REVIEW ARTICLE | Iranian Biomedical Journal 27 (5): 219-246 September 2023

CRISPR-Cas Technology as a Revolutionary Genome Editing tool: Mechanisms and Biomedical Applications

Sahar Ebrahimi[1,2], Mohammad Ali Khosravi[2], Abbasali Raz[3], Morteza Karimipoor[2*], Parviz Parvizi[1*]

[1]Molecular Systematics Laboratory, Parasitology Department, Pasteur Institute of Iran, Tehran, Iran; [2]Molecular Medicine Department, Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran; [3]Malaria and Vector Research Group (MVRG), Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

OPEN ACCESS

Received: 1 February 2023
Accepted: 14 June 2023
Published online: 18 June 2023

Citation:
Ebrahimi S, Khosravi MA, Raz A, Karimipoor M, Parvizi P. CRISPR-Cas Technology as a Revolutionary Genome Editing tool: Mechanisms and Biomedical Applications. *Iranian biomedical journal* 2023; 27(5): 219-246.

ABSTRACT

Programmable nucleases are powerful genomic tools for precise genome editing. These tools precisely recognize, remove, or change DNA at a defined site, thereby stimulating cellular DNA repair pathways that can cause mutations or accurate replacement or deletion/insertion of a sequence. The CRISPR-Cas9 system is the most potent and useful genome editing technique adapted from the immune system of certain bacteria and archaea against viruses and phages. In the past decade, this technology has made notable progress, and at present, it has largely been used in genome manipulation to make precise gene editing in plants, animals, and human cells. In this review, we aimed to explain the basic principles, mechanisms of action, and applications of this system in different areas of medicine, with an emphasize on the detection and treatment of parasitic diseases.
DOI: 10.61186/ibj.27.5.219

Keywords: CRISPR-Cas system, Genome editing, Medicine, Parasitology

Corresponding Author:
Morteza Karimipoor
[2]Molecular Medicine Department, Biotechnology Research Center (BRC), Pasteur Institute of Iran, Tehran, Iran; mortezakarimi@pasteur.ac.ir, mortezakarimi@yahoo.com

Parviz Parvizi
Molecular Systematics Laboratory, Parasitology Department, Pasteur Institute of Iran, Tehran, Iran; parpparvizi@yahoo.com

[DOR: 20.1001.1.1028852.2023.27.5.1.7]

[DOI: 10.61186/ibj.3898]

List of Abbreviations:
AIDS: acquired immunodeficiency syndrome; **AML:** acute myeloid leukemia; **BCL11A:** B-cell Lymphoma 11A; **Cas:** CRISPR-associated protein; **Cas9n:** Cas9 nickase; **cccDNA:** covalently closed circular DNA; **CCHFV:** Crimean-Congo hemorrhagic fever virus; **CEP290:** centrosomal protein 290; **CF:** cystic fibrosis; **CFTR:** cystic fibrosis transmembrane conductance regulator; **CONAN:** Cas3-operated nucleic acid detection; **COVID-19:** the Coronavirus Disease 2019; **CREST:** Cas13a-based, rugged, equitable, scalable testing; **CRISPR:** clustered regularly interspaced short palindromic repeats; **CRISPR-Cas9:** clustered regularly interspaced short palindromic repeats and CRISPR-associated protein 9; **crRNA:** CRISPR RNA; **CSP:** circumsporozoite protein; **CTL:** cytotoxic T-cells; **CXCR4:** C-X-C chemokine receptor type 4; **DSB:** double-strand breaks; **dCas9:** nuclease-deactivated Cas9 or dead Cas9; **DETECTR:** DNA Endonuclease Targeted CRISPR Trans Reporter; **DMD:** Duchenne muscular dystrophy; **Env:** envelope; **FAM:** fluorescein amidite; **FELUDA:** FnCas9 editor-linked uniform detection assay; **fM:** femtomolar; **FnCas9:** *Francisella novicida* Cas9; **FREP1:** fibrinogen-related protein 1; **gRNA:** guide RNA; **HA:** hemagglutinin; **HBB:** β-globin gene; **HbF:** fetal hemoglobin; **HBV:** hepatitis B virus; **HD:** Huntington’s disease; **HDR:** homology-directed repair; **HIV:** human immunodeficiency virus; **HPV:** human papillomavirus; **HSC:** hematopoietic stem cells; **HSF1:** heat shock factor 1; **HSPC:** hematopoietic stem and progenitor cell; **HTT:** huntingtin; **Indel:** insertion or deletion; **IP3R:** inositol 1,4,5-trisphosphate receptor; **iPSC:** induced pluripotent stem cells; **IRD:** inherited retinal diseases; **K13-propeller:** kelch propeller domain of K13 gene; **kDNA:** kinetoplast DNA; **LbCas12a:** *Lachnospiraceae bacterium* Cas12a; **LdMT:** *Leishmania donovani* miltefosine transporter; **LTR:** long terminal repeat; **NA:** neuraminidase; **NHEJ:** non-homologous end joining; **NUC:** nuclease domain; **PAM:** protospacer adjacent motif; **RBD:** RNA-binding domain; **RH4:** reticulocyte binding protein homology 4; **RNAi:** RNA interference; **RPA:** recombinase polymerase amplification; **Rta**: replication and transcription activator; **RT-RPA:** reverse transcriptase recombinase polymerase amplification; **SAM:** synergistic activation mediator; **SARS-CoV-2:** severe acute respiratory syndrome coronavirus 2; **SCD:** sickle cell disease; **sgRNA:** single guide RNA; **SHERLOCK:** specific high-sensitivity enzymatic reporter UnLOCKing; **SpCas9:** Cas9 protein from *Streptococcus pyogenes*; **ssDNA:** single-stranded DNA; **TALEN:** transcription activator-like effector nuclease; **TET1:** ten-eleven translocation; **tracrRNA:** trans-activating CRISPR RNA; **VP64:** virion protein 64; **WT Cas9:** wild-type Cas9; **ZFN:** zinc finger nuclease

