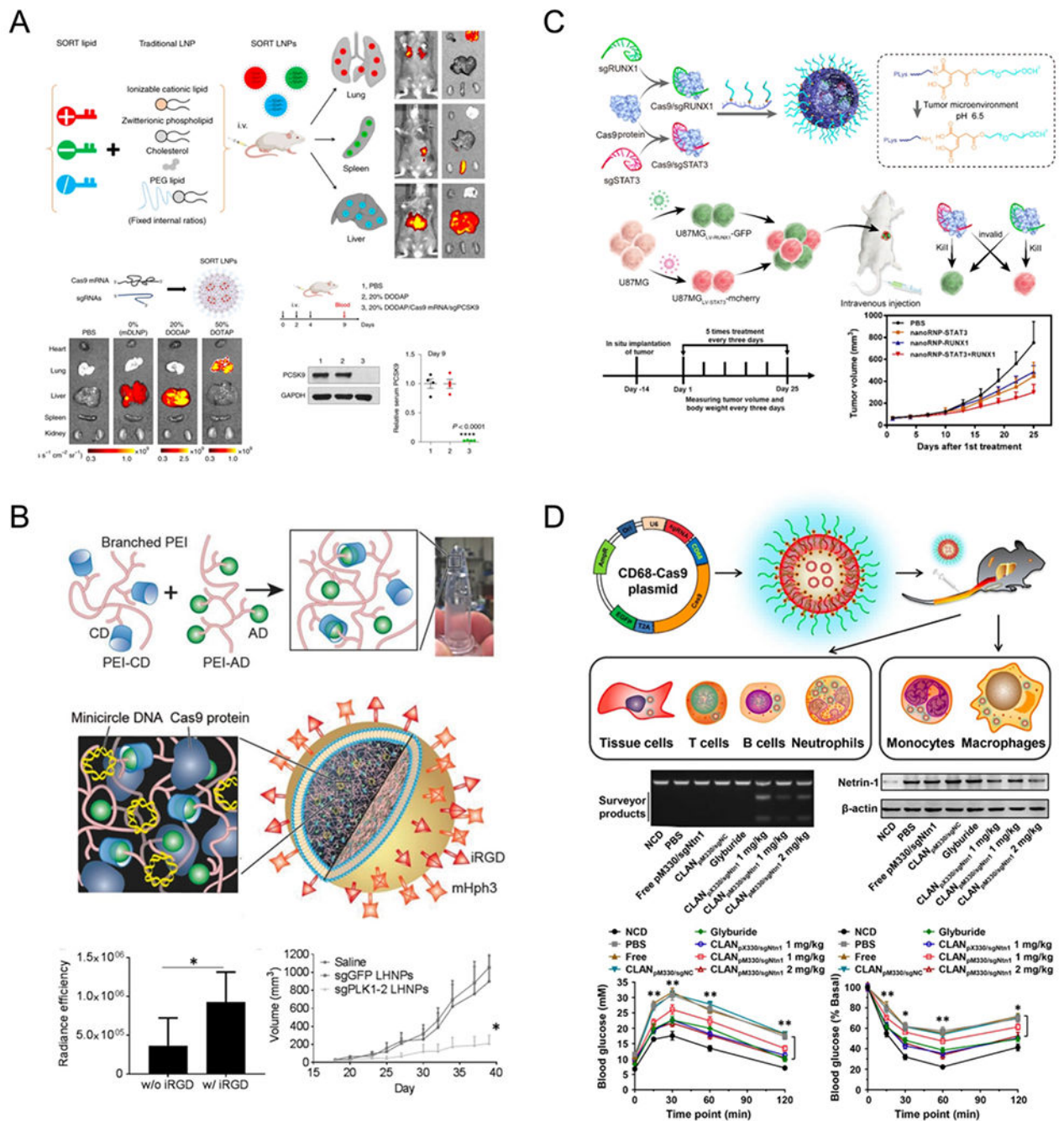


Figure 8. Nonviral delivery systems for genome editing targeting extrahepatic tissues via systemic administration. (A) Selective organ targeting (SORT) enables lipid nanoparticles (LNPs) to be systematically and predictably engineered to deliver mRNA into lung and spleen, in addition to liver. mRNA dendrimer-based lipid nanoparticles (mDLNPs) and SORT LNPs (20% DODAP) induced tdTom fluorescence specifically in the liver and SORT LNPs (50% DOTAP) selectively edited the lung. Co-delivery of Cas9 mRNA and modified sgPCSK9 was achieved with 20% DODAP SORT LNPs in C57BL/6 mice *via* 3 IV injections (days 0,

