Acta Pharmacologica Sinica (2017) 38: 738–753
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Review

Therapeutic gene editing: delivery and regulatory perspectives

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Abstract

Gene-editing technology is an emerging therapeutic modality for manipulating the eukaryotic genome by using target-sequence-specific engineered nucleases. Because of the exceptional advantages that gene-editing technology offers in facilitating the accurate correction of sequences in a genome, gene editing-based therapy is being aggressively developed as a next-generation therapeutic approach to treat a wide range of diseases. However, strategies for precise engineering and delivery of gene-editing nucleases, including zinc finger nucleases, transcription activator-like effector nuclease, and CRISPR/Cas9 (clustered regularly interspaced short palindromic repeats-associated nuclease Cas9), present major obstacles to the development of gene-editing therapies, as with other gene-targeting therapeutics. Currently, viral and non-viral vectors are being studied for the delivery of these nucleases into cells in the form of DNA, mRNA, or proteins. Clinical trials are already ongoing, and *in vivo* studies are actively investigating the applicability of CRISPR/Cas9 techniques. However, the concept of correcting the genome poses major concerns from a regulatory perspective, especially in terms of safety. This review addresses current research trends and delivery strategies for gene editing-based therapeutics in non-clinical and clinical settings and considers the associated regulatory issues.

Keywords: gene editing; zinc finger nuclease; transcription activator-like effector nuclease; CRISPR/Cas9; delivery system; regulation; safety

Acta Pharmacologica Sinica (2017) 38: 738–753; doi: 10.1038/aps.2017.2; published online Apr 10 2017

Introduction

Gene-editing technology has recently emerged as a new treatment modality for a variety of diseases, including hereditary, infectious, and neoplastic diseases. The remarkable progress in nuclease engineering has enabled accurate modification of the eukaryotic genome for therapeutic purposes[1, 2]. Gene-editing nucleases are restriction enzymes with engineered DNA-binding domains that produce double-stranded breaks (DSBs). The three major types of gene-editing nucleases, listed in order of their development, are zinc finger nuclease (ZFN)[3], transcription activator-like effector nuclease (TALEN)[4], and clustered regularly interspaced short palindromic repeats (CRISPR)-associated nuclease Cas9 (CRISPR/Cas9)[5]. The application of CRISPR/Cas9 to genome editing, in particular, has had a tremendous effect on the rate at which genetically engineered materials for gene therapy have been developed, owing to the simplicity of the manufacturing process, as compared with earlier generations of engineered nucleases, including ZFN and TALEN[6].

Gene-editing-based therapy has considerable merits compared with transient expression-modulating gene therapy[1, 2, 5]. For inducing gene expression, functional genes have been delivered to target cells or tissues that lack specific functions. For decreasing gene expression, short nucleic acid sequences have been introduced to silence or interfere with the disease-related gene. However, both strategies have limitations, such as an incomplete therapeutic effect, owing to transient gene silencing and mutagenesis caused by incorrect gene insertion. In contrast, therapeutics based on gene-editing technology can exert permanent and elaborate proofreading effects at the genome level and thus represent a key development in gene therapy.

There are currently dozens of clinical studies of gene-editing-based therapeutics, including *in vivo* and *ex vivo* editing therapies. For the successful translation of gene-editing-based

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Received: 2016-11-14 Accepted: 2017-02-04

therapeutics to the clinic, efficient and safe delivery systems are indispensable[7]. Various delivery systems[7–9] and electroporation techniques[10–13] have been studied to introduce gene-editing nucleases in the form of DNA[14–18], mRNA[19–22], or protein[23–28]. Viral vectors used for *ex vivo* and *in vivo* delivery of nuclease-encoding genes include adeno-associated virus[29–33], adenovirus[34–37], and lentivirus[38–42]. Additionally, non-viral delivery systems, lipid-based nanoparticles[24, 25, 43–48], polymeric nanoparticles[49–52], and cell-penetrating peptides[26, 27] have been investigated.

Currently, there are several guidelines for gene-based therapeutics and cell therapies. These guidelines cover quality control[53–56], safety[56–59], and efficacy[54, 59–61] issues of gene-based therapeutics and may provide a basis for regulatory considerations regarding the evaluation of gene-editing therapeutics. Despite the rapid progress and clinical significance of gene-editing technology, explicit regulatory guidelines focusing on the preclinical and clinical evaluation of gene-editing therapeutics are not yet available. In this review, we address the strategies for delivering gene-editing-based therapeutics and consider the regulatory aspects of nuclease delivery systems.

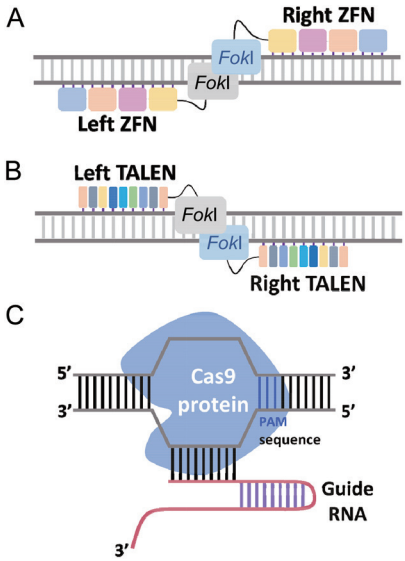

Figure 1. Gene-editing nucleases. Gene-editing nucleases include ZFN (A), TALEN (B), and CRISPR/Cas9 (C).

Gene-editing nucleases

Gene-editing nucleases (Figure 1) are composed of a target-sequence-recognizing domain (guide RNA, in the CRISPR/Cas9 system) and a nuclease[1]. After the programmed nuclease cleaves the target gene, repair of DSBs proceeds through two different mechanisms: non-homologous end joining (NHEJ) and homology-dependent repair (HDR). NHEJ, which eliminates the target region by joining DSBs, can be utilized to silence or correct a pathogenic gene, whereas HDR can introduce a homologous sequence into DSBs, enabling donor DNA to be inserted to either correct an existing gene or add a new one. The properties of gene-editing nucleases and the differences among them are summarized in Table 1.

ZFN (Figure 1A) contains a Cys_2-His_2 zinc finger and *Fok*I nuclease as the DNA-binding and cleavage domains, respectively[62]. Because each zinc finger module (~30 amino acids) recognizes a specific 3-base-pair DNA sequence, several zinc finger modules are typically engineered on the basis of the target sequence. After the binding of zinc finger modules to target DNA, the *Fok*I restriction enzyme mediates DNA cleavage. In general, if more than three modules are engineered, symmetrical *Fok*I dimerization is required for increased target specificity and cleavage efficiency. Although zinc fingers have demonstrated their potential as genetic 'scissors' in mammalian cells, the strategy is limited because the selected target region must meet the requirement of a 5′-GNNGNNGNN-3′ motif. Engineering a zinc finger module for each target gene is also expensive and time consuming.

Table 1. Properties of standard gene-editing nucleases.