[DOI: 10.61186/ibj.3898] [DOR: 20.1001.1.1028852.2023.27.5.1.7] [Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

INTRODUCTION

Genome editing technology is a powerful tool for manipulating the genome, which allows scientists make highly specific changes in the DNA sequence of living organisms, including plants, bacteria, and animals. Gene editing is performed using programmable nucleases to target a specific DNA sequence, in which DNA is inserted, removed, or modified[1-3]. Genome editing nucleases induce targeted DSBs. DSBs are subsequently repaired by cellular mechanisms via NHEJ in the absence of a donor template or HDR in the presence of a donor template. HDR can be used for gene integration or base correction, while NHEJ is used to create Indels, which causes disruption of the target gene[4] (Fig. 1). Gene editing technology has been emerged in the 1990s, and three site-specific genome editing tools, namely ZFN, TALEN, and CRISPR-Cas9, each with its pros and cons, have widely been used (Table 1). The inspiration of the natural zinc fingers leads to the development of the first genome editing tool, ZFN, which is a fusion of a customizable DNA-binding protein and *Fok I* endonuclease to create DSB[5-7]. The discovery of transcription activator-like effectors in late 2009 resulted in the consideration of the TALEN as an alternative platform to ZFNs as engineering programmable DNA-binding proteins. TALEN is also comprised of DNA-binding and *Fok I* cleavage domains. In comparison with the ZFN, TALEN benefits from two important advantages: first, lower toxicity and higher specificity, and second, the simpler design[8,9]. Genome editing technology has taken a leap forward since the discovery of CRISPR-Cas in 1987[10,11].

In comparison with ZFNs and TALENs, CRISPR/Cas9 is not only a versatile, robust, and highly precise genome editing tool, but also is very quick, inexpensive, and extremely simple to use. Unlike ZFN and TALEN that recruit proteins to recognize the targeted sequences in the genomic regions, the CRISPR/Cas system uses a crRNA to recognize the targeted genomic sequence and acts as a scaffold for recruiting the Cas endonuclease to introduce site-specific DSBs[12]. This review provides background knowledge on the history and mechanism of CRISPR/Cas systems, the classification of CRISPR-Cas systems, and the application of CRISPR as a genome editing tool. The application of CRISPR systems has been discussed for treatment and diagnosis, with more focus on infectious diseases and parasites.

