Pharmacology & Therapeutics 209 (2020) 107501

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Recent advances in genome editing of stem cells for drug discovery and therapeutic application

Jaesuk Lee [a,b,c,1], Delger Bayarsaikhan [a,1], Govigerel Bayarsaikhan [a,1], Jin-Soo Kim [b,c,***], Elizabeth Schwarzbach [d,**], Bonghee Lee [a,*]

[a] Lee Gil Ya Cancer and Diabetes Institute, Gachon University, Incheon, Republic of Korea
[b] Department of Chemistry, Seoul National University, Seoul, Republic of Korea
[c] Center for Genome Engineering, Institute for Basic Science, Daejeon, Republic of Korea
[d] The New York Stem Cell Foundation Research Institute, New York, NY, United States

ARTICLE INFO

Available online 13 February 2020

Keywords:
Genome editing
Stem cells
Drug discovery
Therapeutic application

ABSTRACT

Genome engineering technologies right from viral vector-mediated to protein-based editing— which include zinc finger nucleases, TALENs, and CRISPR/Cas systems—have been improved significantly. These technologies have facilitated drug discovery and have resulted in the development of potential curative therapies for many intractable diseases. They can efficiently correct genetic errors; however, these technologies have limitations, such as off-target effects and possible safety issues, which need to be considered when employing these techniques in humans. Significant efforts have been made to overcome these limitations and to accelerate the clinical implementation of these technologies. In this review, we focus on the recent technological advancements in genome engineering and their applications in stem cells to enable efficient discovery of drugs and treatment of intractable diseases.

Contents

Abbreviations: AAVS, Adeno-associated virus integration site; ABE, Adenosine base editor; AGE, Advanced glycation end products; Bp, Base pair; Cas9, CRISPR associated protein 9; CBE, Cytidine base editor; CRISPR, Clustered regularly interspaced short palindrome repeats; CRISPRi, CRISPR interference; crRNA, CRISPR RNA; DMD, Duchenne muscular dystrophy; DNA, Deoxyribonucleic acid; ESC, Embryonic stem cell; FA, Fanconi anemia; FACS, Fluorescence-activated cell sorting; FDA, Food and Drug Administration, United States; GFP, Green fluorescent protein; HIF, Hypoxia inducible factor; HIV, Human immunodeficiency virus; HDR, Homology-directed repair; HSPC, Hematopoietic stem or progenitor cell; iPSC, Induced pluripotent stem cell; mRNA, messenger RNA; MSC, Mesenchymal stem cell; NHEJ, Non-homologous end joining; NIH, US, National Institutes of Health, United States; NOD, Non-obese diabetic; NSC, Neural progenitor stem cell; NSG, NOD/SCID gamma mouse; PAM, Protospacer-adjacent motif; rAAV, Recombinant adeno-associated virus; RNA, Ribonucleic acid; RNP, Ribonucleoprotein; saCas9, *Staphylococcus aureus* Cas9; SCD, Sickle cell disease; SCID, Severe combined immunodeficiency; sgRNA, single guide RNA; TadA, transfer RNA adenine deaminase; TALE, Transcription activator-like effector; TALEN, Transcription activator-like effector nucleases; tracrRNA, Trans-activating crRNA; UGI, Uracil glycosylase inhibitor; UDG, Uracil DNA glycosylase; ZFN, Zinc finger nuclease.

* Correspondence to: Bonghee Lee, Center for Genomics and Proteomics, Lee Gil Ya Cancer and Diabetes Institute, Gachon University, Incheon 21999, Republic of Korea.
** Correspondence to: Elizabeth Schwarzbach, The New York Stem Cell Foundation Research Institute, New York, NY 10019, United States.
*** Correspondence to: Jin-Soo Kim, Department of Chemistry, Seoul National University, Seoul 08826, Republic of Korea.
E-mail addresses: jskim01@snu.ac.kr (J.-S. Kim), eschwarzbach@nyscf.org (E. Schwarzbach), bhlee@gachon.ac.kr (B. Lee).
[1]These authors contributed equally to this work.

https://doi.org/10.1016/j.pharmthera.2020.107501

1. Introduction

Genome editing is widely used in biological research, especially in stem cell research on human diseases, for the identification and validation of novel therapeutic targets, and for the development of novel therapeutic agents (Qi, 2017). Generally, genome editing strategies involve DNA modifications in living organisms; these include "beneficial" deletions, corrections *via* gene replacement, and insertions, and corresponding protocols have been established for introducing these modifications in stem cells during the last decades (Chen & Gonçalves, 2018). Genome editing technologies based on common engineered nuclease-based platforms, such as zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and clustered regularly interspaced short palindrome repeats (CRISPR) in combination with Cas9 RNA-guided endonucleases, utilize the endogenous DNA repair machinery to easily target almost any genomic location (Kim & Kim, 2014; Li et al., 2019).

Currently, two main genome editing-based approaches are widely studied as promising strategies for cellular therapy, (i) *ex vivo* genome editing-based stem or somatic cell therapy, which consists of *in vitro* transduction/correction of therapeutic genes in stem or somatic cells, which are then transplanted back into the recipients (Dever et al., 2016; Torikai et al., 2016); (ii) *in vivo* genome editing in stem or somatic cells, which consists of transduction/correction of therapeutic genes in endogenous cells *via* virus-, protein-, or mRNA-based delivery of the genome editing cargo (Ho, Loh, Chan, & Soh, 2018). The *ex vivo* approach is used more widely than the *in vivo* approach because of associated advantages, such as the ease of editing, safety, and high efficiency, but is limited by the availability of target cells (Tong, Moyo, Lee, Leong, & Bao, 2019).

In this review, we summarize the different widely used techniques for genome editing and the recent advances in *ex vivo* or *in vivo* genome editing in stem cells to treat genetic or intractable diseases. We also discuss the utility, impact, and limitations of using genome editing as a precision medicine.

2. Genome editing technology

Genome editing technology evolved in the late 1970s with advances in biotechnology, which allowed the successful development of site-specific gene modifications in the yeast *Saccharomyces cerevisiae* (Hinnen, Hicks, & Fink, 1978). Current developments in genome editing technologies are aimed at improving the accuracy and efficiency of these technologies. Depending on the underlying working principles, these technologies are classified into (i) traditional genome editing (conventional homologous recombination and single-stranded donor oligonucleotide homologous recombination) and (ii) programmable nuclease utilizing methods, such as the ZFN, TALEN, and CRISPR systems (Gaj, Sirk, Shui, & Liu, 2016). Despite their unique characteristics, programmable nucleases share a common underlying working principle, *i.e.*, cleavage of chromosomal DNA in a site-specific manner that efficiently leads to precise editing (Table 1) (Kim & Kim, 2014).

2.1. ZFNs

ZFNs were the first programmable nucleases to be introduced for genome editing of stem cells. Prior to the introduction of ZFNs in 1996, it was hypothesized that gene therapy was a promising strategy for managing or even curing diseases as random/untargeted integration of normal genes into disease-causing defective genomes decreased the disease symptoms (Chandrasegaran, 2017). ZFNs are composed of two domains, namely, a DNA-binding zinc-finger domain and a nuclease domain derived from the *Fok*I restriction enzyme. The DNA binding domain is able to bind DNA sequences of up to 18 base pair (bp) in length, whereby each of the 3–6 zinc finger motifs is able to recognize a 3–4 bp DNA sequence. The FokI domain then cleaves the genome (Handel, Alwin, & Cathomen, 2009; Schierling et al., 2012).

ZFN target sites consist of guanine-rich 3 bp subsites, namely, 5′-GNN-3′ where N denotes any nucleotide. However, the clinical translation of ZFNs remains challenging owing to complications in designing zinc finger domains specific for DNA triplets, including 5′-ANN-3′, 5′-CNN-3′, and 5′-TNN-3′ (Chandrasegaran & Carroll, 2016; Koo, Lee, & Kim, 2015). ZFN heterodimers are suitable for adeno-associated virus (AAV)-mediated delivery because they can be easily packaged into ~1 kb/monomer (Lee, Sebo, Peng, & Guo, 2015). Several strategies have been employed to reduce off-target effects, such as the use of well-designed heterodimeric ZFNs, protein engineering, and protein delivery based on the permeability characteristics of recipient cells (Kim & Kini, 2017; Liu, Gaj, Wallen, & Barbas, 2015; Miller et al., 2007). The first cell permeable ZFNs were described by Gaj, Guo, Kato, Sirk, and Barbas 3rd (2012).

Since 2009, ZFN technology has been utilized in a small number of clinical trials in the USA and China. Currently, 165 patients with human immunodeficiency virus (HIV) infection, sickle cell anemia, transfusion dependent β-thalassemia, human papillomavirus-related malignant neoplasm, mucopolysaccharidosis, and hemophilia B are participating in phase I or II clinical trials that are testing ZFNs (NIH US, www.clinicaltrials.gov).

2.2. TALENs

TALENs were first used for genome editing following the identification of DNA-recognizing bacterial proteins. TALENs contain a *Fok*I nuclease domain at their carboxyl terminal and DNA-binding TALE-repaeat domains at their amino terminal (Miller et al., 2011). Although their target site capacities vary, ZFNs and TALENs are based on similar principles. For example, both ZFNs and TALENs utilize the FokI endonuclease domain for cleaving the chromosomal DNA, and the process is activated by two distinct DNA binding domains (Gupta & Musunuru, 2014). In TALENs, approximately 12–20 bp around the spacer sequence are disrupted by the FokI nuclease domains (Certo & Morgan, 2016; Kim et al., 2013). The DNA binding domains of the TALE proteins contain repetitive sequence of residues, each comprised of approximately 34 amino acids (Cuculis, Abil, Zhao, & Schroeder, 2015). The amino acid sequences of these subdomains are almost identical, differing only at positions 12 and 13. This region is known as the repeat variable dinucleotide, which plays a major role in defining the nucleotide specificity of TALEN-based genome editing tools (Lee et al., 2015). Several commonly used repeat variable dinucleotides such as HD (cytosine), NI (adenine), and NG (thymine) are present in TALENs (Gaj et al., 2016).

In principle, TALENs can edit any type of DNA sequence (Kim & Kim, 2014). Despite their large size (6 kb per pair), TALENs can be delivered efficiently inside cells using different tools, such as codon divergent repeat variable di-residues (RVDs), adenoviral vectors, or mRNA- or protein-based gene transfer methods (Cermak et al., 2011; Certo & Morgan, 2016 and Qi, 2017).

TALENs are being tested in three phase I interventional clinical trials in China and in the USA. An estimated 262 patients with human papillomavirus-related malignant neoplasms or acute myeloid leukemia are either participating or are expected to participate in these studies (U.S. National Institute of Health, www.clinicaltrials.gov.us).

2.3. CRISPR/Cas

In 1987—with the discovery of an interrupted short tandem repeat sequence of nucleotides—CRISPR was identified in *Escherichia coli* (Ishino, Krupovic, & Forterre, 2018). CRISPR-based genome editing was demonstrated for the first time in 2013, and since then, this technique has developed rapidly (Cho, Kim, Kim, & Kim, 2013; Cong et al., 2013; Jinek et al., 2012; Luo et al., 2016; Mali et al., 2013). So far, numerous CRISPR systems have been identified in bacterial and archaeal

Table 1
Comparison of the key features of the three engineered nucleases, ZFNs, TALENs, and CRISPR/Cas9.

	ZFN	TALEN	CRISPR/Cas9	Reference
Binding principle	Protein-DNA	Protein-DNA	RNA-DNA	Gupta & Musunuru, 2014
Length of target site	2 sites, each 15 or 18 bp	2 sites, each ≥13 bp	1 site, 18 to 20 bp plus 2–5 bp for PAM	Carroll, 2011; Fu et al., 2014; Christian et al., 2010
Endonuclease	FokI	FokI	Cas9	Durai et al., 2005; Christian et al., 2010; Mali et al., 2013; Cong et al., 2013; Cho et al., 2013
Design and construction simplicity	Hard	Easy	Very easy	
Time required for design and construction	7–15 days	5–7 days	1–3 days	Cermak et al., 2011
Targeting specificity	Low	Medium	High	Ranganathan, Wahlin, Maruotti, & Zack, 2014
Targeting efficiency	1–50%	1–50%	>70%	Gupta & Musunuru, 2014
Off-target frequency	High	Low	Low	Pattanayak, Ramirez, Joung, & Liu, 2011; Lee, Chung, et al., 2016
Multiple gene mutations	Limited	Limited	Unlimited	Lin et al., 2014; Kennedy et al., 2014
Cytotoxicity	Moderate	Low	Low	Gupta & Musunuru, 2014
Number of Phase I Clinical Trials	14	6	30	www.clinicaltrials.gov

genomes (Koonin, Makarova, & Zhang, 2017). The well-known CRISPR systems—currently in use—are divided into two major classes and encompass six types (I-VI), depending on their related Cas genes (Cas effectors 1–13) and organization of the corresponding loci. Among these, type I, III, and IV CRISPR/Cas systems belong to Class 1 and the rest are grouped into the Class 2 (Makarova & Koonin, 2015). The type II and type V systems—belonging to Class 2—are used for programmable genome editing. The most commonly used CRISPR/Cas9 system is the type II system, and its biology, mechanism, and applications have been well-documented (Bae, Hur, Kim, & Hur, 2019; Mir, Edraki, Lee, & Sontheimer, 2018).