	ZFN	TALEN	CRISPR/Cas9
DNA binding	Zinc finger protein	TALE protein	Guide RNA
DNA cleavage	*Fok*I	*Fok*I	Cas9
DNA recognition range	18–36 bp (3 bp/Zinc finger module)	30–40 bp (1 bp/TALE module)	22 bp (DNA-RNA base pairing)
Recognition sequence	Sequence containing G base as follows: 5'-GNNGNNGNN-3'	Sequence starting from 5'-T and ending with A-3'	Sequence immediately followed by an adjacent protospacer motif 5'-NGG-3'
Advantages	Sequence-based module engineering Small protein size (<1 kb)	High specificity Accurate recognition by 1 bp Relatively easy selection of target region	Free selection of target region Simple synthesis of guide RNA Multiplexing ability
Limitations	Difficult sequence selection and zinc finger engineering Expensive and time-consuming	Not applicable to methyl cytosine Expensive and time-consuming Large protein size (>3 kb)	Large protein size (>4 kb)

TALEN (Figure 1B), like ZFN, also utilizes *Fok*I nuclease as a cleavage domain[4]. The DNA-binding domain, TALE, which originates from the plant pathogen *Xanthomonas campestris*, contains 34–35 amino acids per module, two of which, termed repeat-variable di-residues, determine the specific DNA base pair recognized. The recognition of a single DNA base pair by one TALE module is beneficial in terms of selecting the target regions; however, the complicated engineering procedures required for the recognition of a wider range of target gene sequences have been a matter of concern. To solve the problem, researchers have developed a TALEN plasmid library that recognizes 14–18 base pairs and can target 18742 human genes[63].

The CRISPR/Cas9 system (Figure 1C) is based on a prokaryotic antiviral mechanism in which bacteria insert a partial gene sequence from an infection source, such as a bacteriophage, into their own genomes to defend against repeat infection[64]. Specifically, CRISPR/Cas9 uses a guide RNA to bind a complementary sequence in a target gene, after which Cas9 recognizes and cuts the target DNA. The CRISPR/Cas9-based system has revolutionized gene editing, and the DNA-binding function of short guide RNA offers the potential for the rapid development of gene-editing-based therapeutics as simple-facile designs of singleguide RNA (sgRNA) design tools and multiplexing ability (several target DNAs in the same cells) become available[65].

In vivo and *ex vivo* gene editing

Therapeutic gene editing can be administered through two basic strategies: (1) direct in vivo delivery of a gene-editing nuclease and (2) delivery of cells engineered *ex vivo* to contain a gene-editing nuclease (Figure 2)[1]. Appropriate delivery systems for *in vivo* gene editing include viral vectors and cationic lipid- or polymer-based non-viral vectors, because the cargo for gene editing usually involves a plasmid vector or mRNA encoding the gene-editing nuclease. Occasionally, naked plasmids have been locally injected into particular tissues, such as eye and muscle. Because systemic injection of gene-editing nucleases can drive gene modifications in multiple tissues, depending on the delivery strategy, the benefits of gene editing can be more readily extended to various diseases, as compared with *ex vivo* gene editing. Similarly, the tissue-wide distribution associated with *in vivo* delivery raises the issue of tailoring the biodistribution and pharmacokinetic profiles of gene-editing delivery carriers to minimize side effects in off-target tissue. *Ex vivo* gene editing requires a somewhat complicated procedure for *ex vivo* genetic modifications before transplantation. Removal of target cells from the patient is the first step in using an *ex vivo* strategy to deliver gene-editing nucleases. Viral vectors have been favored for the delivery of gene-editing systems to hard-to-transfect cells, such as immune cells and stem cells. Moreover, stimulus-based strategies, such as electroporation and magnetofection, are possible for *ex vivo* gene editing, because the selection of a delivery vector for this type of gene editing is not affected by the behavior of the vector *in vivo* (Table 2).

Current strategies for the delivery of gene-editing therapeutics

Gene-editing-based therapy has been actively studied for investigational and non-clinical development (Figure 3). The majority of these studies have been conducted in the United States (54.4%), and the next highest percentage of studies (19.1%) have been conducted in China (Figure 3A). Viral

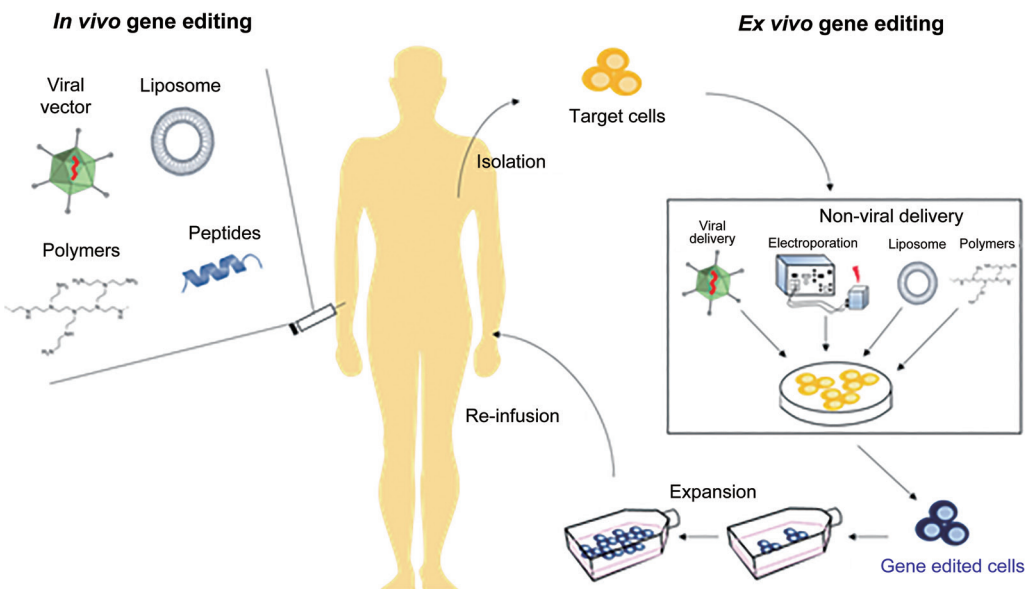

Figure 2. Therapeutic gene-editing strategies. A schematic depiction of *in vivo* and *ex vivo* gene editing is shown. For *in vivo* gene editing, viral or non-viral vectors carrying nucleases are directly injected into the body. For *ex vivo* gene editing, the target cells are isolated and gene-edited with viral or non-viral vectors carrying nucleases, after which gene-edited cells are expanded and reinfused into the body.

Table 2. *In vitro* and *in vivo* gene-editing studies in non-clinical development.