2, 4). After 9 days, ~60% indel at the PCSK9 locus of liver tissue was detected, leading to ~100% PCSK9 protein reduction in mice serum. Reproduced with permission from ref 100. Copyright 2020 Springer Nature. (B) Liposome-templated hydrogel nanoparticles (LHNPs) were core-shell structures, where the core and shell were formed by DOTAP liposomes and PEI hydrogel, respectively. Systemic injection of iRGD conjugated LHNPs targeting polo-like kinase 1 (Plk1) significantly suppressed tumor proliferation in mice bearing U87 flank tumors. Reproduced from ref 101. Copyright 2017 John Wiley & Sons, Inc. (C) Tumor environment responsive copolymer (PLys₁₀₀-CA-mPEG₇₇) were mixed with Cas9 ribonucleoproteins and formed stable nanostructures, named as nanoRNP. Intravenous injection of nanoRNP targeting *STAT3* and *RUNX1* gene loci effectively inhibited tumor growth in a heterogeneous tumor model. Reproduced from ref 102. Copyright 2019 American Chemical Society. (D) Cationic lipid-assisted PEG-*b*-PLGA nanoparticles (CLANs) encapsulating CD68 promoter-driven CRISPR/Cas9 plasmids could be internalized into diverse cells post intravenous injection, but only allowing Cas9 expressed in monocytes and macrophages. Intravenous injection of CLAN_{pM330/sgNtn1} successfully knocked-out at the *Ntn1* gene locus, specifically in the macrophages and monocytes, inhibited the expression of netrin-1 in macrophages and finally improved type 2 diabetes symptoms in the mouse model. Reproduced with permission from ref 103. Copyright 2018 American Chemical Society.

Table 1.

Ongoing clinical trials of CRISPR/Cas9 based gene therapies.

All clinical trials listed in this table are based on [ClinicalTrials.gov](https://clinicaltrials.gov).¹¹⁷ AAVS, adeno-associated viruses; hHSPCs, human hematopoietic stem and progenitor cells; EBV-CTLs, Epstein-Barr virus-specific cytotoxic T lymphocytes; CAR-T, chimeric antigen receptor (CAR) T-cell; iHSCs, induced hematopoietic stem cells; MSCs, mesenchymal stem cells; CISH, cytokine-induced SH2 protein; TIL, tumor infiltrating lymphocytes.

Study Title	Conditions/Disease	Target Gene	Type	Delivery Systems	Phase	Status	ClinicalTrials.gov identifier
Single Ascending Dose Study in Participants With LCA10	Blindness; Leber Congenital Amaurosis 10 Vision Disorders Eye Diseases Retinal Disease	CEP290 (Correction)	Virus (AAV5)	Adeno-associated virus	Phase 1/2	Recruiting	NCT03872479
A Safety and Efficacy Study Evaluating CTX001 in Subjects With Transfusion-Dependent β -Thalassemia	Beta-Thalassemia Thalassemia Genetic Diseases, Inborn Hematologic Diseases Hemoglobinopathies	BCL11A	CD34+ hHSPCs	Ex vivo	Phase 1/2	Recruiting	NCT03655678
A Safety and Efficacy Study Evaluating CTX001 in Subjects With Severe Sickle Cell Disease	Sickle Cell Disease Hematological Diseases Hemoglobinopathies	BCL11A	CD34+ hHSPCs	Ex vivo	Phase 1/2	Recruiting	NCT03745287
PD-1 Knockout EBV-CTLs for Advanced Stage Epstein-Barr Virus (EBV) Associated Malignancies	Stage IV Gastric Carcinoma Stage IV Nasopharyngeal Carcinoma T-Cell Lymphoma Stage IV Stage IV Adult Hodgkin Lymphoma Stage IV Diffuse Large B-Cell Lymphoma	PD-1	EBV-CTLs	Ex vivo	Phase 1/2	Recruiting	NCT03044743
A Study of Metastatic Gastrointestinal Cancers Treated With Tumor Infiltrating Lymphocytes in Which the Gene Encoding the Intracellular Immune Checkpoint CISH Is Inhibited Using CRISPR Genetic Engineering	Gastrointestinal Epithelial Cancer Gastrointestinal Neoplasms Cancer of Gastrointestinal Tract Cancer, Gastrointestinal Cancer, Gastrointestinal Cancer Colo-rectal Cancer Pancreatic Cancer Gall Bladder Cancer Colon Cancer Esophageal Cancer Stomach Cancer	CISH	TIL	Ex vivo	Phase 1/2	Recruiting	NCT04426669
A Safety and Efficacy Study Evaluating CTX110 in Subjects With Relapsed or Refractory B-Cell Malignancies	B-cell Malignancy Non-Hodgkin Lymphoma B-cell Lymphoma	CD19	CAR-T	Ex vivo	Phase 1/2	Recruiting	NCT04035434
A Feasibility and Safety Study of Universal Dual Specificity CD19 and CD20 or CD22 CAR-T Cell Immunotherapy for Relapsed or Refractory Leukemia and Lymphoma	B Cell Leukemia B Cell Lymphoma	CD19 and CD20 or CD22	CAR-T	Ex vivo	Phase 1/2	Recruiting	NCT03398967
A Study Evaluating UCART019 in	B Cell Leukemia B Cell Lymphoma	CD19	CAR-T	Ex vivo	Phase 1/2	Recruiting	NCT03166878