The CRISPR/Cas9 system consists of two components, a Cas9 nuclease and a single guide RNA. The latter consists of sequence specific targeting crRNA and tracrRNA, which bind to Cas9 together with the CRISPR RNA. In total, 18–20 nucleotides can be precisely targeted by the crRNA (Sun, Lutz, & Tao, 2016). There are three different CRISPR/Cas9-based genome editing strategies, 1) plasmid-based CRISPR/Cas9 system, 2) Cas9/mRNA and sgRNA system, and 3) ribonucleoprotein (RNP) complex-based Cas9 and sgRNA system. Each strategy has its advantages and shortcomings (Liu, Zhang, Liu, & Cheng, 2017). Among these strategies, the RNP system is most commonly used for clinical purposes as it is associated with fewer off-target effects, which is the major disadvantage of CRISPR/Cas9 tools. Compared to other systems, the half-life of the RNP system is relatively short, which reduces the risk of undesired genome editing (Farboud et al., 2018). Cas9 RNP system was the first system used in human cells, where it was shown that reduced off-target effects with rapid degradation of corresponding protein and gRNA (Kim, Kim, Cho, Kim, & Kim, 2014). The other advantages of the RNP system are the quick delivery of CRISPR/Cas9 tools, precise genome editing, ease of design, improved safety, and reduced immune responses (Martinez-Lage, Puig-Serra, Menendez, Torres-Ruiz, & Rodriguez-Perales, 2018). Several strategies to reduce the off-target activity of CRISPR/Cas9 are being developed, *e.g.*, systems with smaller or divided variants of Cas9 or using allosterically-regulated Cas9 (Chapman, Gillum, & Kiani, 2017; Shen et al., 2019).

Improvements in CRISPR technology have led to an exponential increase in the access and use of genome editing. Currently, 24 CRISPR-based clinical trials are underway in the USA, China, France, Canada, Germany, and in the UK, in which more than 900 patients are anticipated to participate, according to NIH registration data (clinicaltrials.gov).

However, transplanted CRISPR tools may cause severe adverse effects when they are not properly monitored. To reduce the occurrence of such adverse effects, a Canadian research group has developed anti-CRISPR proteins (Bondy-Denomy, Pawluk, & Davidson, 2013). Currently, more than 20 anti-CRISPR proteins are known to target the type I and type II CRISPR systems. Proteins, AcrF1-AcrF10 and AcrE1-AcrE4 inhibit the type I CRISPR system, while AcrIIA1-AcrIIA5, as well as AcrIIC1-AcrIIC3, act against the type I ICRISPR system (Maxwell, 2017; Pawluk, Davidson, & Maxwell, 2018). A recent study demonstrated the potential application of anti-CRISPR proteins as regulators of mammalian cell behavior, generators of write-protected cells, and regulators of CRISPR-based gene regulatory circuits (Nakamura et al., 2019).

2.4. CRISPR/Cas9-base editors

CRISPR/Cas9 uses programmable nucleases to create double-strand breaks at the target DNA sites for the generation of desired DNA modifications. However, this approach may not function efficiently in non-dividing cells, and may lead to the generation of unwanted indels (such as frameshifts), translocations, or chromosomal rearrangements (Thuronyi et al., 2019). Base editors are a promising alternative DNA editing tool, as they irreversibly convert nucleotides in the targeted window without causing double strand breaks.

The two types of base editors have been reported thus far; these include the cytidine base editors (CBEs) and adenosine base editors (ABEs). Base editors are created by fusing a deaminase with Cas9. The deaminase catalyzes a C to T (G to A) and A to G (T to C) conversion in the target window. Deamination of cytidine results in uridine, which is converted to thymine after DNA replication (Komor, Kim, Packer, Zuris, & Liu, 2016). Various types of deaminases have been used with CBEs, namely, APOBEC1, APOBEC3A CDA1, and FERNY. Studies aiming at the evolution of the CBEs should focus on increasing their efficiency and the targeting window (Thuronyi et al., 2019). Fusion of uracil glycosylase inhibitor (UGI) to the C terminus of the CBE construct (from BE2) protects the resulting uridine from base excision because it allows its glycosylation by the endogenous uracil DNA glycosylase (UDG). This uridine finally gets converted into a thymine after DNA replication (Komor et al., 2016).

The deaminase in the ABE, which converts adenosine to inosine—which is later converted into guanosine (A to G (T to C))—is an evolved version of TadA (TadA*). Bacteria-based evolution is conducted using the ABE7.10 version of TadA*. The entire ABE7.10 construct is similar to the CBE construct, with the exception of UGI. The canonical target window for both CBE and ABE is 4–8 nucleotides at the distal protospacer region of the protospacer adjacent motif (PAM) sequence (Komor et al., 2017).

A primitive base editor consists of a dead form of Cas9 (D10A and H840A) (Gaudelli et al., 2017). However, the nickase form of Cas9 (nCas9; D10A), which creates a nick on the opposite strand, can manipulate the cellular DNA repair machinery, thereby increasing the efficiency of editing and yielding better base editing outcomes. Therefore, the current versions of CBEs and ABEs are constructed with nCas9 (D10A). In addition, both CBE and ABE are constructed with spCas9, which may limit the targets, as spCas9 specifically targets NGG-PAM. Therefore, modified versions of spCas9 (non-NGG PAM, such as NG-PAM and NGA-PAM), saCas9 (NNGRR(T)), and Cpf1 (TTTV PAM) have

been constructed to extend the repertoire of target sites. Such base-editing technologies may be used to treat diseases caused by single nucleotide polymorphisms (SNPs), by correcting the mutation(s). For applications in humans, researchers have modified induced pluripotent stem cells (iPSCs) by delivering CBE and ABE with a 4D-nucleofector system (Zhou et al., 2019). Base editing in mouse embryos was performed for the first time by Kim et al. (2017), and the generation of mutant disease models has been reported in rabbits and pigs (Liu et al., 2018; Xie et al., 2019). Microinjection or electroporation has been used to deliver CBE targeting *DMD, TYR*, *LMNA*, *RAG1, RAG2*, and *IL2RG* to create disease models (Xie et al., 2019).

2.5. Prime editors

Prime editor is a recently developed new gene editing technology based on CRISPR/Cas9 system (Anzalone et al., 2019). Prime editor consists of Cas9 fused with an engineered reverse transcriptase (RT) and specialized guide RNA called prime editing guide RNA (pegRNA). This system enables to create desired genome editing without any double-strand breaks or donor DNA templates. Instead of creating double-strand break and requiring donor DNA templates, it has prime editing guide RNA (pegRNA) to replace sequence of target strand.

The pegRNA has 3′ extended form of typical sgRNA and extended sequence contains primer binding sites (PBS) and RT template sequence for replacement of target sequence. RT template can be used as template of reverse transcriptase for replacement, alike a donor template for HDR in CRISPR/Cas9 system.

Three different versions of prime editors have been developed PE1, PE2 and PE3. Prime editor 1 (PE1) is a primarily designated form of prime editor, it uses wild-type M-MLV RT fused at C terminus of nickase, nCas9(H840A). It creates nick on the target site, and gives 3′-hydroxyl group exposure, which enables RT to replace the RT template of pegRNA into target strand.

Prime editor 2 (PE2) has the same system except engineered RT (D200N/L603W/T330P/T306K/W313F). It substantially raises 1.6- to 5.1 folds in editing efficiency. Prime editor 3 (PE3) system uses additional simple sgRNA to create the nick on non-edited strand to raise further editing efficiency. Which results in 1.5-to 4.2-fold increment compared to PE2. To minimize indels caused by concurrent nicks on both strands, spacer sequences of sgRNA was designed to match to edited sequence. Which results in 13-folds decrease of indel without any decrease in editing efficiency (PE3b) (Anzalone et al., 2019; Cohen, 2019).

In summary, since prime editor is double strand break free, and provides fewer unwanted byproducts such as indels than typical CRISPR/Cas9 system. Also, unlike base editors (CBE and ABE), prime editor mediates targeted all 12 possible base to base conversion in a single nucleotide resolution. Therefore, prime editor can expand scope of genome editing fields, and it can be used for various precise genome editing applications.

2.6. Digenome sequencing

Although the CRISPR/Cas9 system has been widely used in biomedical research, the target specificity of Cas9 in the genome remains an issue. The CRISPR/Cas9 system relies on guide RNAs to target DNA. Few nucleotide mismatches at off-target sites may result in unwanted editing. Therefore, validation of off-target sites is important for limiting the occurrence of off-target editing and ensuring safety (Lee, Cradick, Fine, & Bao, 2016).

Digenome sequencing is based on whole genome sequencing after *in vitro* digestion, which enables profiling of off-target effects at a genome-wide scale. When a whole-genome sample is digested by CRISPR/Cas9, multiple sequences are generated at the cleavage sites, all having the same 5′ ends (for both forward and reverse strands), which represent potential off target sites after DNA editing. In addition, the cleaved strands generated *in vitro* do not undergo DNA repair as other modifications, including indels, are not considered in this method. The sequences of the multiple cleaved strands can be identified computationally, aligned, and visualized using computational tools such as the Integrative Genomics Viewer (IGV) (Kim et al., 2015). Digenome sequencing can be multiplexed, analyzing up to 11 sgRNAs at the same time (Kim, Kim, Park, & Kim, 2016). In addition to histone-free naked genomic DNA, chromatin DNA can also be used for digenome sequencing to account for the effects of chromatin on the off-target ramifications of CRISPR (Kim & Kim, 2018).

Both CBE and ABE can be analyzed using the digenome sequencing. Uridines or inosines on the target strand are removed by adding endonuclease V, and nCas9 generates a nick in the opposite strand. This results in staggered cleavage at the target sites of CBE and ABE. The results of computational analyses are confirmed using IGV to visualize the potential off-target candidate sites (Kim, Kim, Lee, Cho, & Kim, 2019). The samples are subjected to targeted deep sequencing at the potential off-target candidate sites to determine off-target effects.

Thus, digenome sequencing is an efficient, sensitive, versatile method for identifying off-target sites with a low (0.1%) mutation frequency. However, as this technology has been introduced only recently, its applications in stem cell therapy and drug discovery are still limited.

3. Genome-edited stem cells for therapeutic application

With the recent advances in the field of sequence-specific nucleases, including ZFNs, TALENs, and CRISPR/Cas9 nucleases, we are beginning to realize the potential of these genome editing tools in the context of disease treatment. These genome-editing tools can be designed for disrupting any disease-causing gene *via* knock-in, knockout, or point mutations (Byrne, Mali, & Church, 2014). Stem cells are an optimal platform for genome editing technologies owing to their self-renewal capability and ability to secrete endogenous proteins, such as coagulation factor IX, VEGF, FGF-b, Ang1, and sRAGE (Hoke, Salloum, Kass, Das, & Kukreja, 2012; Lee et al., 2019; Liu et al., 2012). However, it has been reported that high passage number adversely affects the self-renewal activity of stem cells (Crisostomo et al., 2006). Therefore, it is crucial to develop therapeutic genome-edited stem cell lines that meet the safety requirements, in addition to fulfilling criteria, such as high efficacy, quality, and reproducibility. As summarized in Table 2, several groups are investigating methods of producing effective combinations of gene editing tools and target cells for the treatment of specific diseases.

3.1. Ex vivo applications

Gene editing methods utilize either *in vivo* or *ex vivo* gene transfer strategies. During the last decade, the *ex vivo* strategies have been investigated more extensively than the *in vivo* ones (Table 2). In *ex vivo* gene editing-based stem cell therapy, defective genes are isolated from patient-derived cells, repaired *in vitro* using gene editing technologies, and then, transplanted back into the patient (Shim et al., 2017). As shown in Fig. 1, pioneering studies on mice have revealed that CRISPR-, TALEN-, or ZFN-edited stem cells can be used to treat various diseases, including HIV, normal and malignant hematopoiesis, X-linked chronic granulomatous disease, sickle cell disease, diseases of the immune system, graft *versus* host disease, and hemophilia A/B (Hoban et al., 2015; Holt et al., 2010; Magis et al., 2018; Mandal et al., 2014; Meng, Han, Jeong, & Kim, 2019; Pavel-Dinu et al., 2019).

3.1.1. Diversity of gene-edited stem cells with respect to therapeutic applications

Currently, several types of gene-edited stem cells are being developed for therapeutic applications. Stem cells can be categorized into the following three groups based on their origin: adult or somatic stem cells, embryonic stem cells (ESCs), and iPSCs (Leventhal, Chen, Negro, & Boehm, 2012). Among these, hematopoietic stem and

progenitor cells (HSPCs), amniotic mesenchymal stem cells, iPSCs and human ESCs, and cardiomyocytes, neural, skin, and muscle cells derived from iPSCs and human ESCs have been extensively studied as therapeutic tools.

3.1.1.1. Application of adult stem cells. Adult stem cells are lineage-restricted tissue-specific stem cells, with a limited ability to differentiate into tissue-specific cell types. As they are stem cells, they naturally tend to possess high self-renewal ability. Due to these features, several types of adult stem cells have been used in gene editing-based therapeutic studies.