Vector	Target gene	Disease	Nuclease	Material type of nucleases	In/Del	*In vitro*/ *In vivo*	Organization	Country	Ref
Lentiviral vectors	CXCR4	HIV	CRISPR/Cas9	DNA	Del	*In vitro*	Wuhan University	China	[38]
	HBV S, X	HBV	CRISPR/Cas9	DNA	Del	*In vitro*	Heinrich Pette Institute	Germany	[40]
	MSRB1	HIV	CRISPR/Cas9	DNA	Del	*In vitro*	Temple University	USA	[39]
	ICP0	HSV	CRISPR/Cas9	DNA	Del	*In vitro*	Temple University	USA	[41]
	RUNX2	Osteosarcoma	CRISPR/Cas9	DNA	Del	*In vitro*	National Cancer Institute	USA	[42]
	HIV long terminal repeats	HIV	CRISPR/Cas9	DNA	Del	*In vitro*	Temple University	USA	[72]
	CCR5	Duchenne muscular dystrophy	ZFN	DNA	In	*In vivo*	Université de Montréal	Canada	[69]
Adenoviral vectors	CCR5	HIV	CRISPR/Cas9	DNA	Del	*In vitro*	Chinese Academy of Sciences	China	[34]
	HBB	Sickle cell disease	CRISPR/Cas9	DNA	In	*In vitro*	University of Alabama at Birmingham	USA	[35]
	CXCR4	HIV	ZFN	DNA	Del	*In vivo*	The Scripps Research Institute	USA	[36]
	CCR5	HIV	ZFN	DNA	Del	*In vivo*	Beckman Research Institute	USA	[37]
Lentivirus and adenovirus	DMD	Duchenne muscular dystrophy	TALEN	DNA	Del	*In vitro*	Leiden University Medical Center	Netherlands	[73]
AAV	MIR137	Neurological disorders	CRISPR/Cas9	DNA	Del	*In vivo*	University of North Carolina at Chapel Hill	USA	[29]
	DMD	Duchenne muscular dystrophy	CRISPR/Cas9	DNA	In/Del	*In vivo*	Harvard University	USA	[30]
	OTC	Hyperammonemia	CRISPR/Cas9	DNA	In/Del	*In vivo*	University of Pennsylvania	USA	[31]
	F9	Hemophilia B	ZFN	DNA	In/Del	*In vivo*	Children's Hospital of Philadelphia	USA	[32]
	G6Pase	Glycogen storage disease type IA	ZFN	DNA	In/Del	*In vivo*	Duke University	USA	[33]
Electroporation	CFTR	Cystic fibrosis	CRISPR/Cas9	DNA	In	*In vitro*	The Salk Institute of Biological Studies	USA	[10]
	ASXL1	Chronic myeloid leukemia	CRISPR/Cas9	DNA	In/Del	*In vivo*	Oxford University Hospital	UK	[15]
	HBB	β-Thalassemia	CRISPR/Cas9, TALEN	DNA	In/Del	*In vitro*	Chinese Academy of medical Sciences	China	[11]
	p17	HIV	TALEN	DNA	Del	*In vitro*	Kyoto Prefectural University of Medicine	Japan	[16]
	Rho	Retinitis pigmentosa	CRISPR/Cas9	DNA	In/Del	*In vivo*	Cedars-Sinai Medical Center	USA	[17]
	GGTA1, CMAH	Acute humoral xenograft rejection	CRISPR/Cas9	DNA	Del	*In vitro*	Indiana University School of Medicine	USA	[18]
	ACVR1	Fibrodysplasia ossificans progressiva syndrome	CRISPR/Cas9	DNA	Del	*In vitro*	Korea Institute of Oriental Medicine	Korea	[14]
	FMR1	Fragile X syndrome	CRISPR/Cas9	DNA	In	*In vitro*	Waisman Center	USA	[74]
	PD-1	Gastric cancer, melanoma	CRISPR/Cas9	DNA	Del	*In vitro*	Medical School of Nanjing University	China	[75]
	EPHA1	Colorectal cancer	CRISPR/Cas9	DNA	Del	*In vitro*	Cardiff University School of Medicine	UK	[76]
	DMD	Duchenne muscular dystrophy	CRISPR/Cas9	DNA	In/Del	*In vivo*	The Ohio State University Wexner Medical Center	USA	[12]
	DMD	Duchenne muscular dystrophy	CRISPR/Cas9	DNA	In/Del	*In vivo*	University of California	USA	[77]
	AAVS1 locus	Chronic granulomatous disease	ZFN	mRNA	In/Del	*In vivo*	National Institutes of Health	USA	[20]

(To be continued)

Vector	Target gene	Disease	Nuclease	Material type of nucleases	In/Del	*In vitro*/ *In vivo*	Organization	Country	Ref
	HBB	Sickle cell disease	ZFN	mRNA	In/Del	*In vivo*	University of California	USA	[21]
	CCR5	HIV	ZFN	DNA	Del	*In vivo*	University of Southern California	USA	[13]
	HBB	Sickle cell disease	ZFN	DNA	In/Del	*In vitro*	Stanford University School of Medicine	USA	[78]
	CCR5	HIV	ZFN	DNA	Del	*In vitro*	Lerdsin General Hospital	Thailand	[79]
	HBB	β-Thalassemia	TALEN	DNA	In/Del	*In vitro*	Guangzhou Institutes of Biomedicine and Health	China	[80]
	AR	Prostate cancer	TALEN	DNA	Del	*In vitro*	Masonic Cancer Center	USA	[81]
	COL7A1	Recessive dystrophic epidermolysis bullosa	TALEN	DNA or mRNA	In/Del	*In vitro*	University of Minnesota	USA	[19]
	F8	Hemophilia A	TALEN	DNA	In/Del	*In vitro*	Severance Biomedical Research Institute	Korea	[82]
	PKLR	Pyruvate kinase deficiency	TALEN	DNA	In/Del	*In vitro*	Centro de Investigaciones Energe´ticas	Spain	[83]
	CCR5	HIV	TALEN	mRNA	Del	*In vitro*	University Medical Centre Hamburg-Eppendorf	Germany	[22]
	DMD	Duchenne muscular dystrophy	ZFN	DNA	In/Del	*In vivo*	Duke University	USA	[84]
	HBB	β-Thalassemia	CRISPR/Cas9	DNA	In/Del	*In vitro*	Guangzhou Medical University	China	[85]
	CCR5	HIV	CRISPR/Cas9	Protein	In/Del	*In vitro*	Seoul National University	Korea	[23]
	NOX2	X-linked chronic granulomatous disease	TALEN	DNA	In	*In vitro*	Hannover Medical School	Germany	[86]
Polymers	CCR5	HIV	ZFN	DNA	Del	*In vitro*	State Key Laboratory of Respiratory Disease	China	[49]
	E6, E7	Cervical cancer	TALEN	DNA	Del	*In vitro*	Huazhong University of Science and Technology	China	[50]
	E7	Cervical cancer	ZFN	DNA	Del	*In vivo*	Huazhong University of Science and Technology	China	[51]
	HBB	Sickle cell anemia	TALEN	DNA	In/Del	*In vitro*	University of Illinois	USA	[52]
Liposomes	HBV genome	HBV	CRISPR/Cas9	DNA	Del	*In vivo*	Soochow University	China	[43]
	HBV genome	HBV	CRISPR/Cas9	DNA	Del	*In vitro*	Tongji University	China	[44]
	HBV genome	HBV	ZFN	DNA	Del	*In vitro*	Drew University	USA	[45]
	CCR5	HIV	ZFN	DNA	Del	*In vitro*	Universitat Auto-noma de Barcelona	Spain	[46]
	HMGB1	Bladder Urothelial Carcinoma	TALEN	DNA	Del	*In vitro*	Loudi Central Hospital of Hunan Province	China	[47]
	CFTR	Cystic Fibrosis	ZFN	DNA	In/Del	*In vitro*	University College Cork	Ireland	[48]
	EGFP	N/A	CRISPR/Cas9, TALEN	Protein	Del	*In vivo*	Harvard University	USA	[24]
	EGFP	N/A	CRISPR/Cas9	Protein	Del	*In vivo*	Tufts University	USA	[25]
Cell penetrating peptide	CCR5	HIV	CRISPR/Cas9	Protein	Del	*In vitro*	Hanyang University	Korea	[26]
	CCR5	HIV	TALEN	Protein	Del	*In vitro*	The Scripps Research Institute	USA	[27]
Microinjection	Cdh23	Age-related hearing loss	CRISPR/Cas9	mRNA	In/Del	*In vivo*	Mary Lyon Centre, MRC Harwell, Harwell, Oxford	UK	[87]
	HBB	Sickle cell anemia	CRISPR/Cas9, TALEN	DNA	In/Del	*In vitro*	Georgia Institute of Technology and Emory University	USA	[88]
Hypothermia	EGFP	N/A	ZFN	Protein	Del	*In vitro*	The Scripps Research Institute	USA	[28]