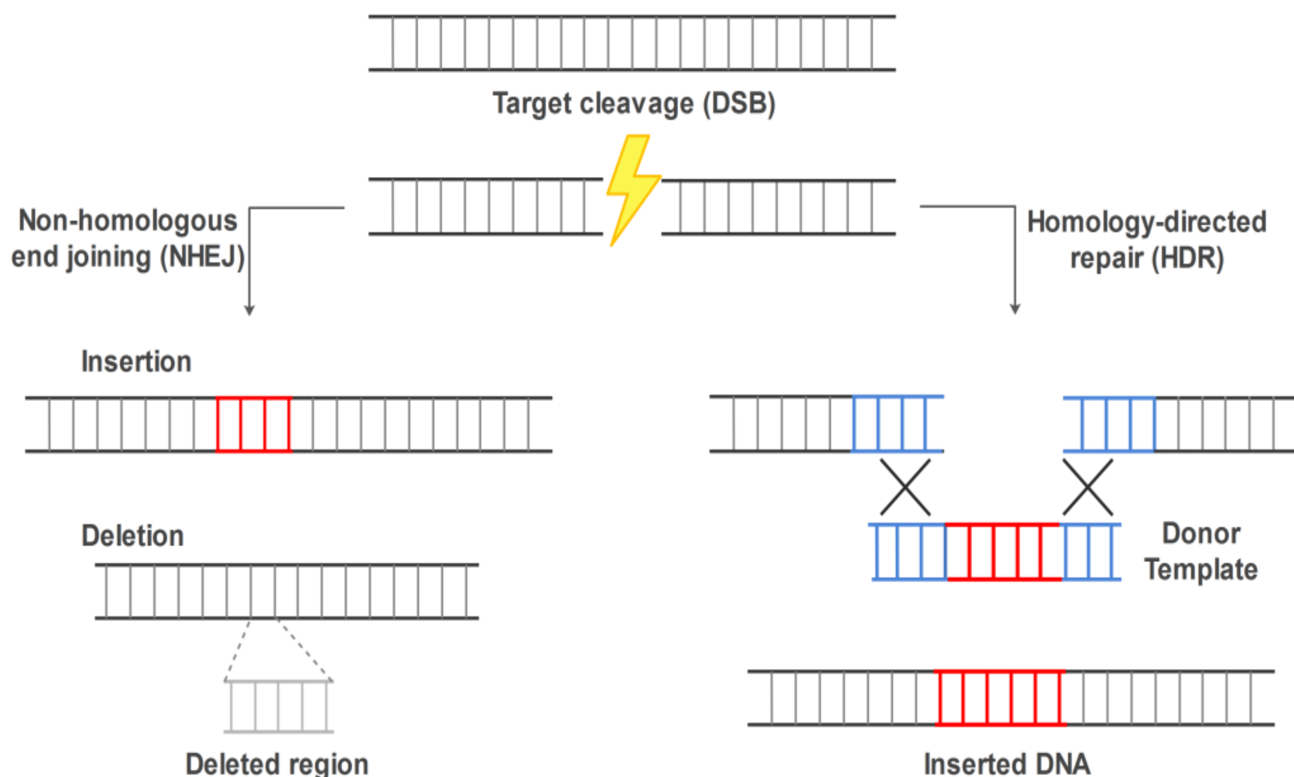

Fig. 1. CRISPR/Cas9-mediated DSB repair mechanism. The Cas9 introduces a DSB in the target DNA. DNA repairing includes the NHEJ and HDR pathways. The NHEJ pathway leads to Indel. The HDR pathway uses homologous donor DNA sequences to create precise insertion between DSB sites.

Table 1. Comparison of the features of three gene editing techniques[13-15].

Genome editing tool	ZFN	TALEN	CRISPR-Cas9
Component	ZFP + FokI Fusion protein	TALE + FokI Fusion protein	gRNA + Cas9 Protein
Nuclease	FokI	FokI	Cas9
DNA-binding domain	Protein	Protein	RNA
Target site size	9-18 bp	30-40 bp	22 bp + PAM sequence
Design availability	More complex	Complex	Very simple
Ease of multiplexing	Low	Low	High
Time	7-10 days	5-7 days	1-3 days
Cost	High	High	Low
Ex vivo delivery	Easy	Easy	Easy
Efficiency	Variable	High	High
Methylation sensitivity	High	High	Low

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

[DOR: 20.1001.1.1028852.2023.27.5.1.7]

[DOI: 10.61186/ibj.3898]

CRISPR/Cas9 System

A brief history of the CRISPR/Cas9 system

CRISPR/Cas systems are repeating DNA sequences in bacteria and archaea and act as adaptive immune response systems that protect the mentioned organisms against invading viruses, such as bacteriophages and plasmids. In 1987, CRISPR systems were first discovered in the *Escherichia coli* genome by a Japanese scientist, Yoshizumi Ishino. This system comprises a set of 29 nucleotide repeats interspaced by five intervening 32-nucleotide non-repetitive sequences in the *Escherichia coli* genome[16]. In 1993, CRISPR clustered repeats were discovered in *Mycobacterium tuberculosis* by van Embden[17] and his team who identified different spacer sequences between the DNA repeats. This clustered array was highly conserved across multiple evolutionary distinct bacterial genomes, and the locus was named CRISPR by Francisco Mojica, a microbiologist at the University of Alicante in Spain, in 2002[18]. In 2010, the CRISPR/Cas system of *Streptococcus thermophilus* was shown to introduce DSBs at a precise position in the target DNA[19]. In 2011, Deltcheva and colleagues[20] reported that tracrRNA, CRISPR-Cas9, and RNase III are essential for maturation of crRNAs in *Streptococcus pyogenes*. One year later, in 2012, it was discovered that the CRISPR system of *Streptococcus pyogenes* serves as a genome editing tool by showing that the Cas9 can be guided by tracrRNA, crRNA, and a synthetic sgRNA to cleave target DNA in vitro[10,21]. Since then, the CRISPR/Cas9 technology has been applied for targeted and precise manipulations of DNA in various cell types and organisms[22-26].

Components of CRISPR/Cas9 system

According to the structure and function of the effector complex (Cas proteins), the CRISPR/Cas system is classified into two main classes with six types. Class I comprises types I, III, and IV. Class II system comprises types II, V and VI. The first and most common type used in genome editing is the type II CRISPR/Cas9 system, which includes three main components: Cas9 protein, crRNA, and tracrRNA. The effector protein of class I consists of a multiprotein complex, while the class II system utilizes the Cas9 protein, which is a single, large, and multidomain Cas protein[27,28]. As an RNA-guided endonuclease, SpCas9 has been adapted for targeted genome editing in a variety of organisms. Cas9 contains two parts: a recognition domain, which is responsible for binding gRNA to target nucleic acid, and a NUC domain, which includes HNH, RuvC lobes, and PAM-interacting domains. In the NUC, the RuvC-like nuclease cleaves the non-complementary (non-target) DNA strand, and the HNH cleaves complementary (target) DNA strand. The PAM-interacting domain is responsible for identifying the PAM sequence and binding to the non-complementary strand of the target DNA[29-31].