HSPCs: Studies conducted during the last few years provide evidence that gene-edited HSPCs have therapeutic potential in the context of blood and immune system diseases in mice. Most of the therapeutic studies using gene-edited adult stem cells are based on the use of HSPCs, as they can differentiate into all mature blood types and are easy to harvest. HSPCs play a crucial role in the production of various types of blood cells with different functions during hematopoiesis (Rieger & Schroeder, 2012). It has been postulated that genetic alterations, such as mutations and epigenetic changes, are responsible for the pathogenesis of malignant hematopoietic diseases, such as myelodysplastic syndrome, myeloproliferative neoplasms, and leukemia (Goldman et al., 2019). However, the exact mechanism by which genes orchestrate normal or malignant hematopoiesis is not completely understood. Advances in genome editing tools can increase our understanding of the functional effects of gene loss. For example, using the Cas9-sgRNA RNP system, genes encoding EED, SUZ12, and DNMT3A were successfully knocked out in mouse and human HSPCs. The disruption rate was greater than 60% in mouse and at least 75% in human HSPCs; this allowed the elucidation of the role played by the genes of interest in disease progression (Gundry et al., 2016).

Numerous abnormalities in genes have been identified that contribute to disease progression (Table 2). Programmable nucleases have been proposed as tools for rectifying genomic mutations, and currently, many new gene editing-based stem cell therapies are under investigation. In this context, most of the studies are designed to repair the target mutations in patient-derived stem cells using one of the three major programmable nucleases. The efficiency of correction is determined by transplanting the cells with the modified genes in appropriate animal disease models. The repopulating capacity and survival rate of the

Table 2
Summary of recent advances of *in vivo* and *ex vivo* pre-clinical models targeting stem cells.

Strategies	Stem cell type	Diseases	Delivery method	Target gene	Model	Reference
CRISPR/Cas9	Hematopoietic stem and progenitor cells	*Ex vivo*				
		Blood and hematopoiesis	Electroporation	Eed, Suz12	Mouse	Gundry et al., 2016
				HBB, CCR5, IL2RG		Bak et al., 2017 Li et al., 2018
		X-linked chronic granulomatous	Electroporation & AAV6	gp91phox	Mouse	De Ravin et al., 2017
		X-linked severe combined immunodeficiency	AAV6	IL2RG	Mouse	Pavel-Dinu et al., 2019
		Sickle cell disease	Electroporation	HBB	Mouse	Magis et al., 2018
		and β-thalassemia	Electroporation	HBB		DeWitt et al., 2016
			Electroporation& AAV6	HBB		Dever et al., 2016
			Electroporation& AAV6	HBB		Pattabhi et al., 2019
		Graft *versus* host disease	Electroporation	HLA-A	Mouse	Torikai et al., 2016
		Acute myeloid leukemia	Electroporation	CD33	Mouse	Kim et al., 2018 Borot et al., 2019
		HIV	Electroporation	B2M, CCR5	Mouse	Mandal et al., 2014
			Electroporation	CCR5		Xu et al., 2017
			Electroporation	GFP		
	iPSc	*Ex vivo*				
		Mucopolysaccharidosis Type 1	Lipofectamine	Idua	Mouse	Miki et al., 2019
		Hemophilia A	Lentiviral vector	FVIII	Mouse	Olgasi et al., 2018
			Electroporation	FVIII		Park et al., 2015
		Diabetes mellitus	Electroporation	STAT3	Mouse	Saarimäki-Vire et al., 2017
		Duchenne muscular dystrophy	Electroporation	DMD	Mouse	Young et al., 2016
		β-thalassemia	–	HBB	Mouse	Ou et al., 2016
	Neural stem cell	*Ex vivo*				
		Brain tumor	Baculoviral vectors	miR-199a/214c	Mouse	Luo, Leenay, & Beisel, 2016
	Skin stem cell	*In vivo*				
		Recessive dystrophic epidermolysis bullosa	Intradermally injection		Mouse	Wu et al., 2017
	Cardiomyocytes and muscle stem cell	*In vivo*				
		Duchenne muscular dystrophy	AAV	DMD	Mouse	Tabebordbar et al., 2016
TALEN	Mesenchymal stem cell	*Ex vivo*				
		Myocardial infraction	Electroporation	IL-10	Mouse	Meng et al., 2019
ZFN	Hematopoietic stem and progenitor cells	*Ex vivo*				
		Fanconi anemia	Lentiviral vector & electroporation	FANCA	Mouse	Diez et al., 2017
		X-linked chronic granulomatous	Electroporation & AAV6	gp91phox	Mouse	De Ravin et al., 2016
		Sickle cell disease	Electroporation	HBB	Mouse	Hoban et al., 2015
		X-linked Severe Combined Immunodeficiency	Electroporation	IL2RG	Mouse	Genovese et al., 2014
		HIV	Electroporation	CCR5	Mouse	Holt et al., 2010
			AAV	CCR5		Li et al., 2013
			Electroporation & AAV	CCR5		Wang et al., 2015
			Electroporation	CCR5		DiGiusto et al., 2016
	Human embryonic stem cells	Myocardial infraction	–	Fluc, NIS, SSTr2	Mouse	Neyrinck et al., 2018
Mega nuclease	Hematopoietic stem and progenitor cells	*Ex vivo*				
		HIV	Electroporation &AAV	CCR5	Mouse	Sather et al., 2015

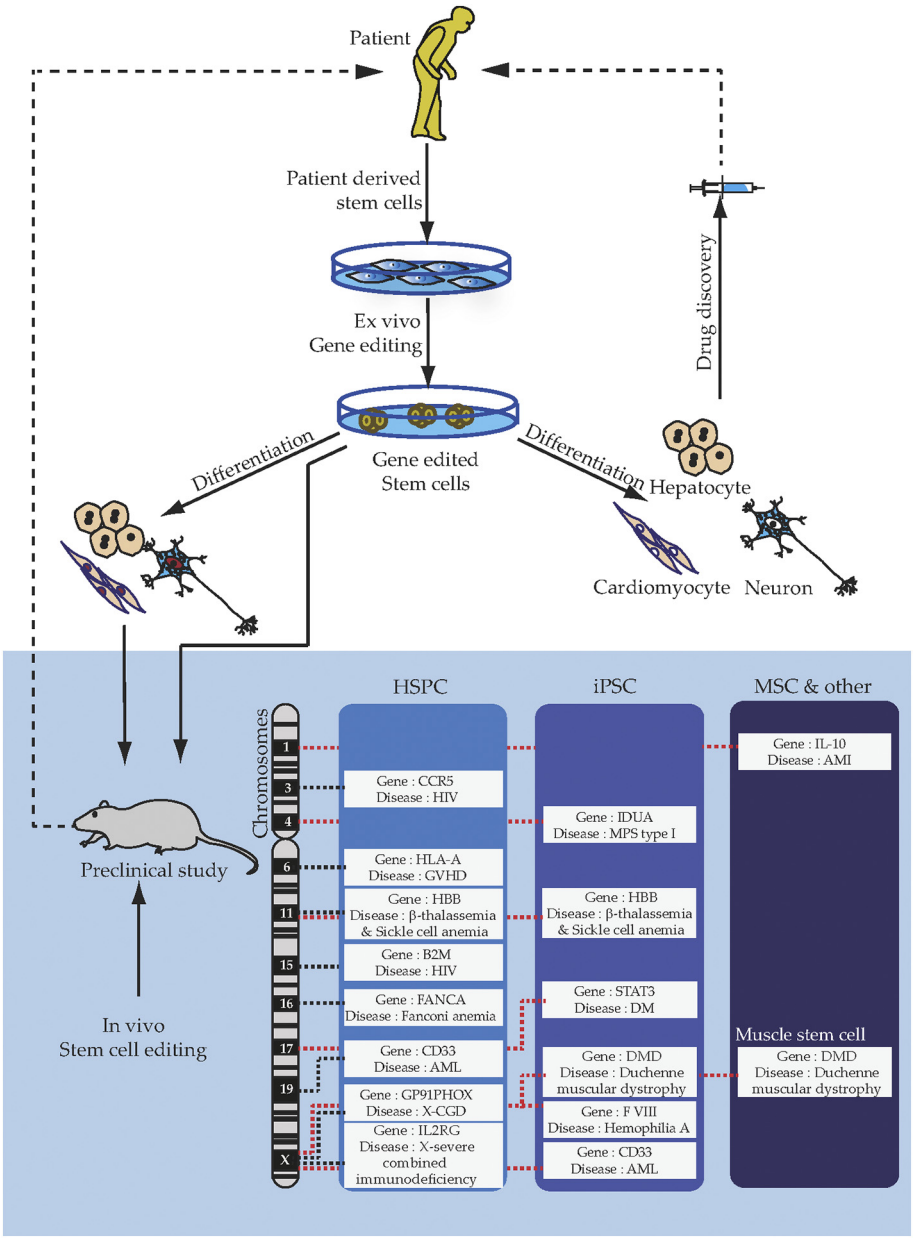

Fig. 1. Representative examples of current preclinical studies using various human gene-edited stem cells.

transplanted cells is then monitored *in vivo* to determine the success of the therapy.

For example, HSPCs derived from patients with X-linked chronic granulomatous disease were used in a study, where CRISPR/Cas9-sgRNA and ZFN systems were introduced to repair the disease-causing genetic mutations. The C676T mutation in *CYBB* was corrected using the CRISPR/Cas9 system and the full-length corrected gene encoding gp91phox was inserted into the AAVS1 safe harbor locus of HSPCs using ZFN. This study demonstrated the feasibility of using gene therapy for correcting monogenic blood disorders using genetically-modified $CD34^+$ hematopoietic stem cells (De Ravin et al., 2016; Qi, 2017).

Gene editing-based stem cell therapy is potentially applicable to other diseases caused by single base pair mutations, such as sickle cell disease (SCD) and β-thalassemia. SCD is one of the most common genetic diseases that causes anemia and multi-organ damage as it affects hemoglobin, which delivers oxygen throughout the body. It is caused by a single point mutation at codon 6 of the human β-globin-coding gene, in which adenine is replaced by thymine, leading to changes in the amino acid sequence of hemoglobin (Inusa et al., 2019). Therefore, researchers have focused on editing this point mutation in patient-derived $CD34^+$ hematopoietic stem and progenitor cells using Cas9 RNP-mediated gene editing with an ssDNA oligonucleotide donor (Collins & Gottlieb, 2018; Dever et al., 2016; DeWitt et al., 2016). Dever et al. (2016) efficiently corrected the β-globulin gene in patient-derived $CD34^+$ HSPCs using CRISPR/Cas9 nuclease and an rAAV6 vector encoding GFP. Subsequently, permanent and multi-lineage reconstitution occurred after transplantation and engraftment of the modified HSPCs in immune deficient non-obese diabetic (NOD) severe combined immunodeficiency (SCID) *Il2rg*$^{-/-}$ (NSG) mice. In addition, a purification scheme, which involved the use of magnetic beads or fluorescent activated cell sorting (FACS), was developed for enriching the population of modified HSPCs with homologous recombination-mediated targeted integration (>85%). This process will be valuable in the clinic for enriching targeted stem cells and eliminating other undesired cells. Furthermore, DeWitt et al. (2016) validated the reduction in the levels of mutated hemoglobin RNA and protein in the corrected HSPCs from patients with sickle cell disease. Recently, Magis et al. (2018) further conducted *in vivo* erythroid differentiation and observed

enrichment of cells harboring corrected β-globulin alleles. Altogether, these results demonstrate that clinical translation of CRISPR/Cas9 for the repair of the sickle cell mutation in human HSPCs may be useful for the development of new gene editing-based treatments for SCD and other hematopoietic diseases.

One of the challenges associated with the clinical translation of single base pair mutation repair is the low level of homology-directed repair (HDR) after transplantation. Studies show that the HDR level is approximately 50% *in vitro*, but it decreases to 5–7% in non-obese diabetic SCID gamma (NSG) mice after transplantation (Dever et al., 2016; DeWitt et al., 2016; Hoban et al., 2015; Lomova et al., 2019). Therefore, the HDR for non-homologous end joining (NHEJ) ratio was introduced by the Lomova lab in 2019 as a metric parameter for the evaluation of approaches aimed at improving gene editing efficiency. While HDR occurs only during the S/G2 phase of the cell cycle, NHEJ occurs throughout the cell cycle and leads to large deletions or genomic rearrangements (Lin, Staahl, et al., 2014). One should remember that the sickle allele can be converted to the more severe β-thalassemia allele due to the generation of indels in the β-globin-encoding gene. Based on these observations, the Lomova lab (2019) (Lomova et al., 2019) used a modified version of Cas9 with reduced nuclease activity in the G1 phase of the cell cycle when HDR cannot occur, and transiently increased the proportion of cells in the S/G2 phase, where HDR occurs preferentially. The results demonstrated that temporal regulation of Cas9 nuclease activity and transient synchronization of human $CD34^+$ cells in the HDR-preferred phase resulted in four-fold improvement in the HDR/NHEJ ratio *in vitro*. Furthermore, the improvement was significant after transplantation of gene-edited stem cells into immune-deficient mice. This strategy for improving gene editing efficiency can be employed in therapeutic approaches against other diseases, where an increased HDR/NHEJ ratio is critical for therapeutic success.

A major disadvantage of the traditional CRISPR/Cas9 system is that it cannot edit several genes simultaneously, and one cannot distinguish gene-edited cells from unmodified cells in the total population. Bak et al. (2017) focused on methods aimed at improving CRISPR/Cas9-mediated genome engineering in human HSPCs by combining CRISPR/Cas9 with a rAAV template delivery method to precisely edit several genes. In addition, they cloned a reporter gene in the donor DNA that enabled the isolation of gene-modified cell populations. In this study, HSPCs were electroporated with RNPs targeting *HBB, CCR5*, and *IL2RG* and transduced with gene-specific rAAV6 donors (HBB-tdTomato, CCR5-tNGFR, and ILRG-GFP). No changes were observed in the proliferation and apoptosis rates of the triple gene-modified groups compared to that in the unmodified group.