(To be continued)

Vector	Target gene	Disease	Nuclease	Material type of nucleases	In/Del	*In vitro*/ *In vivo*	Organization	Country	Ref
Non-viral combination	Factor VIII	Hemophilia A	CRISPR/Cas9	DNA	In/Del	*In vivo*	Yonsei University College of Medicine	Korea	[89]
	E7	Cervical cancer	CRISPR/Cas9	DNA	Del	*In vitro*	Huazhong University of Science and Technology	China	[90]
	FANCC	Fanconi Anemia	CRISPR/Cas9	DNA	Del	*In vitro*	Division of Blood and Marrow Transplantation	USA	[91]
	PSIP1	HIV	TALEN	DNA	Del	*In vitro*	Mayo Clinic College of Medicine	USA	[92]
Viral/non-viral combination	HBB	Sickle cell disease	CRISPR/Cas9, TALEN	mRNA	In/Del	*In vitro*	University of California	USA	[93]
	Fah	Tyrosinemia	CRISPR/Cas9	mRNA	In/Del	*In vivo*	Massachusetts Institute of Technology	USA	[94]

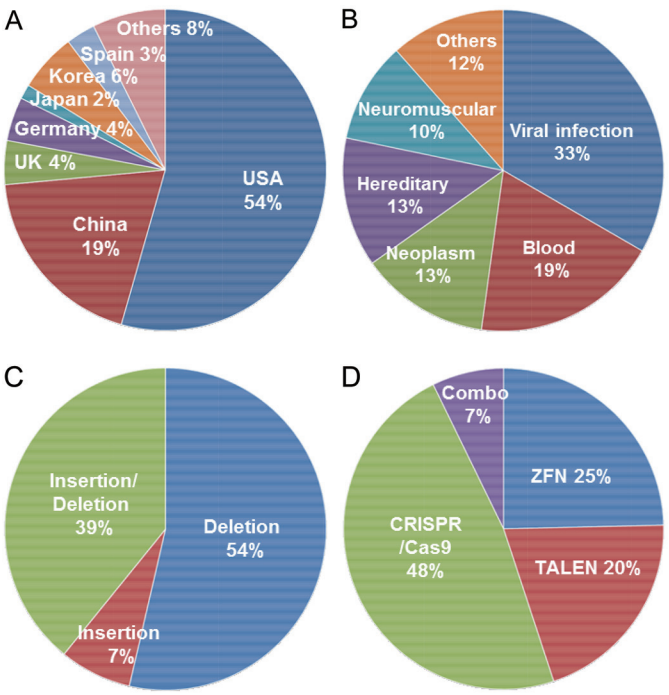

Figure 3. Current status of gene-editing studies in non-clinical development. Therapeutic gene editing in non-clinical development, analyzed by country (A), target disease (B), editing type (C), and nuclease type (D).

infection (33%), blood disease (19%), and neoplasms (13%) have been the major disease targets of therapeutic gene editing (Figure 2). Although CRISPR/Cas9-mediated gene editing is the most recently developed gene-editing tool, it has been the most widely investigated both *in vitro* and *in vivo*, owing to its convenience and accessibility (Figure 3D).

The delivery strategies for gene editing are divided into viral and non-viral vector systems (Figure 4). Unlike traditional gene therapy methods, non-viral vector-based studies have predominated (70%) over viral vector-based studies in investigations of gene-editing therapies (Figure 4A), because electroporation, a non-viral method, is the main approach utilized for *ex vivo* gene editing (39% of all reports). In the case of viral vectors, lentiviruses are most often used, followed by adenovirus-associated virus (AAV) and adenovirus, respectively (Figure 4B). After electroporation, which is by far the most important non-viral method for *ex vivo* gene delivery (56%), the next most common delivery methods are based on lipids (17%) and polymers (8%) (Figure 4C).

Lentiviral vectors have the advantage of being able to integrate into dividing and non-dividing cells; they also have a relatively large capacity (~8 kb), thus facilitating the delivery of large nuclease-coding sequences[66]. Lentiviral vectors have traditionally been used for the transfer of gene-editing components into hard-to-transfect cells, such as T cells[38, 39, 67] and primary cells[38, 68, 69], which are used to treat human immunodeficiency virus (HIV) infections and neuromuscular disease, among others. Compared with adenoviruses, AAVs are less pathogenic and non-immunogenic and are thus suitable for *in vivo* gene editing to exert influences throughout the body[70]. Indeed, most approaches that have used AAV delivery systems have been applied to investigate *in vivo* gene editing in animals.

Among the non-viral delivery systems, electroporation, a traditional gene-transfer method, is the most effective in delivering genetic materials and thus exhibits high transfection efficacy in hard-to-transfect cells[7]. However, this method has limitations for direct application *in vivo*, including cytotoxicity and immune-stimulating effects. Therefore, electroporation has been widely used for *ex vivo* gene editing of immune cells, stem cells, and primary cells[10, 14, 19]. Lipid- or polymer-based non-viral vectors, compared with viral delivery vectors, are relatively safer *in vivo* and can be modified in various ways for target-cell-specific delivery[71]. Because cationic liposomes or polymers usually load nucleic acid cargo via electrostatic interactions, they enable various types of gene-editing components, such as mRNAs and short RNAs, to function immediately in the cytoplasm after cellular entry.

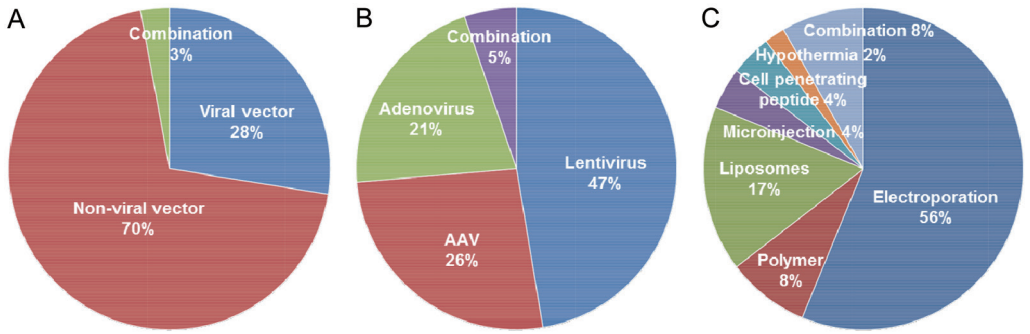

Figure 4. Delivery strategies for gene-editing studies in non-clinical development. Therapeutic gene editing in non-clinical development, analyzed based on delivery strategy (A), viral vector type (B), and non-viral type (C).

Clinical trials of gene-editing-based therapeutics

Currently, 18 gene-editing-based therapeutics are undergoing clinical trials worldwide (www.clinicaltrials.gov) as depicted in Figure 5. ZFN-based approaches, with 11 trials (6 of which are organized by Sangamo Bioscience), represent the majority (61%) of these clinical studies. Among the trials using ZFNs, three are Phase I/II trials targeting the *CCR5* (C–C motif chemokine receptor 5) gene for HIV infection. The remaining eight ZFN studies are Phase I trials varying in terms of target gene, disease, and delivery strategy. In the case of TALEN trials, three Phase I trials of multiplex gene editing targeting *TCRa* (T cell receptor a) and *CD52* to improve the efficacy of chimeric antigen receptor (CAR)-modified T cells targeting CD19 (CD19-CAR-T; UCART19, licensed to Servier) are currently underway. The most recently developed CRISPR/Cas9 system has recently been used in clinical trials in China designed to knock out *PDCD1*(programmed cell death protein 1) as a treatment for multiple cancers.