Mechanisms of CRISPR/Cas9-based genome editing

The critical part of the CRISPR/Cas9 tool is sgRNA, which is a combination of crRNA and tracrRNA. The crRNA is a 20-bp sequence complementary to the target DNA site (also called protospacer), located at the 5′ end of sgRNA, and tracrRNA serves as a binding scaffold for the Cas9 endonuclease. Easily programmable sgRNA can effectively recognize specific sequences and guide the Cas9 endonuclease to the target site by simple Watson–Crick base pairing and makes DSBs 3 or 4-bp upstream of the PAM sequence at a target site[10]. The specific binding of CRISPR/cas9, besides the 20 bp complementarity sequence of gRNA, requires

[DOI: 10.61186/ibj.3898] [DOR: 20.1001.1.1028852.2023.27.5.1.7] [Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

a PAM sequence, i.e. a short and conserved sequence of 2-5 bp length located next to the target site. The PAM sequence and its size depend on the bacterial species. The PAM sequence for *Streptococcus pyogenes* Cas9 is 5′-NGG-3′[21-27,32-35], for *Streptococcus thermophiles* is 5'-NGGN G-3' or 5'-NNAGAAW-3'[36,37], for *Staphylococcus aureus* is 5'-NNGRRT-3' or 5'-NNGRR(N)-3'[38,39], for *Neisseria meningitidis* is 5'-NNNRRT-3' or 5'-NNNNGMTT-3'[40], and for *Francisella novicida* (FnCpf1) is 5'-NGG-3'[41]. Nucleotide bases are represented by N; G shows guanine, R indicates purine A or G, M represents nucleotide A or C, and W denotes weak bond A or T.

Different Cas types

In order to enhance precise genome editing using the CRISPR-Cas system, several other Cas endonuclease proteins and Cas9 variants with different features and PAM specificities have been studied and developed as gene editing tools. Three variants of the Cas9 as genome editors have been designed and used so far. The first, WT Cas9, cleaves double-stranded DNA at a target site using HNH and RuvC-like NUCs[42-45]. Various methods have been developed to increase on-target efficiency and reduce potential off-target mutagenesis of WT Cas9 such as shortened and modified sgRNAs, purified Cas9 ribonucleoproteins, engineered Cas9 protein, *FokI*-Cas9 fusion nucleases, and paired catalytic mutant Cas9n. WT Cas9 can be converted into second variant Cas9n by introducing point mutations in one of the NUCs (RuvC^{D10A} or HNH^{H840A}). Cas9n produces single-strand breaks rather than a DSB. To produce DSBs with Cas9n, specific binding of two gRNAs, placed on opposite strands, is required[46-48]. Cas9^{D10A} nickase was successfully exploited to target genes for the DNA-damage response proteins MDC1, 53BP1, RIF1 and P53, and Lamin A/C (the nuclear architecture proteins) in three different human cell lines[45]. The third variant of SpCas9 is nuclease-deactivated Cas9, called dCas9, which is produced through D10A and H840A mutations at the HNH and RuvC NUCs, respectively. The dCas-9 nuclease does not have DNA cleavage activity, but its DNA-binding activity has been still retained[49]. Thus, the dCas9 can be utilized to precisely and specifically bind to the targeted site within the genome, without cutting the DNA. The CRISPR/dCas9 DNA-targeting technique has various applications in many areas. For instance, the dCas9 protein can be fused with a variety of functionally active domains to directly modify transcription without genetically changing the DNA sequence[50-52]. Effector domains may include epigenetic repressing domains to create CRISPR inhibitors, such as Krüppel-associated Box or Sin3a-interacting domain, that silence the expression of the target gene by interfering with transcriptional initiation (via obstruction of RNA polymerase binding and elongation[53-55]. The CRISPR/dCas9 can also be fused to the transcription activator domains to produce a CRISPR activator for gene activation by recruiting transcription factors to the target gene. Until date, many transcriptional activators have been developed. VP64 (4× fusion of the VP16 transcriptional activation protein derived from herpes simplex virus) is a widely used activator domain for modest gene activation[56-59] (Fig. 2A). VPR is a tripartite complex consist of VP64, P65, and Rta fused with dCas9 in order to activate transcription[60] (Fig. 2B). SAM is a combination of dCas9/VP64 protein engineered with aptamers, which binds to MS2, HSF1, and p65 proteins. dCas9-VP64-SAM acts as a strong transcriptional activator[61,62] (Fig. 2C). SunTag system uses up to 24 repeats of VP64 instead of a single copy at each target site. Thus, dCas9-SunTag recruits more transcriptional machinery to the targeted gene. The limitation of this platform is the complexity of its construction[63,64] (Fig. 2D). Epigenetic modifications are a wide range of changes in gene expression without altering the DNA sequence. Epigenetic dysregulation is correlated with alterations in gene expression levels and disease states. Recently, targeted editing of the epigenome has become feasible using the CRISPR/Cas9 system. The dCas9-TET1 system is a dCas9 fused with the TET1 protein that is employed for DNA demethylation by oxidizing 5-methylcytosine to 5-hydroxymethylcytosine. Targeted DNA methylation at the specific site is achievable by dCas9-DNA methyltransferase[65-67].