ZFNs are also being investigated for the treatment of sickle cell diseases in addition to other approaches, such as the combination of Cas9 RNP with a DNA oligonucleotide donor. Hoban et al. (2015) delivered engineered ZFNs to HSPCs with a lentiviral vector and donor templates and observed that modified $CD34^+$ cells have the capacity to differentiate into different cell types, including erythroid, myeloid, and lymphoid cells, both *in vitro* and *in vivo*. This study showed that the production of wild-type hemoglobin in SCD patients can be enabled by correcting the sickle cell disease-causing mutation in bone marrow $CD34^+$ cells; this may be a step toward the development of an effective SCD therapy. The therapeutic activity of ZFN-mediated HSPCs can also be extended to Fanconi anemia (FA), which leads to bone marrow failure, malformations, and chromosome fragility due to mutations in FA genes (Bogliolo & Surralles, 2015). Diez et al. (2017) used ZFNs for correcting the *FANCA* gene in $CD34^+$ cells isolated from patients with FA. The results demonstrated that it is feasible to develop a treatment for bone marrow failure disorders based on the correction of the disease phenotype in hematopoietic $CD34^+$ cells from FA-A patients.

The success of transplantation of organs and cells, such as hematopoietic stem cells from umbilical cord blood, depends on the similarity extent between the human leukocyte antigens (HLA) of the host and the donor. Torikai et al. (2016) conducted genetic editing of *HLA* genes in hematopoietic stem cells to increase the chances of successful donor-matched transplantation by eliminating HLA-A expression. For this purpose, they targeted the *HLA-A* alleles in HSPCs using ZFNs, which resulted in increased match at *HLA-B/C/DRB1*. An additional factor determining the success of transplantation is the homing efficiency of the injected cells. Genovese et al. (2014) corrected *ILL2RG* using ZFNs in human umbilical cord blood-derived $CD34^+$ cells transplanted into NOD-SCID-*Il2rg* (NSG) mice to check long-term efficiency. Eight weeks after transplantation, surviving human cells could be detected in the spleen and bone marrow of NSG mice using polymerase chain reaction (PCR). In addition, the low off-target activity of the ZFN was confirmed, showing high specificity for *IL2RG*.

ZFN-, TALEN-, and CRISPR/Cas9-mediated gene-edited HSPCs are also being considered for therapeutic approaches against HIV, a lentivirus that infects human immune cells, such as helper T cells, dendritic cells, and macrophages. The patients suffer from progressive immune system failure, which causes life-threatening infections and cancer. CCR5 is the major co-receptor used for the entry of HIV-1 into the target cells (Ellwanger, Kaminski, & Bogo Chies, 2019; Miller, Cairns, Bridges, & Sarver, 2000). Disruption of *CCR5* is key for generating HIV-1-resistant HSPCs.

HSPCs have been used in many studies because of their unique properties. For example, the entire hematopoietic system can be efficiently reconstituted using a small number of genetically modified HSPCs (Yu, Natanson, & Dunbar, 2016). Programmable nucleases are considered promising tools for editing HSPCs. In 2014, Mandal et al. introduced for the first time CRISPR/Cas9 nuclease-mediated editing of *B2M* and *CCR5* in human primary HSPCs in an animal model. They demonstrated that CRISPR/Cas9 can be used to ablate genes of clinical significance in $CD4^+$ T cells and $CD34^+$ HSPCs, with an efficiency that is therapeutically meaningful in several clinical settings, such as the treatment of HIV. Their demonstration that CRISPR/Cas9-targeted $CD34^+$ HSPCs retain multi-lineage potential *in vitro* and *in vivo*, combined with very high on-target and minimal off-target mutation rates, suggests that CRISPR/Cas9 may have broad applicability, enabling gene and cell-based therapies of blood disorders.

In addition to CRISPR/Cas9, similar studies using ZFNs and TALENs have demonstrated the promising applications of gene therapy for various diseases, including HIV-1 infections. Since individuals homozygous for a 32 bp deletion in *CCR5* are resistant to infections by CCR5-tropic HIV-1 infection, Holt et al. (2010) generated HIV-resistant progeny using a ZFN and human fetal liver-derived HSPCs. They noted that a homozygous *CCR5* mutation provides genetic resistance to HIV-1 infection, while heterozygosity is associated with delayed progression, without any additional apparent immunological dysfunction. Transplantation of ZFN-edited HSPCs into NOD/SCID/*IL2r* gamma (null) mice significantly reduced HIV-1 levels compared to the control mice, indicating that the $CCR5^{-/-}$ progeny supported the use of ZFN-modified autologous hematopoietic stem cells as a clinical approach for treating HIV-1. In other words, a single dose of HIV-resistant HSPCs may contribute significantly to the treatment of and protection against HIV-1 infection.

A comparable study was also conducted by Li et al. in 2013 to solve the problem of low frequency of naturally occurring CCR5 donors. They studied the effect of protein kinase C activators on the disruption of *CCR5* expression in HSPCs using a recombinant adenoviral vector encoding a *CCR5*-specific pair of ZFNs in HIV-1-challenged NSG mice. Their results showed that pretreatment of HSPCs with protein kinase C activators may increase *CCR5* disruption (> 25%), thereby facilitating the development of *CCR5*-disrupted cell lines.

Gene correction or knock-in in HSPCs is effort-intensive. For example, relatively high amounts of nucleases and donor DNA are required, which may be cytotoxic for HSPCs. To overcome this issue, Wang et al. (2015) delivered the mRNAs encoding for ZFNs in HSPCs *via* electroporation and then transduced the donor DNA *via* an AAV vector. The donor DNA harbored a GFP-coding gene in the *CCR5* region, which disrupted the endogenous *CCR5* to generate HIV-1-resistant HSPCs. The

combination of ZFN mRNA and donor DNA delivered *via* the AAV6 vector showed increased site-specific insertion in both mobilized blood HSPCs and fetal liver HSPCs. DiGiusto et al. (2016) developed a strategy for gene therapy of HIV-1 infection by deleting *CCR5* in human HSPCs with ZFNs, and a positive outcome was obtained. Currently, this strategy, which exhibits higher efficacy and safety, is being tested in a Phase I/II clinical trial in USA (NCT02500849).

CCR5 blocking therapy has also been conducted using mega nucleases. For example, in a preclinical study, Sather et al. (2015) modified the *CCR5* locus of adult mobilized $CD34^+$ blood stem cells using the AAV donor template and megaTAL nuclease, which is a hybrid of the TALE DNA binding domain and an engineered sequence-specific homing endonuclease. Emphasizing the benefits of sequence specificity and reduced cytotoxicity associated with megaTAL nucleases, the authors highlighted the feasibility of highly efficient targeted integration of sequences coding chimeric antigen receptors into the *CCR5* locus in $CD34^+$ cells.

Although the *ex vivo* disruption of *CCR5* has been successfully demonstrated by several research groups, the clinical translation of these approaches is challenging, especially the possibility of efficient gene editing with high specificity and long-standing therapeutic effects. To overcome such difficulties, Xu et al. (2017) utilized developments in programmable nucleases to introduce a long-term CRISPR/Cas9-mediated *CCR5* ablation system in HSPCs. The results demonstrated robust *CCR5* disruption efficiency in secondary transplanted repopulating hematopoietic cells and HIV-1 resistance.

To summarize, *ex vivo* editing in HSPCs is one of the major approaches studied to develop therapeutic approaches against systemic intractable diseases, and it may yield promising results in the near future.

Mesenchymal stem cells (MSCs): MSCs consist a major adult stem cell population of diverse origin in the human body, and play crucial roles in stem cell-based therapies owing to their abundance, low oncogenic characteristics, moderate potential of differentiation into other cell types, and paracrine effects (Fitzsimmons, Mazurek, Soos, & Simmons, 2018; Murphy, Moncivais, & Caplan, 2013). Although randomized controlled clinical trials have not yet demonstrated their safety and efficacy, wild-type MSCs have been extensively used for treating tissue injury or inflammation-related diseases, such as myocardial infarction, graft *versus* host disease, liver cirrhosis, spinal cord injury, osteoarthritis, or rheumatoid arthritis (Wei et al., 2013).

MSC-based stem cell therapy has been developed extensively for several decades, and some efficacy has been demonstrated if the MSCs are allowed to differentiate into a relevant cell type or applied to a relevant disease. However, clinical trials using MSC-based therapy are limited, and most have failed because of the limited viability of these cells in pathological lesions and of safety issues (*e.g.*, arrhythmias). For example, only 1% of MSCs were observed in inflamed hearts 4 days after transplantation (Toma, Pittenger, Cahill, Byrne, & Kessler, 2002). Thus, it is essential to develop a method that can sustain the survival of the transplanted cells and lead to efficient MSC-based therapies, as well as improve the inflammatory niche.

Toward this objective, inflammatory molecules such as AGE-albumin have been successfully introduced for obtaining higher survival and efficacy levels in neurological and inflammatory diseases (Bayarsaikhan et al., 2016; Lee et al., 2019; Son et al., 2017). Such gene edited-MSC-based therapy has provided significant therapeutic benefits for myocardial infarction in mice. It is well known that cardiac dysfunction is strongly associated with inflammation, and that MSCs possess anti-inflammatory properties because of interleukin 10 (IL-10) secretion (Timmers et al., 2008). Moreover, the rapid development of gene editing technology allows the generation of functionally improved stem cells. In this context, Meng et al. (2019) edited the safe harbor genomic site of amniotic MSCs using a TALEN to develop a long-term IL-10 secreting system. Their results showed that interleukin knock-in in MSCs can strongly suppress inflammatory cells (CD68 and F4/80 positive) and reduce infarct size by supporting the left ventricular function. The strong inhibitory activity of these gene-edited MSCs against apoptosis in the infarcted myocardium supports a promising and highly effective approach for modified stem cell-based treatment.

Neural stem cells (NSCs): NSCs can differentiate into major nerve cell types such as oligodendrocytes, neurons, and astrocytes. Currently, NSCs are being classified into three subgroups based on their sources: (i) NSCs directly isolated from the developing/adult central neural system; (ii) *in vitro* cultivated NSCs; and (iii) ESC- or iPSC-derived NSCs. The latter may help to resolve ethical and immune-related problems, and constitute a novel source of NSCs for clinical applications (Shoemaker & Kornblum, 2016).

As NSCs are vital constituents of the nervous system, research for the development of NSC-based treatments for neurological diseases has been extensively conducted since the 1990s. For example, NSC-based therapeutic approaches with some success have been reported for Parkinson's disease, Huntington's disease, stroke, and spinal cord injuries in mice (Zhao & Moore, 2018). Although these studies used non-edited NSCs, genome-edited NSCs have been used as gene delivery vectors by Luo et al. in 2016. This group has previously shown that iPSC-derived NSCs harbor certain microRNAs, which are negative regulators of hypoxia-induced cell migration by degrading hypoxia inducible factor (HIF)-1α and abrogating c-Met signaling, and are co-expressed from the *miR-199a/214* cluster. Based on these results, the authors targeted the *miR-199a/214* cluster in iPSC-derived NSCs using the CRISPR interference (CRISPRi) tool and suppressed microRNA expression. The ensuing enhanced tumor tropism of iPSC-derived NSCs was a novel application of CRISPRi in NSC-based tumor targeted gene therapy. In other words, it is possible to deliver therapeutic genes because of the rapid NSC migration into brain tumors. Genome-edited NSCs hold promise for the treatment of neurodegenerative diseases as they offer protection against the diseases and support regeneration.

3.1.1.2. Application of ESCs. ESCs are pluripotent stem cells with unlimited self-renewal capacity and ability to differentiate into any cell type of the body. According to the NIH database, approximately 60 ESC-related clinical trials are either ongoing or have been completed. Despite their potential, the number of trials is extremely low because of the ethical issues raised in many countries (Ismail, 2015). Therefore, ESC-related research now aims at identifying more efficient methods for the development of stem cells from human embryos (Shand, Berg, & Bogue, 2012).

A Belgian research group conducted a study to develop imaging reporters and suicide genes (human somatostatin receptor type 2 and human sodium iodide symporter) for monitoring cell survival during transplantation of ESC-derived cardiomyocytes (Collins & Gottlieb, 2018). However, in this study, where human ESCs harboring a radionuclide imaging reporter gene were created using ZFN tools, no improvement in myocardial infarction was observed. Nonetheless, this study contributed to the clinical translation of stem cell reporter gene-based imaging and our understanding of the safety switch mechanism regulating undesired teratoma development during cell therapy.

The combination of the superior pluripotency and high proliferation of ESCs with the specificity of genome editing can lead to highly efficient stem cell therapies for many intractable diseases once ethical and safety issues are surpassed.

3.1.1.3. Application of iPSCs. iPSCs are pluripotent stem cells derived from the reprogramming of adult somatic cells using specific pluripotent indicator genes, such as *OCT3/4, SOX2, c-MYC*, and *KlF4* (Takahashi & Yamanaka, 2006). iPSCs are valuable substitutes of ESCs in terms of pluripotency and differentiation ability. In addition, iPSCs are novel cell sources for disease model generation, drug development, and cytotoxicity studies as they may originate from patients (Narsinh et al., 2011; Omole & Fakoya, 2018; Takahashi & Yamanaka, 2006). Furthermore, iPSCs are more frequently being utilized in the field of

biomedicine as they possess the same properties as ESCs, while their use is ethically accepted.