The gene-editing delivery strategies used in clinical trials can be classified into three categories: viral vectors (6 studies), electroporation (6 studies), and naked plasmids (1 study) (Table 3). The *ex vivo* gene-editing strategies targeting CCR5 to inhibit HIV entry use adenoviral vectors (4 studies) or the electroporation method (3 studies) for T cell transfection. Electroporation of TALEN mRNA (3 studies) has also been used for *TCRa/CD52* knockout in the trials of CD19-CAR-T. Although information on vectors is not yet available for the four trials using *ex vivo* CRISPR/Cas9 gene editing, viral vectors or electroporation methods are presumably used on the basis of their efficient T cell transfection. *In vivo* administration of AAV vectors has also been used in hepatocyte-targeted gene-editing therapy for the treatment of hemophilia B and MPS I. A *CCR5*-knockout T cell line (SB-728-T), a leading therapeutic gene-editing candidate, has been constructed by adenoviral delivery of ZFN[95]. In a Phase I safety study (NCT00842634), 12 HIV-infected patients received a single infusion of 5×10^9–10×10^9 *CCR5*-knockout T cells. The blood

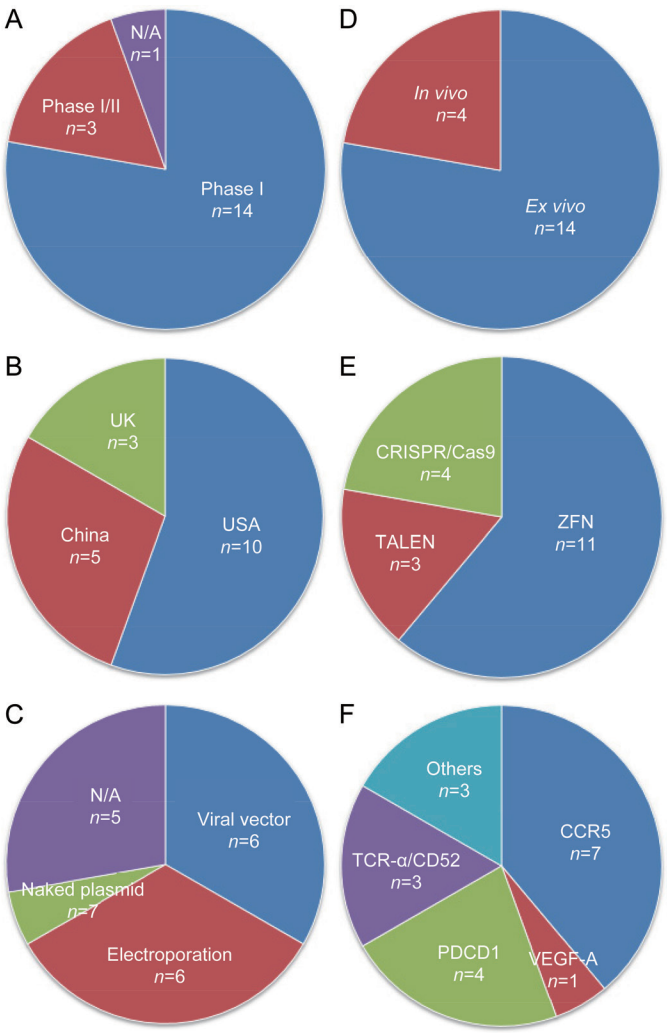

Figure 5. Current status of therapeutic gene editing clinical trials. Clinical studies of therapeutic gene editing, analyzed by phase (A), country (B), delivery vector (C), editing strategy (D), nuclease type (E), and target gene (F).

Table 3. Therapeutic gene editing in clinical trials.

Vector	Phases	Target gene	Disease	Nuclease	*Ex/In vivo*	Interventions	Enrollment	Country	Organization	NCT number
Adenoviral vectors	I	CCR5	HIV	ZFN	*Ex vivo*	ZFN-modified CD4+T cells	12	USA	University of Pennsylvania	NCT00842634
	I	CCR5	HIV	ZFN	*Ex vivo*	ZFN-modified CD4+T cells	19	USA	Sangamo Biosciences	NCT01044654
	I/II	CCR5	HIV	ZFN	*Ex vivo*	ZFN-modified CD4+T cells	21	USA	Sangamo Biosciences	NCT01252641
	I/II	CCR5	HIV	ZFN	*Ex vivo*	ZFN-modified CD4+T cells	26	USA	Sangamo Biosciences	NCT01543152
AAV vectors	I	Factor IX	Hemophilia B	ZFN	*In vivo*	ZFP-encoding rAAV(SB-FIX)	9	USA	Sangamo Biosciences	NCT02695160
	I	IDUA	MPS I	ZFN	*In vivo*	ZFP-encoding rAAV (SB-318)	9	USA	Sangamo Biosciences	NCT02702115
Electroporation (mRNA)	I	TCR-α/CD52	Relapsed/ refractory B acute lymphoblastic leukemia	TALEN	*Ex vivo*	TALEN-modified CD19-CAR-T	10	UK	Institut de Recherches Internationales Servier	NCT02808442
	I/II	CCR5	HIV	ZFN	*Ex vivo*	ZFN-modified T cells+ cyclophosphamide	12	USA	Sangamo Biosciences	NCT02225665
	I	CCR5	HIV	ZFN	*Ex vivo*	ZFN-modified CD4+T cell+ cyclophosphamide	15	USA	University of Pennsylvania	NCT02388594
	I	CCR5	HIV	ZFN	*Ex vivo*	ZFN-modified HSPC+busulfan	12	USA	City of Hope Medical Center	NCT02500849
	N/A	TCR-α/CD52	ALM	TALEN	*Ex vivo*	TALEN-modified anti-CD19 CAR-T	200	UK	Institut de Recherches Internationales Servier	NCT02735083
	I	TCR-α/CD52	ALM	TALEN	*Ex vivo*	TALEN-modified anti-CD19 CAR-T	12	UK	Servier	NCT02746952
Naked plasmid	I	VEGF-A	Intermittent claudication	ZFN	*In vivo*	DNA Plasmid Vector	10	USA	National Heart, Lung, and Blood Institute (NHLBI)	NCT00080392
N/A	I	E7	HPV-related malignant neoplasm	ZFN	*In vivo*	ZFN-603 and ZFN-758	20	China	Huazhong University of Science and Technology	NCT02800369
	I	PDCD1	Metastatic Non-small cell lung cancer	CRISPR/ Cas9	*Ex vivo*	CRISPR/Cas9-edited T cells+ cyclophosphamide+Interleukin-2	15	China	Sichuan University	NCT02793856
	I	PDCD1	Invasive bladder cancer stage IV	CRISPR/ Cas9	*Ex vivo*	CRISPR/Cas9-edited T cells+ cyclophosphamide+interleukin-2	20	China	Peking University	NCT02863913
	I	PDCD1	Metastatic renal cell carcinoma	CRISPR/ Cas9	*Ex vivo*	CRISPR/Cas9-edited T cells+ cyclophosphamide+interleukin-2	20	China	Peking University	NCT02867332
	I	PDCD1	Hormone refractory prostate cancer	CRISPR/ Cas9	*Ex vivo*	CRISPR/Cas9-edited T cells+ cyclophosphamide+interleukin-2	20	China	Peking University	NCT02867345

ALM, Advanced Lymphoid Malignancies.

concentration of SB-728-T was consistently maintained, exhibiting a mean half-life of 48 weeks (loss rate, 1.81 cells/d). In comparison, the loss rate of the unmodified T cells was 7.25 cells per day. In the preliminary stage of a Phase II trial, an adenoviral delivery strategy also induced additional CD8-mediated immune stimulation, thus suggesting an adjuvant-like effect of the adenovirus used for gene editing.