CRISPR-Cas12a (Cpf1)

CRISPR-SpCas9 from *Streptococcus pyogenes* is type II, subtype II-A of CRISPR/Cas Class 2 systems, which is the first and most popular genome editing tool. Class II type V is divided into four subtypes (V-A, V-B, V-C, and V-U). The CRISPR-Cas12a from *Prevotella* and *Francisella 1* bacteria belongs to class II, type V, and subtype V-A CRISPR system. Cas12a is an endonuclease enzyme, which comprises ~1,300 amino acids, and it is a little smaller than SpCas9 with 1,368 amino acids. There are some differences between Cas12a and Cas9 proteins. Cas12a, in contrast to Cas9, does not require a tracrRNA for the biogenesis of mature crRNA. Unlike Cas9, Cas12a recognizes 5' T-rich PAM sequences "TTN/TTTN/TTTV" (N=A, T, C, G; V=A, C, G) to generate DSBs. Cas12a cleaves targeted sequence, 18-23 nt in the downstream of the PAM in a staggered pattern (4 or 5 nt overhang), to create sticky ends, contrary to blunt ends produced by Cas9. Cas9 possesses two endonuclease domains (RuvC and HNH),

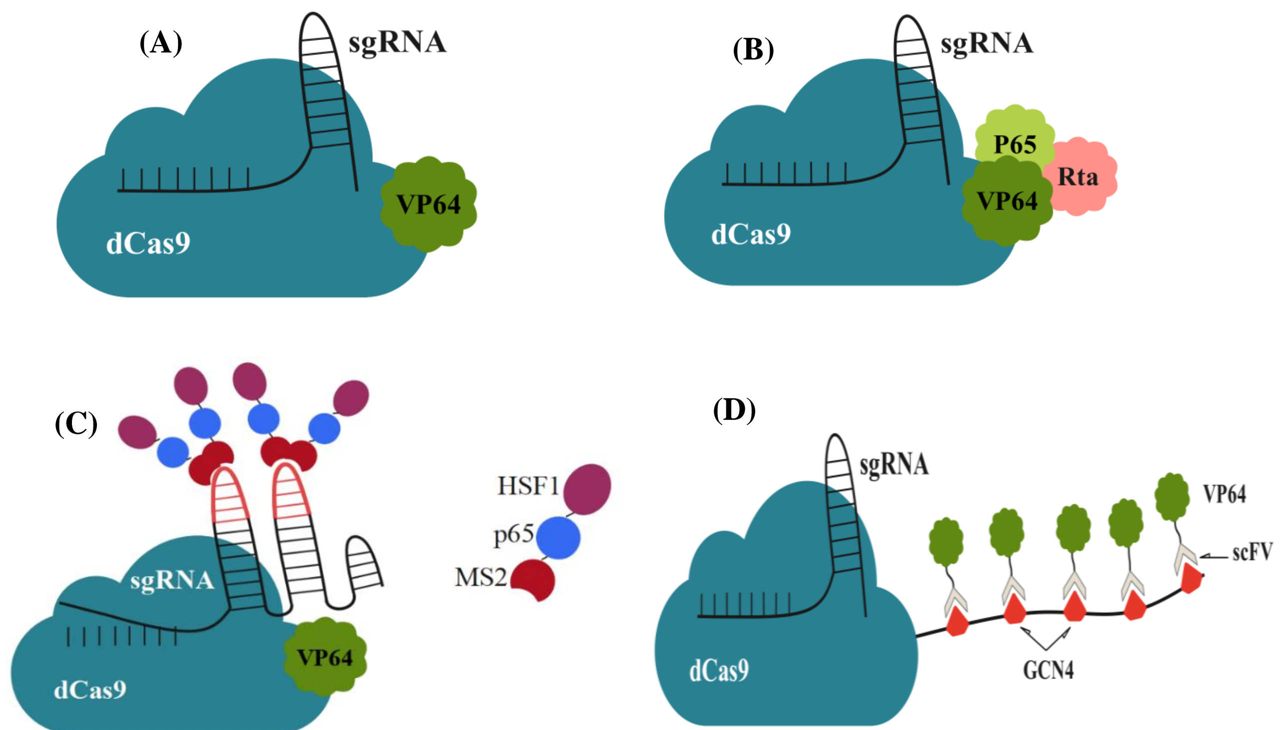

Fig. 2. Gene activation by engineered CRISPR systems. (A) The dCas9-VP64 was generated by fusing dCas9 with VP64, a strong transcriptional activator domain (four tandem copies of VP16, herpes simplex viral protein 16). (B) The VPR system is dCas9-VP64, which is fused with two other strong transcriptional activators (p65 and Rta). The VPR system is stronger than the dCas9-VP64 in gene activation. (C) The SAM system is a dCas9-VP64 with engineered sgRNAs fused with MS2, HSF1, and p65 proteins, which shows the highest levels of gene activation. (D) The SunTag system comprises dCas9 fused to tandem GCN4 peptide repeats, a single chain variable fragment (scFv) antibody, and multiple copies of the VP64.