Study Title	Conditions/Disease	Target Gene	Type	Delivery Systems	Phase	Status	ClinicalTrials.gov identifier
Patients With Relapsed or Refractory CD19+ Leukemia and Lymphoma							
A Safety and Efficacy Study Evaluating CTX120 in Subjects With Relapsed or Refractory Multiple Myeloma	Multiple Myeloma	BCMA	CAR-T	Ex vivo	Phase 1	Recruiting	NCT04244656
Study of PD-1 Gene-knocked Out Mesothelin-directed CAR-T Cells With the Conditioning of PC in Mesothelin Positive Multiple Solid Tumors	Solid Tumor, Adult	PD-1	CAR-T	Ex vivo	Phase 1	Recruiting	NCT03747965
Cell Therapy for High Risk T-Cell Malignancies Using CD7-Specific CAR Expressed On Autologous T Cells	T-cell Acute Lymphoblastic Leukemia T-cell Acute Lymphoblastic Lymphoma T-non-Hodgkin Lymphoma	CD-7	CAR-T	Ex vivo	Phase 1	Not yet recruiting	NCT03690011
Study of CRISPR-Cas9 Mediated PD-1 and TCR Gene-knocked Out Mesothelin-directed CAR-T Cells in Patients With Mesothelin Positive Multiple Solid Tumors.	Solid Tumor, Adult	PD-1/TCR	CAR-T	Ex vivo	Phase 1	Recruiting	NCT03545815
CRISPR (HPK1) Edited CD19-specific CAR-T Cells (XYF19 CAR-T Cells) for CD 19+ Leukemia or Lymphoma	Leukemia Lymphocytic Acute (ALL) in Relapse Leukemia Lymphocytic Acute (All) Refractory Lymphoma, B-Cell CD 19 Positive	CD19	CAR-T	Ex vivo	Phase 1	Recruiting	NCT04037566
A Safety and Efficacy Study Evaluating CTX130 in Subjects With Relapsed or Refractory Renal Cell Carcinoma	Renal Cell Carcinoma	CD70	CAR-T	Ex vivo	Phase 1	Recruiting	NCT04438083
TACE Combined With PD-1 Knockout Engineered T Cell in Advanced Hepatocellular Carcinoma	Advanced Hepatocellular Carcinoma	PD-1	T cells	Ex vivo	Phase 1	Recruiting	NCT04417764
PD-1 Knockout Engineered T Cells for Metastatic Non-small Cell Lung Cancer	Metastatic Non-small Cell Lung Cancer	PD-1	T cells	Ex vivo	Phase 1	Active, not recruiting	NCT02793856
A Safety and Efficacy Study of TALEN and CRISPR/Cas9 in the Treatment of HPV-related Cervical Intraepithelial Neoplasial	Human Papillomavirus-Related Malignant Neoplasm	E6/E7	Plasmid	Gel	Phase 1	Unknown	NCT03057912

Study Title	Conditions/Disease	Target Gene	Type	Delivery Systems	Phase	Status	ClinicalTrials.gov identifier
Cell Therapy for High Risk T-Cell Malignancies Using CD7-Specific CAR Expressed On Autologous T Cells	T-cell Acute Lymphoblastic Leukemia T-cell Acute Lymphoblastic Lymphoma T-non-Hodgkin Lymphoma	CD7	CAR-T	Ex vivo	Phase 1	Not yet recruiting	NCT03690011
iHSCs With the Gene Correction of HBB Intervent Subjects With β -thalassemia Mutations	Thalassemia	HBB (Correction)	iHSCs	Ex vivo	Early Phase 1	Not yet recruiting	NCT03728322

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