Regulatory perspectives

With the growing number of studies investigating gene-editing-based therapeutics comes the need to address regulatory concerns that might affect clinical applications[54, 60, 96]. Although it is not possible to clearly outline all the potential regulatory concerns relevant to gene-editing-based therapeutics, the major issues include safety, efficacy, and quality control. In fact, the genetic materials for delivery of gene-editing nucleases are not markedly different from those for conventional *ex vivo*/*in vivo* gene therapies. Thus, quality control and efficacy evaluations of *in vivo* gene-editing therapeutics can be considered in the context of existing gene-therapy guidelines[54–61, 97]. Similarly, existing gene/cell therapy guidelines can be applied to *ex vivo* gene-editing therapeutics. The existing guidelines relevant for gene-editing therapeutics issued by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) are summarized in Table 4. However, novel gene-editing mechanisms using exogenous nucleases raise significant new safety concerns.

Gene-editing-based therapeutics introduce a new degree of complexity to delivery methods and delivery systems[98]. Depending on the mode of delivery, the risk levels and concerns can vary[99]. Overall, the risk is higher for direct *in vivo* delivery compared with *ex vivo* delivery of gene-edited cell therapeutics. The delivery system itself can also affect risk levels. The use of viral vectors, compared with proteins or mRNAs delivered through non-viral vectors or electroporation, may raise greater safety concerns. Because of the high risks associated with *in vivo* gene editing at the current stage of development, most gene-editing-based therapeutics currently in clinical trials use the *ex vivo* gene editing strategy. In this review, we will focus greater attention on the *ex vivo* delivery form of gene-editing therapeutics.

Safety

Safety is the most important issue from a regulatory perspective. The working mechanism of nuclease-mediated genome editing presents a double-edged sword: whereas it provides a therapeutic effect that is unprecedented in its power, it also bears major safety concerns[100]. Because gene-editing thera-

Table 4. Relevant guidance documents for gene-editing therapeutics.

Guidance titles	Regulatory agencies	Year
Guidance for industry: FDA guidance for human somatic cell therapy and gene therapy	FDA	1998
Quality, preclinical and clinical aspects of gene transfer medicinal products	EMA	2001
Guideline on development and manufacture of lentiviral vectors	EMA	2005
Guidance for industry: gene therapy clinical trials-observing participants for delayed adverse events	FDA	2006
Guidance for industry: supplemental guidance on testing for replication competent retrovirus in retroviral vector based gene therapy products and during follow-up of patients in clinical trials using retroviral vectors	FDA	2006
Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors	EMA	2007
Guideline on potency testing of cell based immunotherapy medicinal products for the treatment of cancer	EMA	2007
Guidance for FDA reviewers and sponsors: content and review of chemistry, manufacturing, and control (CMC) information for human gene therapy investigational new drug applications (INDs)	FDA	2008
Guideline on the non-clinical studies required before first clinical use of gene therapy medicinal products	EMA	2008
Guideline on human cell-based medicinal products	EMA	2008
Guideline on safety and efficacy follow-up- risk management of advanced therapy medicinal products	EMA	2008
ICH consideration oncolytic viruses	EMA	2009
Follow-up of patients administered with gene therapy medicinal products	EMA	2009
Quality, non-clinical and clinical issues relating specifically to recombinant adeno-associated viral vectors	EMA	2010
Guideline on the minimum quality and non-clinical data for certification of advanced therapy medicinal products	EMA	2010
Guideline on immunogenicity assessment of monoclonal antibodies intended for *in vivo* clinical use	EMA	2010
Guidance for industry: potency test for cellular and gene therapy products	FDA	2011
Guideline on quality, non-clinical and clinical aspects of medicinal products containing genetically modified cells	EMA	2012
Preclinical assessment of investigational cellular and gene therapy products	FDA	2013
Guideline on the risk-based approach according to annex I, part IV of directive 2001/83/EC applied to advanced therapy medicinal products	EMA	2013
Guideline on similar biological medicinal products containing biotechnology-derived proteins as active substance: non-clinical and clinical issues	EMA	2013
Management of clinical risks deriving from insertional mutagenesis	EMA	2013
Guidance of industry: considerations for the design of early-phase clinical trials of cellular and gene therapy products	FDA	2015

peutics act by creating DSBs in genomic DNA, the risks of off-target toxicity at unintended sites are higher than those associated with other gene therapeutics that do not induce chromosomal insertions or genome alterations. Moreover, unlike chemicals and antibody-based drugs, the genome-level action of gene-editing therapeutics evokes concerns about the selection of relevant animal models for safety studies. For *in vivo* gene-editing therapeutics, the binding specificity of the designed nucleases is governed by specific sequences in the genome. Because the mouse genome has substantial differences compared with the human genome, preclinical safety studies, especially for *in vivo* gene-editing therapeutics, should be conducted in humanized animal models that mimic the human genome.

Off-target genotoxicity

Although the engineered nucleases possess a targeting specificity for accurate gene editing, unintended interactions of the nucleases and the consequent cleavage of non-target sites nonetheless occur[101]. "Off-target" genotoxicity can be defined as toxic side effects caused by unintended gene cleavage at non-target sites. The CRISPR/Cas9 system has a relatively higher chance of generating such 'off-target' cleavages, because sgRNA can bind a mismatched sequence with a partial complementation. The off-target effect has been regarded as a major concern for clinical application of gene editing therapeutics. Because gene-editing technology, by nature, modifies the genome, the most important safety issue is the possibility of genotoxicity through the modification of non-target genes[101]. Alterations of the genome at non-target loci are classified primarily as large-scale translocations or deletions and small-scale insertions/deletion (indels). A variety of appropriate methods are available for studies designed to test the occurrence of large- and small-scale off-target genotoxicity. Functional studies of gene-edited cells would be suitable for initial off-target genotoxicity studies. These functional studies could include an examination of changes in the viability, proliferation, and cell-cycle behavior of gene-edited cells.

If no changes in the functional behavior of the gene-edited cells are observed, the next step would be to test for large- and small-scale off-target effects. Large-scale genomic changes include translocation, deletion, and inversion[70]. These large-scale genomic changes can be detected by using array comparative genomic hybridization techniques and genotyping in gene-edited cells used in *ex vivo* therapeutics or in biopsied cells from *in vivo* therapeutic applications.

If no evidence of large-scale gene modification is found, the next step would be to test small-scale indel frequencies at off-target loci. In silico surveys, whole-exome profiling, and whole-genome profiling can be used to test the occurrence of off-target indels in gene-edited therapeutics. Whole-exome profiling can reveal changes in the exons encoding all proteins and should be performed for any delivery form. Recently, whole-exome profiling has been used to test the off-target effects of CRISPR/Cas nucleases[102]. DNA fragments derived from viral vectors or plasmid DNA, compared with proteins or mRNA delivered using non-viral vectors or electroporation, may have higher risks of insertion into DSBs at off-target sites.