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

[DOR: 20.1001.1.1028852.2023.27.5.1.7]

[DOI: 10.61186/ibj.3898]

while Cas12a lacks the HNH NUC[68-73]. Because Cas12a has ssDNA cleavage activity, it can be employed for nucleic acid detection and genome manipulation. Chen et al.[74] combined the LbCas12a with RPA, a sensitive and selective isothermal amplification technique, to develop a rapid method known as DETECTR. This method has been exploited for nucleic acid detection of two high-risk types of HPV 16 and 18 with attomolar sensitivity. In this study, the extracted viral DNA was amplified by isothermal amplification. crRNAs are designed to target the hypervariable loop V region of the L1 gene of HPV16 and 18. The crRNA and target DNA binding trigger LbCas12a proteins to cleave the target and nontarget DNA in which fluorophores and quenchers are connected to different sides of ssDNA[74].

CRISPR-Cas13a

CRISPR-Cas13a, previously known as CRISPR-C2c2, from *Leptotrichia* species, belongs to the type VI class II CRISPR-Cas system. CRISPR-Cas13a is the most recently identified editing tool with RNA-guided ribonuclease activity, which can be programmed to cut single-stranded RNA molecules. The CRISPR-Cas13a system contains an effector protein with two predicted conserved higher eukaryotes and prokaryotes nucleotide-binding RNase domains, which are responsible for preferentially cutting the targeted RNA at uracils. Previous studies have shown that Cas13a is an applicable and effective protein for cutting and editing of targeted RNA in both prokaryotic and eukaryotic cells, as well as nucleic acids detection with high sensitivity[75-80]. In 2017, Gootenberg et al.[75] developed a novel CRISPR/Cas13a-based nucleic acid detection platform called SHERLOCK, which is created by combining the collateral cleavage effect of Cas13a with T7 transcription and RPA for detection of Zika and dengue viruses with attomolar sensitivity and specificity. The RPA-SHERLOCK platform was also used by Li et al.[81] to detect the RNA-based CCHFV, a severe hemorrhagic fever virus. Within 30-40 minutes, the CCHFV was identified using a rapid, sensitive, and precise method with a limit of detection of 1 copy/µl. In a different study published in 2019, Liu et al.[82] used the SHERLOCK platform (CRISPR/Cas13a, RT-RPA, and T7 transcription) to detect Avian Influenza A (H7N9)

virus nucleic acid by targeting its HA and NA genes. The platform successfully detected one femtomole of HA and NA within 50 minutes.

CRISPR-Cas14

The newly discovered Cas14, with 400 to 700 amino acids, two folds shorter than Cas9 (~1400 amino acids), is the smallest and most compact RNA-guided nuclease recognized till now. Cas14 was found in the DPANN, a superphylum of archaea that contains the smallest cell and genome size compared to other archaea. Cas14 binds to ssDNA and cuts it without requiring a PAM flanking the target DNA sequence. The non-specific ssDNA trans-cleavage activity of Cas14 has enabled more efficient and robust applications of this system in the development of DNA detection platforms, such as diagnosis of microbial infections, ssDNA viruses, and detection/modification of cancer cells[83-85].

CRISPR-Cas12b

CRISPR-Cas12b (C2c1) is an intriguing genetic engineering tool/genome editing system, which changes the molecular biology landscape. It is an RNA-guided nuclease derived from the Type II CRISPR-Cas bacterial adaptive immune system. Discovered in the early 2000s, the tool has revolutionized the development and application of genome editing technology. At the structural level, Cas12b is a 20-25 kDa single polypeptide that consists of two separate domains: the RuvC and HNH. The RuvC domain is responsible for unwinding the targeted DNA sequence, while the HNH domain performs the cutting action. Working in concert, the two domains can quickly identify a specific DNA sequence and cut it out to convert a single base pair mutation. In terms of clinical application, Cas12b has been repurposed to enable mammalian genome editing. Numerous studies have demonstrated its ability to introduce the desired mutations at the single base pair resolution in the genomes of living mammals. In addition to genome editing, the versatile nature of Cas12b can also be used to detect viruses at ultrasensitive scales. By employing the C2c1-based diagnostic system, the detection of viruses can be achieved with high accuracy, specificity, and speed. Most recently, the Cas12b platform has been used to develop genetic circuitries via combinatorial assembly and engineering. This progress paves the way for the development of new approaches to gene therapy, gene manipulation, and the creation of designer organisms[86-89]. As the body of knowledge surrounding C2c1 continues to expand, the opportunities for broadening its uses increase. By its broadening scope and its efficient and precise delivery of genetic modifications, it is no wonder that Cas12b is now a central figure in modern molecular biology and genetics. This ongoing progress of the Cas12b technology promises to revolutionize the biomedical industry at the cutting edge of genome engineering.