To date, nearly one hundred iPSC-based therapies have been successfully enrolled in clinical trials and yielded positive outcomes in preclinical animal studies. Developments in iPSC and programmable nuclease-based tools have synergistically enhanced the power of gene editing-based stem cell therapies and hold the promise for breakthrough discoveries in this century.

In several studies, CRISPR tools have been utilized to edit iPSCs. This includes a therapeutic study on hemophilia A (classical hemophilia) using genetically modified iPSCs. Hemophilia A is an X-linked genetic and bleeding disorder caused by the lack or deficiency of the functional coagulation factor VIII. Repetitive injection of recombinant factor VIII is the current therapy for patients with hemophilia A. However, this is not an optimal treatment option as some of the patients develop anti-FVIII neutralizing antibodies after receiving the injections. In addition, the need for repeated injections and the associated high cost of the therapy are economic and quality of life burdens for the patients. On the other hand, gene-corrected stem cells can result in life-long correction. Hence, FVIII gene-edited stem cell therapy offers the potential for significant improvement over the traditional approach for treating hemophilia A (Park et al., 2015).

One of the key steps in gene-edited iPSC therapy is the accurate delivery of the gene into patients *via* viral or non-viral methods. Delivery of the gene encoding the functional coagulation factor FVIII *via* viral vectors is challenging because of the large size of the gene. A South Korean research team (Park et al., 2015) reported for the first time that transplantation of endothelial cells derived from FVIII gene-corrected patient-specific iPSCs is the most efficient and suitable treatment for hemophilia A. In that study, they corrected the FVIII gene of patient-derived iPSCs using CRISPR/Cas9 nucleases and differentiated them into endothelial cells, which were then transplanted into a mouse model of hemophilia A. The positive outcomes of the study demonstrated the possibilities of functional correction of large chromosomal rearrangements using CRIPSR and contributed to advances in therapeutic utilization of patient-derived iPSCs.

A similar pioneering study was conducted by Olgasi and colleagues in 2018. Their study aimed at reducing the formation of anti-FVIII neutralizing antibodies during gene-edited stem cell therapy. To solve this problem, they generated patient-specific disease-free iPSCs from peripheral blood $CD34^+$ cells and differentiated them into functional endothelial cells secreting FVIII, which were then transplanted into immune-deficient null HA mice. The transplanted FVIII-expressing endothelial cells engrafted and proliferated in the liver along the sinusoids, resulting in long-term stable therapeutic activity of FVIII even at low concentrations.

Duchenne muscular dystrophy (DMD) is another example of an X-linked fatal genetic disorder, which may be rectified using CRISPR-edited iPSCs. It is caused by the absence of the dystrophin protein, which plays a crucial role in muscle cells. To date, there is no cure for this disease, except surgical and physical therapies to alleviate some of the symptoms. Approximately 60% of the mutations (frameshift) causing DMD occur between exons 45 and 55, and correcting these mutations or modifying *DMD* are promising treatment options for DMD patients (Echigoya, Lim, Nakamura, & Yokota, 2018). The largest CRISPR/Cas9-mediated deletion (725 kb) in DMD was performed in a pioneering study by Young et al. (2016), in which the authors removed exons 45–55 and NHEJ to reframe dystrophin and create a functional protein with a stable internal deletion. The gene-edited iPSCs were differentiated into cardiomyocytes and skeletal muscle cells and transplanted into mice models of the disease. Engraftment of correctly localized dystrophin and β-dystroglycan illustrated that *in situ* or *ex vivo* gene correction strategies may be potential options for DMD therapy in the near future.

The combination of CRISPR/Cas9 and iPSC technology has also been applied for the genetic therapy of β-thalassemia (Ou et al., 2016). Autologous transplantation of *ex vivo*-corrected patient-derived hematopoietic stem cells was used to treat β-thalassemia, although it is difficult and time-consuming to correct all hematopoietic stem cells. On the other hand, generation of an *HBB* gene-corrected β-thalassemia patient-derived iPSC line and its differentiation to hematopoietic stem cells for autologous transplantation resulted in a more effective and rapid method of treating β-thalassemia. Ou's et al. also observed high levels of hematopoietic stem cell differentiation of gene-edited iPSCs both *in vivo* and *in vitro*.

Furthermore, the metabolic disease mucopolysaccharidosis was targeted using CRISPR-edited iPSC therapy. Miki et al. (2019) reported iPSC derivation and *ex vivo* gene correction using a mouse model of mucopolysaccharidosis type 1. In this context, they corrected the gene encoding α-L–iduronidase in defective iPSCs using the CRISPR/Cas9 system. The authors applied CRISPR to remove the neomycin-resistant gene cassette in exon VI of the α-L–iduronidase gene of defective iPSCs and repaired the gene with donor DNA template without any indel mutation. The pluripotency and differentiation capacity of iPSCs render them major players for drug discovery programs.

3.2. In vivo application: stem cell targeting

In vivo genome editing in endogenous stem cells is performed using either viral vectors or a mixture of viral vectors and lipid nanoparticles (Zhao & Huang, 2014). Clinical trials utilizing *in vivo* genome-edited stem cells have not yet been registered in the FDA database. Owing to efficacy and safety issues, the *in vivo* genome editing approach is scarcely used in the field of stem cell therapy. CRISPR-induced skin stem cells, as well as cardio myocytes and muscle stem cells, are examples of cells used in preclinical studies to assess the safety and therapeutic effects of *in vivo* gene editing.

Wu et al. (2017) used *in vivo* electrophoretic delivery of the CRISPR/Cas9 system to edit collagen VII, defects in which cause recessive dystrophic epidermolysis bullosa. In that study, the gene editing system (Cas9/sgRNA RNP) was first injected into epithelial stem cells of a mouse disease model, followed by electroporation, resulting in the partial restoration of collagen VII function.

Issues related to the off-target activity, low diffusion, or poor stability of the genome editing tools *in vivo* render the efficiency of their viral delivery low. Therefore, extensive studies are being conducted to enhance the *in vivo* transfection efficiency of programmable nucleases *via* viral delivery methods, which can follow either local or systemic transfer strategies. To improve viral-mediated gene delivery, several strategies have been proposed, such as reduction in the size of Cas9 and use of a dual vector system for CRISPR/Cas9 tools. The reason for this is the 4.7 kb packaging capacity of the AAV genome (Grieger & Samulski, 2005). Following this approach, *in vivo* correction of *DMD* in muscle stem cells using CRISPR/Cas9 has been attempted to treat DMD (Tabebordbar et al., 2016). In summary, the therapeutic application of AAV-based traditional approaches is limited for the treatment of diseases such as hemophilia A and DMD caused by mutations in large parts of genes. Thus, the dual vector system was developed as an alternative solution for such problems. Accordingly, Tabebordbar et al. (2016) co-delivered AAVS-SaCas9 and AAV9-Dmd23 gRNAs into the *DMD* locus of muscle stem cells, as well as differentiated muscle fibers and cardiomyocytes *in vivo*. As a result, targeted gene modifications lead to functional dystrophin expression.

As the rate of *in vivo* delivery of programmable nucleases is not sufficiently high and the number of targeted stem cells in the patient is low, current research focuses on the development of therapeutic approaches supporting the recovery of low amounts of proteins/gene-edited cells.

3.3. Clinical application

According to the WHO definition, a clinical trial is any research study that prospectively assigns human participants or groups of people to

one or more heath related interventions to evaluate the effects in health outcomes (International standard for clinical trial registries by World Health Organization, 2012). As of 2019, more than 300,000 studies have been registered as clinical trials, and of them, approximately 7400 cases include stem cell application and less than 10 involve gene-edited stem cell therapy (U.S., NIH, www.clinicaltrials.gov). These data suggest that the clinical translation of genome-edited stem cell therapies is still emerging. The mapping of clinical trials applying stem cells shows USA, Europe, and East Asia as leaders in the field. Middle East countries and Canada are following. Currently published clinical trials including gene-edited stem cells, are shown in Table 3.

When disease is caused by a known mutation, genome-edited stem cell therapy has advantages over traditional stem cell therapies, for example, (i) functional improvement, (ii) mutation correction, (iii) homing and survival improvement, (iv) human leukocyte antigen matching, and (v) rapid and highly efficient treatment. With the aid of gene editing tools, stem cells can be functionally improved through the introduction of genes encoding for therapeutically active proteins that will be overexpressed during the treatment. Secondly, the precision of the gene editing tools ensures the success of *ex vivo* and *in vivo* gene correction, as described previously. Thirdly, the homing and viability properties of stem cells can be improved by gene editing tools. During stem cell transplantation, most of the cells cannot survive and undergo complete engraftment or migrate to the damaged area. A variety of growth factors, nucleic acids, and chemokines can improve the viability and homing properties of transplanted MSCs and iPSCs (Baek, Tu, Zoldan, & Suggs, 2016). These molecules can be secreted and synthesized from the transplanted stem cells themselves, when they are genetically edited by programmable nucleases, such as CRISPR/Cas9, ZFN, and TALEN, through knock in, knock out or correction of the responsible genes. Fourthly, the problem of immune rejection with non-autologous stem cell therapies is solved by human leukocyte antigen (HLA) matching through genome editing. Finding HLA-matched donor stem cells is time consuming, and their practical application is complicated and requires significant effort because of HLA diversity. Recently, gene-editing tools have been used to match HLA types between donor and patients to overcome these challenges and provide patients with HLA allele-matched stem cells in a short time period. For instance, CRISPR/Cas9 successfully knocked out the heterozygous HLA-B from iPSCs to develop homozygous HLA-A iPSCs (Jang et al., 2019). Taken together, the advantages of genome-edited stem cells enhance the promptness and efficiency of stem cell therapies.

In the field of genome-edited stem cell therapy, TALEN tools do not yet exist, but CRISPR and ZFN systems are being introduced in some countries. Currently, more than 120 people are estimated to participate in these clinical trials, and approximately 80% of them are involved in clinical studies of CRISPR-edited stem cells (HSPCs and MSCs), while the remaining 20% are in clinical trials of ZFN-mediated HSPCs (US NIH, www.clinicaltrials.gov).

Several therapies based on of genome-edited stem cells have been developed for applications in human diseases, such as the CTX001, ST-400, BIVV003, CCR5, and Epi-KAB therapies, according to currently conducted clinical trials (Fig. 2).

CTX001 therapy: This therapy has three stages: (i) isolating patient-derived bone marrow HSPCs, (ii) *ex vivo* genome editing by CRISPR/Cas9, and (iii) autologous transplantation. The patient-derived HSPCs are edited *ex vivo* at the erythroid lineage-specific enhancer of the BCL11A gene, and this modification allows production of high amounts of fetal hemoglobin containing erythrocytes, which helps the patients to overcome hemoglobin deficiencies originated from genetic blood diseases, such as SCD and β-thalassemia (Gonzalez-Romero et al., 2019). In this therapy, autologous transplantation of genome-edited HSPCs is conducted *via* single infusion of CTX001 through a central venous catheter. According to clinicaltrials.gov, approximately 90 patients are estimated to participate in CTX001-based clinical trials.

ST-400 or BIVV003 therapies: Similar to the CTX001 therapy, STP-400/BIVV003 uses patient-derived HSPCs and the Sangamo's ZFN technology. Depending on the disease, ZFN-edited HSPC therapies are named ST-400 (when applied for β-thalassemia) or BIVV003 (when applied for Sickle cell disease) (Holmes et al., 2017). The therapies target sickle cell disease or β-thalassemia by suppressing fetal hemoglobin in erythrocytes, thereby enhancing the expression of the BCL11A gene, which has been precisely disrupted by ZFN in patient-derived HSPCs. ST-400 and BIVV003 are combined with chemotherapy to allow for the accommodation of the new cells in the bone marrow of the patient. Currently, 14 patients are estimated to participate in clinical trials assessing the safety and efficacy of ST-400 and BIV003 therapies in inherited blood diseases (NIH U.S., www.clinincaltrials.gov).

CCR5 gene modification therapies: As we discussed previously, it has been shown that disruption of the CCR5 gene can be a promising approach for the long-term treatment of HIV infection. Therefore, clinical studies of genome-edited stem cell therapies against HIV infection are aiming at the disruption of the CCR5 gene. All these therapies use HSPCs and a similar gene editing strategy (targeted to CCR5 gene), but they differ on the employed genome-editing tools. Ongoing clinical trials use either the CRISPR/Cas9 system or the ZFN system. In these therapies, *ex-vivo* editing of patient-derived HSPCs is followed by autologous transplantation. According to the National Institute of Health (USA) database, there are 17 participants in clinical trials for HIV genome-edited stem cell therapies. In both cases, additional antiviral therapy is applied to achieve undetectable HIV levels in the patient's peripheral blood. In the ZFN-based therapy, busulfan is used to support the engraftment of-CCR5 disrupted HSPCs.

Epi-KAB therapy: Epigenome editing in Kabuki syndrome therapy utilizes patient-derived MSCs and the CRISPR/Cas9 system. Mutations in either the KMT2D or KDM6A genes can cause Kabuki syndrome (Tsai et al., 2018). The Epi-KAB therapy has been developed for the disease caused by the KMT2D mutation to restore MLL4 activity. Patient-derived fibroblasts are reprogrammed into MSCs and genome editing is conducted *ex vivo* prior to their autologous transplantation. The plan is to enroll 4 patients in the Phase I study (US NIH, www.clinicaltrials.gov).