Thus, whole-genome profiling may be a necessary adjunct for applications in which nucleases are delivered by viral vectors or plasmid DNA. For genome-wide profiling, Guide-seq[103] and Digenome-seq[104] systems have been used to assess off-target cleavage by CRISPR/Cas nucleases. A T7 endonuclease 1 cleavage assay has also been used to verify small-scale off-target indels and mutations introduced by CRISPR/Cas nucleases[105].

Gene-editing therapeutics use several pathways for editing genes, each with its own benefits. After the cleavage of genomic DNA by ZFN, TALEN or CRISPR/Cas9, the resulting DSBs are repaired by either NHEJ or HDR mechanisms. In the latter mechanism, both crossover and non-crossover pathways are possible. If NHEJ is the major pathway, indels and mutations may be the predominant genotoxicities. If HDR and gene addition are the major pathways, the possibility of off-target insertion should be carefully monitored.

Recent studies have reported that the frequency of small-scale indels varies depending on cell type[106]. For comparison with other studies and for evaluating the consistencies of gene-editing techniques, the frequencies of small-scale indels must be quantified for both *ex vivo* gene-edited cells and *in vivo*-biopsied cells.

Immunogenicity

The immunogenicity of gene-editing therapeutics is an important consideration that must be assessed regardless of the delivery method and nuclease type. ZFN, TALEN, and CRISPR/Cas9 are all exogenous and foreign to the human body. Notably, intracellular processing and presentation of these antigens by major histocompatibility molecules have not yet been studied. However, given the foreign nature of bacterial nucleases such as TALEN and CRISPR/Cas9, the induction of antibodies against these nucleases should be investigated[107]. In addition to inducing humoral immune responses, the presentation of antigens derived from these foreign nucleases with major histocompatibility complex I molecules on gene-edited cell surfaces may also evoke T-cell immune responses. Moreover, the possibility of autoimmunity to autologous and gene-edited cell therapeutics should be addressed.

When viral vectors are used for *in vivo* gene editing, the development of antibodies and T-cell immune responses against the viral vectors can limit the repeated use of the same viral vectors[108]. Indeed, the possibility of host immune responses caused by the bacterial origin of CRISPR/Cas9 proteins and viral vectors has been suggested to be one of the hurdles for the use of *in vivo* gene editing in viral-vector-based therapy[109]. AAV vectors have been most widely used in gene editing, because AAV vector was authorized for the delivery of Glybera, the first approved gene therapeutic in Europe. However, previous studies have shown that AAV vectors can induce antibodies and T-cell responses[110, 111]; impurities in viral vector preparations, such as host cell proteins and other

contaminants, can also influence the immunogenicity of the recombinant viral vectors that encode nucleases[112]. In addition, the virus capsid protein along with prolonged expression of the encoded nucleases after the delivery of *in vivo* viral-vector-based gene-editing therapeutics can induce antibodies and T-cell immune responses. Although non-viral vectors such as polymeric nanoparticles and lipid nanoparticles are considered to be less immunogenic than viral vectors[109], the immunogenicity of the expressed nucleases should also be studied, as should the induction of antibodies against the proteins or peptides assembled within the nanoparticles that are used for *in vivo* non-viral delivery of nucleases and guide RNA.

Pharmacokinetics and biodistribution

The pharmacokinetics of gene-editing therapeutics may differ between *ex vivo* and *in vivo* strategies[113, 114]. For *ex vivo* gene-editing therapeutics, pharmacokinetic studies of the time-dependent profiles of gene-edited cells must be performed. In addition to the level of gene-edited cells in the blood, the DNA levels of the gene-modified segments should be monitored through quantitative polymerase chain reactions. For *in vivo* gene-editing therapeutics, the form of the delivery system is expected to affect the pharmacokinetic profile. If viral vectors are used for the *in vivo* delivery of gene-editing nucleases, the pharmacokinetic profiles of the vectors should be traced by using relevant markers and by monitoring the encoded nuclease gene[115]. If protein forms of the nucleases are used for their *in vivo* delivery, levels of the protein nucleases should be monitored in the blood possibly by using enzyme-linked immunosorbent assays[116]. If mRNA or naked plasmid DNA is used for the *in vivo* delivery of nucleases via electroporation, the introduced forms of nuclease-encoding nucleic acids in the blood should also be quantified.

Studies of the *in vivo* distribution of *ex vivo* gene-editing therapeutics must address two aspects: the *in vivo* fate of the gene-edited cells and the distribution of nucleases used for gene modification. If *ex vivo* modifications are performed with protein forms of the nucleases, the distribution of nuclease protein levels would be relevant. If *ex vivo* modifications are performed with the mRNA form of nucleases, distribution studies would be best performed by following mRNA and protein levels in each organ. Moreover, if *ex vivo* modifications are performed with the DNA form of nucleases, distribution studies should investigate the DNA levels. Finally, if the *ex vivo* modification is performed with a viral vector encoding nuclease DNA, the persistence of foreign nuclease DNA in the body should be studied as well.

The *in vivo* distribution of *in vivo* gene-editing therapeutics may require more extensive investigations, owing to the systemic circulation of nucleases administered by various delivery vectors[57, 58, 117]. Currently, one of the major target diseases of gene-editing therapeutics is cancer. Thus, it would be important to know the target distribution and gene-editing effects of nucleases in cancer tissues. However, the distribution and modification of genes in normal tissues by systemically injected nucleases may cause severe side effects. Especially in cases where *in vivo* gene editing is performed with viral vectors encoding nucleases and additional genes, distribution studies should test the DNA levels of nucleases and inserted genes in all organs and germ cells.

Tumorigenicity

The tumorigenicity of gene-edited therapeutics is an important aspect that must be investigated. Gene-modified $CD34^+$ cells transduced with a retroviral vector carrying a gene encoding interleukin-2 receptor gamma have been clinically studied for the treatment of X-linked severe combined immunodeficiency. In that study, the retrovirus inserted itself near proto-oncogenes, thus resulting in the development of T-cell leukemia in four patients[118]. The potential tumorigenicity of induced pluripotent stem cells is considered to be one of the major hurdles for stem cell therapies[119]. In the case of induced pluripotent stem cells, retroviral gene insertion has been discussed as one of the factors that contribute to tumorigenicity. These studies have raised concerns about the tumorigenicity risks of insertional gene modifications at off-target sites. Therefore, strong evidence for the lack of tumorigenicity needs to be provided by using animal models before initiation of clinical trials. The use of Onco-chip[120] to screen for changes in oncogenic protein expression would provide relevant supplementary data.

Safety of delivery systems

The safety of delivery systems for nucleases and other components of gene-editing systems should be studied separately[121]. For *in vivo* gene-editing viral vectors, in addition to immunogenicity, chromosomal integration and duration of expression should be monitored. The possibility of viral shedding in the cases in which viral vectors are used for *in vivo* gene-editing therapeutics must be carefully addressed. For viral vectors that are capable of gene editing, sexual transmission to spouses may cause unwanted gene editing. Moreover, the germline transmission of viral vectors should also be monitored.