CRISPR-Cas7-11

The CRISPR-Cas7-11 (CRISPR-Cas III-E) is an advanced type of CRISPR-Cas technology capable of programmable RNA targeting with a single-protein effector. CRISPR-Cas7-11 is unique in its design and utility compared to other CRISPR-Cas systems and has been the subject of intense study to further understand its structure and underlying engineering. The Cas7-11 protein is a single molecule of a complex design composed of three subunits: a CRISPR-associated protein, a subunit of structure (CSE), and an RBD. The core of the protein is the CSE, which forms an RNA-binding platform for gRNA molecules. An RBD, composes of four distinct alpha-helical domains, interacts with the CSE and the gRNA to form a platform for executing programmable RNA cleavage. The CRISPR-Cas7-11 system works by targeting foreign RNA molecules. The gRNA attached to the CSE of the Cas7-11 protein is programmed to recognize an mRNA molecule of interest. Once the mRNA is identified, the RBD inflates and interacts with the target RNA, leading to its cleavage. This action results in an mRNA degradation pathway, with the cleavage of the targeted mRNA molecules precluding translation of the sequence and resulting in the loss of expression of the target protein. The unique architecture of Cas7-11 and its programmable RNA targeting ability have enabled it to be utilized in applications related to RNA manipulation and gene editing[90,91]. The Cas7-11 system has already been used in research on the issues relating to transcriptome inhibition, target gene expression manipulation, and RNAi in a single-protein system. Further development of the protein has enabled more efficient localization of the various CRISPR-associated RNAs at different sites and further optimized the system for target genes and mRNA editing. The CRISPR-Cas7-11 technology has the potential to revolutionize gene editing and RNA manipulation due to its efficient and single-protein design. With further study of the CRISPR-Cas7-11 system, scientists may be able to develop new tools and techniques for precision genetic engineering, disease treatment, and even synthetic biology. One of the unique features of the CRISPR-Cas7-11 system is the use of a single protein, known as Cas7, to target and cut specific RNA molecules. This view is in contrast to other CRISPR systems, such as CRISPR-Cas9, which requires multiple proteins and a gRNA to achieve the same function. The simplicity of the CRISPR-Cas7-11 system makes it easier to engineer and use in a wide range of

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

applications. Another advantage of the CRISPR-Cas7-11 system is its ability to target RNA molecules that are not translated into proteins. These include regulatory RNAs that play an important role in gene expression and cell signaling. By manipulating these non-coding RNAs, scientists could develop new therapies for diseases that are difficult to treat using traditional gene editing techniques. However, there are still many challenges to be overcome before that the full potential of the CRISPR-Cas7-11 system can be realized. These challenges include improving the specificity and accuracy of the system to minimize off-target effects, as well as developing delivery methods that can efficiently introduce the system into cells and tissues[92]. Despite these challenges, the CRISPR-Cas7-11 system represents a promising avenue for genetic engineering and RNA manipulation that could have far-reaching implications for medicine, industry, and beyond.

Different types of Cas proteins

By comparing and contrasting the different Cas proteins, researchers can gain a better understanding of their individual functions, and how they work together. This knowledge can then be used to develop new applications for CRISPR-Cas technology, which has the potential to revolutionize genetic engineering. It can also help scientists explore new treatments for genetic diseases and create more effective therapies. Each type of Cas protein has a different function and structure. For instance, Cas9 proteins have a DNA cutting function, while Cas12a proteins possess a DNA cleaving function[34,69,78,85,93,94] (Table 2).

Applications of CRISPR-Cas Systems

In just ten years, the RNA-programmable site-specific CRISPR–Cas genome editing tool has become one of the most famous discoveries in biology and has an enormous impact on the life sciences. Scientists have applied CRISPR systems in many areas, including the medical field, biotechnology, and agriculture. Herein, we review some of the main applications of CRISPR technology.

Establishing cell and animal models of human diseases

The development of cellular and animal models is one of the early applications of the CRISPR-Cas system, which is useful to realize the reason behind diverse diseases and clarify molecular pathways used for more effective therapeutic strategies. Traditional methods for generation of cell and animal models of human diseases are complex, costly and time-consuming. Since the discovery of CRISPR-Cas systems, the generation of genetically modified cell and animal models has become significantly simpler in a highly efficient approach. Genetically modified animal models created using CRISPR technology, have a wide range of applications, such as their use in pharmaceutical and biotechnological production. They can also be used to study specific genes, investigate their functions and evaluate the mechanisms and progression of diseases in order to assess potential therapies. As one of the most common models for biological research, as well as animal studies (rats, Caenorhabditis elegans, drosophila, zebrafish, frogs, pigs, goats, and cynomolgus monkey models), the CRISPR-Cas tool has already been used to manipulate genes in mice[95-100]. So far, modeling diseases with CRISPR have been created for lung, pancreatic, brain, and hematopoietic cancers[101-107], cardiovascular disorders, cardiomyopathy[108], muscular dystrophy[109], HD[110], albinism[111], obesity[112], hemophilia B[113], and infectious diseases. In addition, the CRISPR-Cas system has been used to produce in vitro models for a wide variety of diseases. CRISPR-Cas can edit multiple sites,