Table 3
Summary of recent advances in clinical trials assessing *ex vivo* stem cell therapy.

Strategies	Diseases	Stem cell type	Target gene	NCT numbers	Status	Phase	Purpose	Study start
CRISPR/Cas9	Kabuki Syndrome 1	MSCs	KMT2D	03855631	Active, not recruiting	N/A	Treatment	2019
	β-Thalassemia	HSPCs	BCL11A	03655678	Recruiting	Phase 1&2	Treatment	2018
	Sickle Cell Disease	HSPCs	BCL11A	03745287	Recruiting	Phase 1&2	Treatment	2018
	HIV	HSPCs	CCR5	03164135	Recruiting	N/A	Treatment	2017
ZFN	β-thalassemia	HSPCs	BCL11A	03432364	Recruiting	Phase 1&2	Treatment	2018
	Sickle Cell Disease	HSPCs	BCL11A	03653247	Recruiting	Phase 1&2	Treatment	2018
	HIV	HSPCs	CCR5	02500849	Active, not recruiting	Phase 1	Treatment	2015
TALEN				N/A				

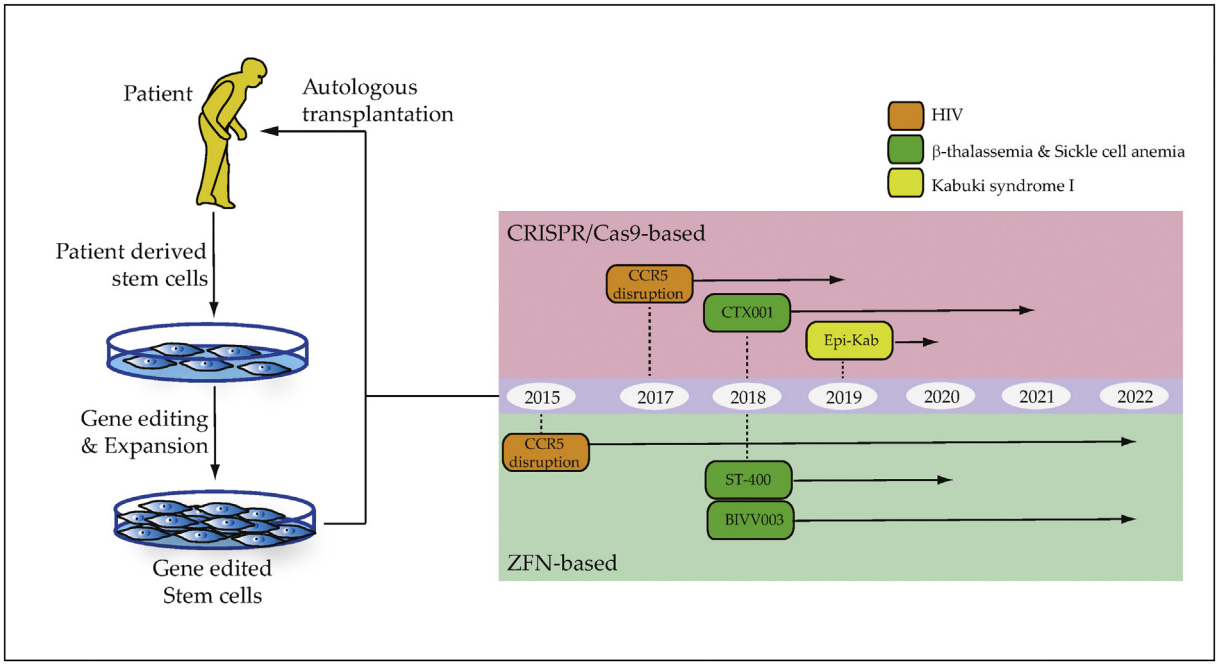

Fig. 2. Timeline of current human clinical trials using gene-edited stem cells.

These therapies have several advantages over conventional methods. For example, it is beneficial, both in terms of time and burden to patients, since genome-edited HSPCs show high and long-lasting efficacy. Additionally, the side effects and off-target effects on HSPCs have been optimized in the applied technologies (Holmes et al., 2017). There are still some issues that raise concern, including the pain associated with the bone marrow extraction procedure. This may be avoided in the near future with allogenic transplantation using universal and safe genome-edited stem cells.

4. Conclusion

With the recent advances in *ex vivo* and *in vivo* genome editing technologies that can correct genetic mutations in targeted stem cells, a new therapeutic modality has emerged that holds great promise for the treatment of intractable diseases. As summarized in this review, various studies have shown that genetic code editing results in priming of stem cells for better therapeutic efficacy, delayed disease progression, and protection against genetically-driven diseases.

However, the challenges associated with the strategies for precise *ex vivo* and *in vivo* editing and the delivery of gene-editing endonucleases must be addressed before genome editing is approved as a therapeutic tool and becomes incorporated into clinical practices. Recently, significant on-target mutagenesis, large deletions, and DNA breaks introduced using gRNA/Cas9 have been reported to result in deletions extending over many kilobases. Furthermore, several studies have revealed complexities in using CRISPR/Cas9-based genome editing because of the creation of DNA deletions, inversions, and duplications.

Many promising clinical trials are currently underway to investigate the clinical applicability and translatability of *in vivo* genome editing to achieve a permanent cure for certain diseases. With the current advances in *in vivo* genome editing, there is an imperative to evaluate its potential as a therapeutic platform and identify strategies to overcome its limitations. Currently, several approaches, including gene therapy, are being developed for patients with intractable diseases, such as cancer. In the near future, these patients may be treated with gene editing-based therapies, allowing the realization of the medical potential of the scientific advances in genome editing (Collins & Gottlieb, 2018).

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This research was supported by the Bio & Medical Technology Development Program of the National Research Foundation (NRF) funded by the Korean government (MSIT) (No. 2017M3A9B4028208/2017M3A9B4061408).

References

Anzalone, A. V., Randolph, P. B., Davis, J. R., Sousa, A. A., Koblan, L. W., Levy, J. M., ... Liu, D. R. (2019). Search-and-replace genome editing without double-strand breaks or donor DNA. *Nature 576*, 149–157.

Bae, T., Hur, J. W., Kim, D., & Hur, J. K. (2019). Recent trends in CRISPR/Cas system: Genome, epigenome, and transcriptome editing and CRISPR delivery systems. *Genes and Genomics 41*, 871–877.

Baek, K., Tu, C., Zoldan, J., & Suggs, L. J. (2016). Gene transfection for stem cell therapy. *Current Stem Cell Reports 2*, 52–61.

Bak, R. O., Dever, D. P., Reinisch, A., Cruz Hernandez, D., Majeti, R., & Porteus, M. H. (2017). Multiplexed genetic engineering of human hematopoietic stem and progenitor cells using CRISPR/Cas9 and AAV6. *Elife 6*, e27873.

Bayarsaikhan, E., Bayarsaikhan, D., Lee, J., Son, M., Oh, S., Moon, J., ... Lee, B. (2016). Microglial AGE-albumin is critical for neuronal death in Parkinson's disease: a possible implication for theranostics. *International Journal of Nanomedicine 10*, 281–292.

Bogliolo, M., & Surralles, J. (2015). Fanconi anemia: A model disease for studies on human genetics and advanced therapeutics. *Current Opinion in Genetics and Development 33*, 32–40.

Bondy-Denomy, J., Pawluk, A., & Davidson, A. R. (2013). Bacteriophage genes that inactivate the CRISPR/Cas bacterial immune system. *Nature 493*, 429–432.

Borot, F., Wang, H., Ma, Y., Jafarov, T., Raza, A., Ali, A. M., & Mukherjee, S. (2019). Gene-edited stem cells enable CD33-directed immune therapy for myeloid malignancies. *Proceedings of the National Academy of Sciences of the United States of America 116*, 11978–11987.

Byrne, S. M., Mali, P., & Church, G. M. (2014). Genome editing in human stem cells. *Methods in Enzymology 546*, 119–138.

Carroll, D. (2011). Genome engineering with ZFN. *Genetics 188*, 773–782.

Cermak, T., Doyle, E. L., Christian, M., Wang, L., Zhang, Y., Schmidt, C., ... Voytas, D. F. (2011). Efficient design and assembly of custom TALEN and other TAL effector-based constructs for DNA targeting. *Nucleic Acids Research 39*, e82.

Certo, M. T., & Morgan, R. A. (2016). Salient features of endonuclease platforms for therapeutic genome editing. *Molecular therapy: the journal of the American Society of Gene Therapy 24*, 422–429.

Chandrasegaran, S. (2017). Recent advances in the use of ZFN-mediated gene editing for human gene therapy. *Cell and gene therapy insights 3*, 33–41.

Chandrasegaran, S., & Carroll, D. (2016). Origins of programmable nucleases for genome engineering. *Journal of Molecular Biology 428*, 963–989.

Chapman, J. E., Gillum, D., & Kiani, S. (2017). Approaches to reduce CRISPR off-target effects for safer genome editing. *Applied Biosafety 22*, 7–13.

Chen, X., & Gonçalves, M. (2018). DNA, RNA, and protein tools for editing the genetic information in human cells. *iScience 6*, 247–263.

Cho, S. W., Kim, S., Kim, J. M., & Kim, J. S. (2013). Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. *Nature Biotechnology 31*, 230–232.

Christian, M., Cermak, T., Doyle, E. L., Schmidt, C., Zhang, F., Hummel, A., ... Voytas, D. F. (2010). Targeting DNA double strand breaks with TAL effector nucleases. *Genetics 186*, 757–761.

Cohen, J. (2019). Prime editing promises to be a cut above CRISPR. *Science 366*, 406.

Collins, F. S., & Gottlieb, S. (2018). The next phase of human gene-therapy oversight. *The New England Journal of Medicine 379*, 1393–1395.

Cong, L., Ran, F. A., Coz, D., Lin, S., Barretto, R., Habib, N., ... Zhang, F. (2013). Multiplex genome engineering using CRISPR/Cas systems. *Science 339*, 819–832.

Crisostomo, P. R., Wang, M., Wairiuko, G. M., Morrell, E. D., Terrell, A. M., Seshadri, P., ... Meldrum, D. R. (2006). High passage number of stem cells adversely affects stem cell activation and myocardial protection. *Shock 26*, 575–580.

Cuculis, L., Abil, Z., Zhao, H., & Schroeder, C. M. (2015). Direct observation of TALE protein dynamics reveals a two-state search mechanism. *Nature Communications 6*, 7277.

De Ravin, S. S., Li, L., Wu, X., Choi, U., Allen, C., Koontz, S., ... Malech, H. L. (2017). CRISPR/Cas9 gene repair of hematopoietic stem cells from patients with X-linked chronic granulomatous disease. *Science Translational Medicine 9* eaah3480.

De Ravin, S. S., Reik, A., Liu, P. Q., Li, L., Wu, X., Su, L., ... Malech, H. L. (2016). Targeted gene addition in human CD34(+) hematopoietic cells for correction of X-linked chronic granulomatous disease. *Nature Biotechnology 34*, 424–429.

Dever, D. P., Bak, R. O., Reinisch, A., Camarena, J., Washington, G., Nicolas, C. E., ... Porteus, M. H. (2016). CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. *Nature 539*, 384–389.

DeWitt, M. A., Magis, W., Bray, N. L., Wang, T., Berman, J. R., Urbinati, F., ... Corn, J. E. (2016). Selection-free genome editing of the sickle mutation in human adult hematopoietic stem/progenitor cells. *Science Translational Medicine 8* 360ra134.

Diez, B., Genovese, P., Roman-Rodriguez, F. J., Alvarez, L., Schiroli, G., Ugalde, L., ... naldini, L., Bueren, J.A., and Rio, P. (2017). Therapeutic gene editing in CD34+ hematopoietic progenitors from Fanconi anemia patients. *EMBO Molecular Medicine 9*, 1574–1588.

DiGiusto, D. L., Cannon, P. M., Holmes, M. C., Li, L., Rao, A., Wang, J., ... Zaia, J. A. (2016). Preclinical development and qualification of ZFN-mediated CCR5 disruption in human hematopoietic stem/progenitor cells. *Molecular Therapy. Methods and Clinical Development 3*, 16067.

Durai, S., Mani, M., Kandavelou, K., Wu, J., Porteus, M. H., & Chandrasegaran, S. (2005). Zinc finger nucleases: Custom designed molecular scissors for genome engineering of plant and mammalian cells. *Nucleic Acids Research 33*, 5978–5990.

Echigoya, Y., Lim, K., Nakamura, A., & Yokota, T. (2018). Multiple exon skipping in the Duchenne muscular dystrophy hot spots: Prospects and challenges. *Journal of personalized medicine 8*, 41.

Ellwanger, J. H., Kaminski, L. V., & Bogo Chies, J. A. (2019). CCR5 gene editing – Revisiting pros and cons of CCR5 absence. *Infection, Genetics and Evolution 68*, 218–220.

Farboud, B., Jarvis, E., Roth, T. L., Shin, J., Corn, J. E., Marson, A., ... Hochstrasser, M. L. (2018). Enhanced genome editing with Cas9 ribonucleoprotein in diverse cells and organisms. *Journal of Visualized Experiments 135*, e57350.