Although the safety profiles of polymer- or lipid-based non-viral nanoparticles are more favorable because they do not insert into chromosomes and because they exhibit shorter *in vivo* half-lives than viral vectors, the relevant *in vivo* toxicities and inflammation issues must be addressed. In the case of physical device-based delivery methods, such as electroporation, risk factors such as device-mediated infection, inflammation, and tissue damage should be considered.

Efficacy

Depending on the purpose and disease target, the models used to demonstrate the efficacy of gene-editing therapeutics may vary. However, similarly to procedures used to address safety concerns, efficacy studies should use humanized animal models. Factors that should be considered in efficacy studies include, at minimum, the selection of dose, establishment of relevant animal disease models, efficiency of gene editing, and duration of efficacy[1].

Dose units

Unlike chemical drugs, for which doses can be similarly expressed in grams, the dose units for different gene-editing therapeutics may differ depending on the type of delivery method. For *ex vivo* gene-editing therapeutics, the population of gene-edited cells among the total injected cells should be determined[95]. For *in vivo* gene-editing therapeutics, the injection dose may vary depending on the form in which nucleases are delivered. In cases in which nucleases are delivered in protein, mRNA, or plasmid DNA forms, the therapeutic effects should be studied on the basis of the administered dose in grams of protein or nucleic acid per body weight. In the case of nucleases delivered by viral vectors, the therapeutic effects should be studied on the basis of the administered doses according to viral titers.

Dose-efficacy correlation

Unlike chemical drugs, which generally show gradually increasing dose effects until a saturating dose is reached, gene-editing therapeutics may show rapid turn-on or turn-off effects over narrow dose ranges. As seen in the case of recently emerging RNA interference (RNAi)-based drugs that act at the mRNA level, rapid degradation of siRNA in the cytoplasmic environment necessitates high doses of injected siRNA. However, the action of gene-editing therapeutics at the genome level may result in an increase in potency, given that a single correction of the genome may result in perpetual correction of cellular protein levels[1]. Indel-based *ex vivo* gene-editing therapeutics may have 'on' or 'off' effects depending on the occurrence of DSBs at the target locus. Thus, the efficacy of gene-editing therapeutics should be studied at various dose units, depending on the method of nuclease delivery.

Efficiency of gene editing

The efficiency of gene editing must be quantified statistically. The efficiency of NHEJ- and HDR-mediated DSB repair is known to vary according to cell type and cell state[1]. Additionally, the efficiency of HDR-mediated gene addition has been reported to depend on the selected target gene[122]. Quantification of gene-editing efficiency would be helpful in comparing the efficiency of various vectors and standardizing the relationship between editing efficiency and therapeutic efficacy. Currently, the efficiency of *in vivo* HDR is known to be low[123].

Duration of gene editing

Spontaneous mutations and instability of the genome may abrogate the therapeutic effects of gene editing. The duration of gene deletion, site-specific correction, or insertion at the target locus after treatment with gene-editing therapeutics should be monitored over months[124]. Especially in the case of *in vivo* viral-vector-based gene-editing therapeutics, the prolonged expression of nucleases in the body can reintroduce DSBs at nearby target sites and lead to instability[125]. The duration of gene editing should thus be more carefully monitored in the case of *in vivo* viral-vector-based gene-editing therapeutics.

Animal models for efficacy tests

Compared with chromosome-insertional viral-vector-based gene therapy, gene editing-based therapeutics have a novel ability to elaborately and controllably modify the genome by recognizing target gene sequences for deletion or insertion. In principle, humanized animal models should be used for studying the on-target efficacy of gene-editing therapeutics. Because the establishment of humanized animal models other than rodents may be difficult, non-human primates might be used, although their genomes differ from the human genome. When non-human primates are used as large animal models, in silico-based studies should be conducted to validate the similarity of target gene sequences between primates and humans[124].

Quality control

Quality control of gene-editing therapeutics may not differ substantially from existing guidelines on gene therapeutics and gene-modified cell therapeutics. The quality control-related guidance for gene and cell therapeutics issued by the FDA and EMA are summarized in Table 4. The main factors to consider for quality control include purity and the absence of microbial contamination and endotoxins. Especially in the case of *ex vivo* gene-editing therapeutics, the viability and fraction of gene-modified cells must be controlled.

Characterization

For *ex vivo* gene-editing therapeutics, the phenotypes of gene-edited cells should be characterized by using relevant markers. For *in vivo* gene-editing therapeutics, delivery vectors should be physicochemically characterized, and especially when non-viral chemical vectors are used, the sizes, zeta potentials, and nuclease/vector ratios must be determined[59, 60, 126].

Purity

For *ex vivo* gene-editing therapeutics, consistent amounts of gene-edited cells should be produced with minimal batch-to-batch variations. Currently, autologous cells are isolated for *ex vivo* gene-editing therapeutics and are injected back into patients. However, transfection and gene-editing efficiencies may vary depending on the age and health of the individual. The ranges of acceptable gene-edited cell populations among total cells should be determined on the basis of statistical analysis of a sufficient number of patients[124]. For *in vivo* gene editing, therapeutics using viral vectors for delivery of nuclease genes should be tested for purity. Regardless of the *ex vivo* or *in vivo* gene-editing strategy, the injectable forms should be free of endotoxins.

Sterility of gene-editing therapeutics

For *ex vivo* gene-editing therapeutics, autologous cells are harvested, and nucleases and other gene-editing components such as single guide RNA, are introduced, after which the gene-edited cells are administered back into patients. Because the cells for *ex vivo* gene-editing therapeutics are processed

outside the body, microbial studies should be performed. In the case of *in vivo* gene-editing therapeutics, viral vectors should be tested and shown to be free of pathogenic microbial and mycoplasma contamination[55, 124].

Viability of ex vivo therapeutics

In the case of *ex vivo* therapeutics, the viability of the gene-edited cells should be tested and consistently monitored. For this purpose, viability studies capable of differentiating gene-edited cells from non-edited cells in the total cell population should be designed and used[53, 124].

Manufacturing processes

The process for manufacturing gene-editing therapeutics should be validated to ensure the sterility and consistency of products[54, 56, 124]. For *ex vivo* gene-editing therapeutics, the procedure for isolating cells from patients, transduction of the cells with gene-editing delivery systems, and cultivation of the gene-edited cells should be validated. Additionally, quality assurance assays should be performed. For *in vivo* viral vector-based gene-editing therapeutics, the processes for amplifying, purifying, identifying, and quantifying viral vectors should be validated. For *in vivo* non-viral vector-based gene-editing therapeutics, the acceptable size ranges of non-viral vectors, nuclease loading, and processes for identifying nuclease-loaded non-viral vectors must be validated.

Conclusions

Gene editing is a rapidly growing technology in the overall field of biotechnology, thus reflecting its potential as an innovative genetic manipulation tool. Indeed, gene-editing therapy has attracted substantial interest from international pharmaceutical companies and has undergone a profusion of clinical trials within a relatively short period. Although gene-editing therapeutics have undergone remarkable progress, considerations of regulatory issues have not kept up with the pace of development. For clinical applications, safety issues should be thoroughly considered on the basis of gene-editing strategies, including *ex vivo*/*in vivo* methods, type of genetic materials, and delivery vectors. Given the potential for off-target genotoxicity and other crucial considerations, the release of new regulations tailored to gene-editing therapeutics would facilitate the development of this potent new treatment modality from bench-to-bedside.

Acknowledgements

This research was supported by a grant (No 16172MFDS206) from the Ministry of Food and Drug Safety in 2016 and from the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education (No NRF-2016R1A6A3A11930441).

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