Fitzsimmons, R. E. B., Mazurek, M. S., Soos, A., & Simmons, C. A. (2018). Mesenchymal stromal/stem cells in regenerative medicine and tissue engineering. *Stem Cells International 2018*, 8031718.

Gaj, T., Guo, J., Kato, Y., Sirk, S. J., & Barbas, C. F., 3rd (2012). Targeted gene knockout by direct delivery of zinc-finger nuclease proteins. *Nature Methods 9*, 805–807.

Gaj, T., Sirk, S. J., Shui, S. L., & Liu, J. (2016). Genome-editing technologies: Principles and applications. *Cold Spring Harbor Perspectives in Biology 8*, a023754.

Gaudelli, N. M., Komor, A. C., Rees, H. A., Packer, M. S., Badran, A. H., Bryson, D. I., & Liu, D. R. (2017). Programmable base editing of A•T to G•C in genomic DNA without DNA cleavage. *Nature 551*, 464–471.

Genovese, P., Schiroli, G., Escobar, G., Tomaso, T. D., Firrito, C., Calabria, A., ... Naldini, L. (2014). Targeted genome editing in human repopulating haematopoietic stem cells. *Nature 510*, 235–240.

Goldman, S. L., Hassan, C., Khunte, M., Soldatenko, A., Jong, Y., Afshinnekoo, E., & Mason, C. E. (2019). Epigenetic modifications in acute myeloid leukemia: Prognosis, treatment, and heterogeneity. *Frontiers in Genetics 10*, 133.

Gonzalez-Romero, E., Martinez-Valiente, C., Garcia-Ruiz, C., Vazquez-Manrique, R. P., Cervera, J., & Sanjuan-Pla, A. (2019). CRISPR to fix bad blood: A new tool in basic and clinical hematology. *Haematologica 104*, 881–893.

Grieger, J. C., & Samulski, R. J. (2005). Packaging capacity of adeno-associated virus serotypes: Impact of larger genomes on infectivity and postentry steps. *Journal of Virology 79*, 9933–9944.

Gundry, M. C., Brunetti, L., Lin, A., Mayle, A. E., Kitano, A., Wagner, D., ... Nakada, D. (2016). Highly efficient genome editing of murine and human hematopoietic progenitor cells by CRISPR/Cas9. *Cell Reports 17*, 1453–1461.

Gupta, R. M., & Musunuru, K. (2014). Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR/Cas9. *The Journal of Clinical Investigation 124*, 4154–4161.

Handel, E. M., Alwin, S., & Cathomen, T. (2009). Expanding or restricting the target site repertoire of zinc-finger nucleases: The inter-domain linker as a major determinant of target site selectivity. *Molecular therapy: The Journal of the American Society of Gene Therapy 17*, 104–111.

Hinnen, A., Hicks, J. B., & Fink, G. R. (1978). Transformation of yeast. *Proceedings of the National Academy of Sciences of the United States of America 75*, 1929–1933.

Ho, B. X., Loh, S., Chan, W. K., & Soh, B. S. (2018). In vivo genome editing as a therapeutic approach. *International Journal of Molecular Sciences 19*, 2721.

Hoban, M. D., Cost, G. J., Mendel, M. C., Romero, Z., Kaufman, M. L., Joglekar, A. V., ... Kohn, D. B. (2015). Correction of the sickle cell disease mutation in human hematopoietic stem/progenitor cells. *Blood 125*, 2597–2604.

Hoke, N. N., Salloum, F. N., Kass, D. A., Das, A., & Kukreja, R. C. (2012). Preconditioning by phosphodiesterase −5 inhibition improves therapeutic efficacy of adipose-derived stem cells following myocardial infarction in mice. *Stem Cells 30*, 326–335.

Holmes, M. C., Reik, A., Rebar, E. J., Miller, J. C., Zhou, Y., Zhang, L., ... Vaidya, S. (2017). A potential therapy for Beta-Thalassemia (ST-400) and sickle cell disease (BIVV003). *Blood 130*, 2066.

Holt, N., Wang, J., Kim, K., Feridman, G., Wang, X., Taupin, V., ... Cannon, P. M. (2010). Human hematopoietic stem/progenitor cells modified by zinc-finger nucleases targeted to CCR5 control HIV-1 in vivo. *Nature Biotechnology 28*, 839–847.

Inusa, B. P. D., Hsu, L. L., Kohli, N., Patel, A., Ominu-Evbota, K., Annie, K. A., & Atoyebi, W. (2019). Sickle cell disease—Genetics, pathophysiology, clinical presentation and treatment. *International Journal of Neonatal Screening 5*, 20.

Ishino, Y., Krupovic, M., & Forterre, P. (2018). History of CRISPR/Cas from encounter with mysterious repeated sequence to genome editing technology. *Journal of Bacterology 200* e00580–17.

Ismail, A. (2015). Stem cell research and ethics: An update. *Oman Medical Journal 30*, 1–2.

Jang, Y., Choi, J., Park, N., Kang, J., Kim, M., Kim, Y., & Ju, J. H. (2019). Development of immunocompatible pluripotent stem cells via CRISPR-based human leukocyte antigen engineering. *Experimental and Molecular Medicine 51*, 3.

Jinek, M., Chylinski, K., Fonfara, I., Hauer, M., Doudna, J. A., & Charpentier, E. (2012). A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science 337*, 816–821.

Kennedy, E. M., Kornepati, A. V., Goldstein, M., Bogerd, H. P., Poling, B. C., Whisnant, A. W., ... Cullen, B. R. (2014). Inactivation of the human papillomavirus E6 or E7 gene in cervical carcinoma cells by using a bacterial CRISPR/Cas RNA-guided endonuclease. *Journal of Virology 88*, 11965–11972.

Kim, D., Bae, S., Park, J., Kim, E., Kim, S., Yu, H. R., ... Kim, J. S. (2015). Digenome-seq: Genome-wide profiling of CRISPR/Cas9 off-target effects in human cells. *Nature Methods 12*, 237–243.

Kim, D., Kim, D. E., Lee, G., Cho, S. I., & Kim, J. S. (2019). Genome-wide target specificity of CRISPR RNA-guided adenine base editors. *Nature Biotechnology 37*, 430–435.

Kim, D., & Kim, J. S. (2018). DIG-seq: A genome-wide CRISPR off-target profiling method using chromatin DNA. *Genome Research 28*, 1894–1900.

Kim, D., Kim, S., Park, J., & Kim, J. S. (2016). Genome-wide target specifities of CRISPR -Cas9 nucleases revealed by multiplex Digenome-seq. *Genome Research 26*, 406–415.

Kim, H., & Kim, J. S. (2014). A guide to genome engineering with programmable nucleases. *Nature Reviews Genetics 15*, 321–334.

Kim, K., Ryu, S. M., Kim, S. T., Baek, G., Kim, D., Lim, K., ... Kim, J. S. (2017). Highly efficient RNA-guided base editing in mouse embryos. *Nature Biotechnology 35*, 435–437.

Kim, M. S., & Kini, A. G. (2017). Engineering and application of zinc finger proteins and TALEs for biomedical research. *Molecules and Cells 40*, 533–541.

Kim, M. Y., Yu, K. R., Kenderian, S. S., Ruella, M., Chen, S., Shin, T. H., ... Gill, S. (2018). Genetic inactivation of CD33 in hematopoietic stem cells to enable CAR T cell immunotherapy for acute myeloid leukemia. *Cell 173*, 1439–1453.

Kim, S., Kim, D., Cho, S. W., Kim, J., & Kim, J. S. (2014). Highly efficient RNA-guided genome editing in human cells via delivery of purified Cas9 ribonucleoproteins. *Genome Research 24*, 1012–1019.

Kim, Y., Kweon, J., Kim, A., Chon, J. K., Yoo, J. Y., Kim, H. J., ... Kim, J. S. (2013). A library of TAL effector nuclease spanning the human genome. *Nature Biotechnology 31*, 251–258.

Komor, A. C., Kim, Y. B., Packer, Y. B., Zuris, J. A., & Liu, D. R. (2016). Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. *Nature 533*, 420–424.

Komor, A. C., Zhao, K. T., Packer, M. S., Gaudelli, N. M., Waterbury, A. L., Koblan, L. W., ... Liu, D. R. (2017). Improved base excision repair inhibition and bacteriophage Mu Gam protein yields C:G-to-T:A base editors with higher efficiency and product purity. *Science Advances 3* eaao4774.

Koo, T., Lee, J., & Kim, J. S. (2015). Measuring and reducing off-target activities of programmable nucleases including CRISPR/Cas9. *Molecules and Cells 38*, 475–481.

Koonin, E. V., Makarova, K. S., & Zhang, F. (2017). Diversity, classification and evolution of CRISPR/Cas systems. *Current Opinion in Microbiology 37*, 67–78.

Lee, C. M., Cradick, T. J., Fine, E. J., & Bao, G. (2016). Nuclease target site selection for maximizing on target activity and minimizing off target effects in genome editing. *Molecular Therapy 24*, 475–487.

Lee, H. B., Sebo, Z. L., Peng, Y., & Guo, Y. (2015). An optimized TALEN application for mutagenesis and screening in Drosophila melanogaster. *Cellular Logistics 5*, e1023423.

Lee, J., Bayarsaikhan, D., Arivazhagan, R., Park, H., Lim, B., Gwak, P., ... Lee, B. (2019). CRISPR/Cas9 edited sRAGE-MSCs protect neuronal death in Parkinson's disease model. *International Journal of Stem Cells 12*, 114–124.

Lee, J., Chung, J. H., Kim, H. M., Kim, D. W., & Kim, H. (2016). Designed nucleases for targeted genome editing. *Plant Biotechnology Journal 14*, 448–462.

Leventhal, A., Chen, G., Negro, A., & Boehm, M. (2012). The benefits and risks of stem cell technology. *Oral Diseases 18*, 217–222.

Li, C., Psatha, N., Sova, P., et al. (2018). Reactivation of γ-Globin in Adult β-YAC Mice After Ex Vivo and in Vivo Hematopoietic Stem Cell Genome Editing. *Blood*, 2915–2928. https://doi.org/10.1182/blood-2018-03-838540.

Li, L., Krymskaya, L., Wang, J., Henley, J., Rao, A., Cao, L. F., ... DiGiusto, D. L. (2013). Genomic editing of the HIV-1 coreceptor CCR5 in adult hematopoietic stem and progenitor cells using zinc finger nucleases. *Molecular Therapy 21*, 1259–1269.

Li, Q., Qin, Z., Wang, Q., Xu, T., Yang, Y., & He, Z. (2019). Applications of genome editing technology in animal disease modeling and gene therapy. *Computational and Structural Biotechnology Journal 17*, 689–698.

Lin, S., Staahl, B. T., Alla, R. K., & Doudna, J. A. (2014). Enhanced homology-directed human genome engineering by controlled timing of CRISPR/Cas9 delivery. *eLife 3*, e04766.

Lin, S. R., Yang, H. C., Kuo, Y. T., Liu, C. J., Yang, T. Y., Sung, K. C., ... Chen, P. J. (2014). The CRISPR/Cas9 system facilitates clearance of the intrahepatic HBV templates in vivo. *Molecular therapy, Nucleic acids 3*, e186.

Liu, C., Zhang, L., Liu, H., & Cheng, K. (2017). Delivery strategies of the CRISPR/Cas9 gene-editing system for therapeutic applications. *Journal of Controlled Release 266*, 17–26.

Liu, J., Gaj, T., Wallen, M. C., & Barbas, C. F. (2015). Improved cell-penetrating zinc-finger nuclease proteins for precision genome engineering. Molecular therapy. *Nucleic Acids 4*, e232.

Liu, X., Wu, Y., Li, Z., Yang, J., Xue, J., Hu, Y., Feng, M., Niu, W., Yang, Q., Lei., M, Xia, J., Wu, L., and Liang, D. (2012). Targeting of the human coagulation Facor IX gene at rDNA locus of human embryonc stem cells. PLoS One 7, e37071.

Liu, Z., Chen, M., Chen, S., Deng, J., Song, Y., Lai, L., & Li, Z. (2018). Highly efficient RNA-guided base editing in rabbit. *Nature Communications 8*, 2717.

Lomova, A., Clark, D. N., Campo-Fernandez, B., Flores-Bjurstrom, C., Kaufman, M. L., Fitz-Gibbon, S., ... Kohn, D. B. (2019). Improving gene editing outcomes in human hematopoietic stem and progenitor cells by temporal control of DNA repair. *Stem Cells 37*, 284–294.

Luo, M. L., Leenay, R. T., & Beisel, C. L. (2016). Current and future prospects for CRISPR-based tools in bacteria. *Biotechnology and Bioengineering 113*, 930–943.

Luo, Y., Xu, X., An, X., Sun, X., Wang, S., & Zhu, D. (2016). Targeted inhibition of the miR-199a/214 cluster by CRISPR interference augments the tumor tropism of human induced pluripotent stem cell – Derived neural stem cells under hypoxic condition. *Stem Cells International 2016*, 3598542.

Magis, W., Dewitt, M. A., Wyman, S. K., Vu, J. T.,Heo, S.-J., Shao, S. J., Hennig, F., Romero, Z.G., Campo-Fernandez, B., Mcneill, M., Rettig, G. R., Sun, Y., Lau, P. J., Wang, Y., Behlke, M.A., Kohn, D. B., Boffelli, D., Walters, M. C., Corn, J. E. and Martin, D. I. K. (2018). In vivo selection for corrected b-globin alleles after CRISPR/Cas9 editing in human sickle hematopoietic stem cells enhances therapeutic potential. BioRxiv, 432716.

Makarova, K. S., & Koonin, E. V. (2015). Annotation and classification of CRISPR/Cas systems. *Methods in Molecular Biology 1311*, 47–75.

Mali, P., Yang, L., Esvelt, K. M., Aach, J., Guell, M., DiCarlo, J. E., ... Church, G. M. (2013). RNA-guided human genome engineering via Cas9. *Science 339*, 823–826.

Mandal, P. K., Ferreira, L. M., Collins, R., Meissner, T. B., Boutwell, C. L., Friesen, M., ... Cowan, C. A. (2014). Efficient ablation of genes in human hematopoietic stem and effector cells using CRISPR/Cas9. *Cell Stem Cell 15*, 643–652.

Martinez-Lage, M., Puig-Serra, P., Menendez, P., Torres-Ruiz, R., & Rodriguez-Perales, S. (2018). CRISPR/Cas9 for Cancer therapy: Hopes and challenges. *Biomedicines 6*, 105.

Maxwell, K. L. (2017). The anti-CRISPR story: A battle for survival. *Molecular Cell 68*, 8–14.

Meng, D., Han, S., Jeong, I. S., & Kim, S. (2019). Interleukin 10 – Secreting MSCs via TALEN mediated gene editing attenuates left ventricular remodeling after myocardial infarction. (2019). *Cellular Physiology and Biochemistry 52*, 728–741.

Miki, T., Vazquez, L., Yanuaria, L., Lopez, O., Garcia, I. M., Ohashi, K., & Rodriguez, N. S. (2019). Induced pluripotent stem cell derivation and ex vivo gene correction using a mucopolysaccharidosis type 1 disease mouse model. *Stem Cells International 2019*, 6978303.

Miller, J. C., Holmes, M. C., Wand, J., Guschin, D. Y., Lee, Y. L., Rupniewski, I., ... Rebar, E. J. (2007). An improved zinc-finger nuclease architecture for highly specific genome editing. *Nature Biotechnology 25*, 778–785.

Miller, J. C., Tan, S., Qiao, G., Barlow, K. A., Wang, J., Xia, D. F., ... Rebar, E. J. (2011). A TALE nuclease architecture for efficient genome editing. *Nature Biotechnology 29*, 143–148.

Miller, R. J., Cairns, J. S., Bridges, S., & Sarver, N. (2000). Human immunodeficiency virus and AIDS: Insights from animal lentiviruses. *Journal of Virology 74*, 7187–7195.

Mir, A., Edraki, A., Lee, J., & Sontheimer, E. J. (2018). Type II-C CRISPR/Cas9 biology, mechanism, and application. *ASC Chemical Biology 13*, 357–365.

Murphy, M. B., Moncivais, K., & Caplan, A. I. (2013). Mesenchymal stem cells: Environmentally responsive therapeutics for regenerative medicine. *Experimental and Molecular Medicine 45*, e54.

Nakamura, M., Srinivasan, P., Chavez, M., Carter, M. A., Dominguez, A. A., Russa, M. L., ... Qi, L. S. (2019). Anti-CRISPR-mediated control of gene editing and synthetic circuits in eukaryotic cells. *Nature Communications 10*, 194.

Narsinh, K. H., Sun, N., Sanchez-Freire, V., Lee, A. S., Almeida, P., Hu, S., ... Wu, J. C. (2011). Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells. *The Journal of Clinical Investigation 121*, 1217–1221.

Neyrinck, K., Breuls, N., Holvoet, B., Oosterlinck, W., Wolfs, E., Vanbilloen, H., ... Deroose, C. M. (2018). The human somatostatin receptor type 2 as an imaging and suicide reporter gene for pluripotent stem cell-derived therapy of myocardial infarction. *Theranostics 8*, 2799–2813.

Olgasi, C., Talmon, M., Merlin, S., Cucci, A., Richaud-Patin, Y., Ranaldo, G., ... Follenzi, A. (2018). Patient-specific iPSC-derived endothelial cells provide long-term phenotypic correction of hemophilia A. *Stem Cell Reports 11*, 1391–1406.

Omole, A. E., & Fakoya, A. O. J. (2018). Ten years of progress and promise of induced pluripotent stem cells: Historical origins, characteristics, mechanisms, limitations, and potential applications. *PeerJ 6*, e4370.

Ou, Z., Niu, X., He, W., Chen, Y., Song, B., Xian, Y., ... Sun, X. (2016). The combination of CRISPR/Cas9 and iPSC technologies in the gene therapy of human β-thalassemia in mice. *Scientific Reports 6*, 32463.

Park, C. Y., Kim, D. H., Son, J. S., Sung, J. J., Lee, J., Bae, S., ... Kim, J. S. (2015). Functional correction of large factor VIII gene chromosomal inversions in hemophilia A patient-derived iPSCs using CRISPR/Cas9. *Cell Stem Cell 17*, 213–220.

Pattabhi, S., Lotti, S. N., & Berger, M. P. (2019). In Vivo Outcome of Homology-Directed Repair at the HBB Gene in HSC Using Alternative Donor Template Delivery Methods. *Nucleic Acids*. https://doi.org/10.1016/j.omtn.2019.05.025.

Pattanayak, V., Ramirez, C. L., Joung, J. K., & Liu, D. R. (2011). Revealing off-target cleavage specificities of zinc finger nucleases by in vitro selection. *Nature Methods 7*, 765–770.

Pavel-Dinu, M., Wiebking, V., Dejene, B. T., Srifa, W., Mantri, S., Nicolas, C. E., ... Porteus, M. H. (2019). Gene correction for SCID-X1 in long-term hematopoietic stem cells. *Nature Communications 10*, 1634.

Pawluk, A., Davidson, A. R., & Maxwell, K. L. (2018). Anti-CRISPR: Discovery, mechanism and function. *Nature Reviews Microbiology 16*, 12–17.

Qi, Y. (2017). Genome editing is revolutionizing biology. *Cell & Bioscience 7*, 35.

Ranganathan, V., Wahlin, K., Maruotti, J., & Zack, D. J. (2014). Expansion of the CRISPR/Cas9 genome targeting space through the use of H1 promoter-expressed guide RNAs. *Nature Communications 8*, 4516.

Rieger, M. A., & Schroeder, T. (2012). Hematopoiesis. *Cold Spring Harbor Perspectives in Biology 4*, a008250.

Fu, Y., Sander, J. D., Reyon, D., Cascio, V. M., & Joung, J. K. (2014). Improving CRISPR/Cas nuclease specificity using truncated guide RNAs. *Nature Biotechnology 32*, 279–284.

Saarimäki-Vire, J., Balboa, D., Russell, M. A., et al. (2017). An Activating STAT3 Mutation Causes Neonatal Diabetes Through Premature Induction of Pancreatic Differentiation. *Cell Reports 19*, 281–294. https://doi.org/10.1016/j.celrep.2017.03.055.

Sather, B. D., Romano Ibarra, G. S., Sommer, K., Curinga, G., Hale, M., Khan, I. F., ... Rawling, D. J. (2015). Efficient modification of CCR5 in primary human hematopoietic cells using a megaTAL nuclease and AAV donor template. *Science Translational Medicine 7* 307ra156.

Schierling, B., Dannemann, N., Gabsalilow, L., Wende, W., Cathomen, T., & Pingoud, A. (2012). A novel zinc-finger nuclease platform with a sequence-specific cleavage module. *Nucleic Acids Research 40*, 2623–2638.

Shand, J., Berg, J., & Bogue, C. (2012). Human embryonic stem cell (hESC) and human embryo research. *Pediatrics 130*, 972–977.

Shen, C. C., Hsu, M. N., Chang, C. W., Lin, M. W., Hwu, J. H., Tu, Y., & Hu, Y. C. (2019). Synthetic switch to minimize CRISPR off-target effects by self-restricting Cas9 transcription and translation. *Nucleic Acids Research 47*, e13.

Shim, G., Kim, D., Park, G. T., Jin, H., Suh, S. K., & Oh, Y. K. (2017). Therapeutic gene editing: Delivery and regulatory perspectives. *Acta Pharmacologica Sinica 38*, 738–753.

Shoemaker, L. D., & Kornblum, H. I. (2016). Neural stem cells (NSCs) and proteomics. *Molecular and Cellular Proteomics 15*, 344–354.

Son, M., Kang, W. C., Oh, S., Bayarsaikhan, D., Ahn, H., Lee, J., ... Lee, B. (2017). Advanced glycation end-product (AGE)-albumin from activated macrophage is critical in human mesenchymal stem cells survival and post-ischemic reperfusion injury. *Scientific Reports 7*, 11593.

Sun, L., Lutz, B. M., & Tao, Y. X. (2016). The CRISPR/Cas9 system for gene editing and its potential application in pain research. *Translational Perioperative and Pain Medicine 1*, 22–23.

Tabebordbar, M., Zhu, K., Cheng, J. K. W., Chew, W. L., Widrick, J. J., Yan, W. X., ... Wagers, A. J. (2016). In vivo gene editing in dystrophic mouse muscle and muscle stem cells. *Science 351*, 407–411.

Takahashi, K., & Yamanaka, S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell 126*, 663–676.

Thuronyi, B. W., Koblan, L. W., Levy, J. M., Yeh, W. H., Zheng, C., Newby, G. A., ... Liu, D. R. (2019). Continuous evolution of base editors with expanded target compatibility and improved activity. *Nature Biotechnology 37*, 1070–1079.

Timmers, L., Sluijter, J. P., van Keulen, J. K., Hoefer, I. E., Nederhoff, M. G., Goumans, M. J., ... de Kleijn, D. P. (2008). Toll-like receptor 4 mediates maladaptive left ventricular remodeling and impairs cardiac function after myocardial infarction. *Circulation Research 102*, 257–264.

Toma, C., Pittenger, M. F., Cahill, K. S., Byrne, B. J., & Kessler, P. D. (2002). Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation 105*, 93–98.

Tong, S., Moyo, B., Lee, C. M., Leong, K., & Bao, G. (2019). Engineered materials for in vivo delivery of genome –editing machinery. *Nature Reviews Materials 4*, 726–737.

Torikai, H., Mi, M., Gragert, L., Maiers, M., Najjar, A., Ang, S., ... Cooper, L. J. N. (2016). Genetic editing of HLA expression in hematopoietic stem cells to broaden their human application. *Scientific Reports 6*, 21757.

Tsai, I. C., McKnight, K., McKinstry, S. U., Maynard, A. T., Tan, P. L., Golzio, C., ... Katsanis, N. (2018). Small molecule inhibition of RAS/MAPK signaling ameliorates developmental pathologies of Kabuki syndrome. *Scientific Reports 8*, 10779.

Wang, J., Exline, C. M., DeClercq, J. J., Llewellyn, G. N., Hayward, S. B., Li, P. W., ... Cannon, P. M. (2015). Homology-driven genome editing in hematopoietic stem and progenitor cells using ZFN mRNA and AAV6 donors. *Nature Biotechnology 33*, 1256–1263.

Wei, X., Yang, X., Han, Z. P., Qu, F. F., Shao, L., & Shi, Y. F. (2013). Mesenchymal stem cells: A new trend for cell therapy. *Acta Pharmacologica Sinica 34*, 747–754.

World Health Organization (2012). *International standards for clinical trial registries.* World Health Organization.

Wu, W., Lu, Z., Li, F., Wang, W., Qian, N., Duan, J., ... Chen, T. (2017). Efficient in vivo gene editing using ribonucleoproteins in skin stem cells of recessive dystrophic epidermolysis bullosa mouse model. *Proceedings of the National Academy of Sciences of the United States of America 114*, 1660–1665.

Xie, J., Ge, W., Li, N., Liu, Q., Chen, F., Yang, X., ... Lai, L. (2019). Efficient base editing for multiple genes and loci in pigs using base editors. *Nature Communications 10*, 2852.

Xu, L., Yang, H., Gao, Y., Chen, Z., Xie, L., Liu, Y., ... Deng, H. (2017). CRISPR/Cas9-mediated CCR5 ablation in human hematopoietic stem/progenitor cells confers HIV-1 resistance in vivo. *Molecular Therapy 25*, 1782–1789.

Young, C. S., Hicks, M. R., Ermolova, N. V., Nakano, H., Jan, M., Younesi, S., ... Pyle, A. D. (2016). A single CRISPR/Cas9 deletion strategy that targets the majority of DMD patients restores dystrophin function in hiPSC-derived muscle cells. *Cell Stem Cell 18*, 533–540.

Yu, K. R., Natanson, H., & Dunbar, C. E. (2016). Gene editing of human hematopoietic stem and progenitor cells: Promise and potential hurdles. *Human Gene Therapy 27*, 729–740.

Zhao, X., & Moore, D. L. (2018). Neural stem cells: Developmental mechanisms and disease modeling. *Cell and Tissue Research 371*, 1–6.

Zhao, Y., & Huang, L. (2014). Lipid nanoparticles for gene delivery. *Advances in Genetics 88*, 13–36.

Zhou, C., Sun, Y., Yan, R., Liu, Y., Zuo, E., Gu, C., ... Wei.Y., Hu, X., Zeng, R., Li, Y., Zhou, H., Guo, F., and Yang, H. (2019). Off-target RNA mutation induced by DNA base editing and its elimination by mutagenesis. *Nature 571*, 275–278.