Table 2. Comparison of different types of Cas proteins and their characterization

Cas protein	Source	Amino acid size	Protein size (kDa)	PAM sequence	Cutting site	Nucleic acid cleavage
Cas9	*Streptococcus pyogenes*	1,368	10-14	NGG	~3 bp 5′ of PAM	DNA blunt ends
Cas12a	*Acidaminococcus* sp.	1,348	7-8	TTTV	~12 bp 5′ of PAM	DNA staggered end
Cas12b	*Lachnospiraceae bacterium* ND2006	1,365	6-7	TTTN	~3 bp 5′ of PAM	DNA blunt ends
Cas13a	*Leptotrichia shahii*	1,204	8-9	GNNG	~4 bp 5′ of PAM	DNA staggered end
Cas13b	*Lachnospiraceae bacterium* A2-165	1,138	8-9	GNNN	~4 bp 5′ of PAM	DNA blunt ends
Cas14	*Neisseria meningitidis*	1,204	2-3	ATTT	~4 bp 5′ of PAM	DNA staggered end

Table 3. Common CRISPR/Cas9 delivery strategies

		Methods	Delivery format	Efficiency	Safety	Cost	Advantages	Disadvantages
Delivery strategies	**Chemical and physical delivery methods**	Lipid nanoparticles	DNA, mRNA protein	Low	Low	Low	FDA-approved; Low stress to the cells	Requires extensive optimization, variable efficiency
		Electroporation	DNA, mRNA protein	High	Low	High	Easy to operate	Cell viability issue; in vivo work difficult
		Microinjection	DNA, mRNA protein	Low	Low	High	Direct delivery; Dosage more controllable	Technical challenging; in vivo work not feasible
		Cell penetrating peptide	Protein	Low	Low	Low	No risk of virus	Requires extensive optimization, variable efficiency
		Gold nanoparticle	Protein	Medium	Low	Medium	No risk of virus	
	Viral delivery methods	Lentivirus	DNA	High	High	Low	Large cloning capacity	Random integration; insertional mutagenesis
		Adenovirus	DNA	Medium	Medium	Medium	Non-integrating	Immune response
		Adeno-associated virus	DNA	Medium	Low	Medium	Non-integrating	Limited cloning capacity
		Extracellular vesicle	Protein	Medium	Low	Low	Non-integrating; multiplexible; all-in-one format	Limited quantification method

simultaneously, by delivery of several gRNAs and Cas endonuclease using different strategies (Table 3). This unique advantage makes this tool an applicable technique for producing a cancer model with complexity similar to that occurs in humans. Matano et al.[114] generated a colorectal cancer model by targeting multiple genes in the human colon epithelium using the CRISPR-Cas9 genome-editing tool. Heckl et al.[104] generated a model of AML by using the CRISPR-Cas9 to introduce multiple gene mutations, including epigenetic modifiers, transcription factors, and cytokine signaling genes in mouse HSCs. The iPSCs are highly attractive cell resources in disease modeling and regenerative medicine based on their unlimited self-renewal and multiple differentiation capability. In recent years, numerous novel disease models have been generated by CRISPR-Cas9-edited iPSCs, including Parkinson's, Niemann-Pick type C, and SCD, as well as β-thalassemia, Rett syndrome, cystic fibrosis, and α1-antitrypsin deficiency[115-117].

Therapeutic application of CRISPR-Cas system

The CRISPR-Cas, as a great potential genome editing tool, has been employed in a wide range of therapeutic disorders, such as cancer[118,119], as well as genetic, infectious and neurodegenerative diseases (Table 4). Hemoglobinopathies, such as β-thalassemia and SCD are a group of autosomal recessive hereditary single-gene diseases caused by absence/reduced β-globin chain synthesis or production of a structurally unstable β-globin chain in the hemoglobin tetramer. β-thalassemia, a prevalent monogenic and inherited blood disorder, is globally caused by more than 300 different point mutations and small Indels within the human *HBB* gene[120,121]. In recent years, the only available treatment for β-thalassemia and SCD has been allogeneic bone marrow transplantation. However, the limited availability of human leukocyte antigen-matched donors makes it a challenging and restricted approach. Thus, there is no defined therapy for people suffering from β-thalassemia and SCD. Alternatively, allogeneic HSC

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

Table 4. Applications of CRISPR/Cas technology as therapeutic strategies

Disease	Target genes	Editing method	Reference
β-thalassemia	*HBB*	Deletion mutation	[128]
	BCL11A	Gene deletion	[129,130]
SCD	*HBB*	Insertion	[131]
	BCL11A	NHEJ/Indel	[132]
Hemophilia A	*F8*	Inversion-corrected iPSC cells	[133]
Hemophilia B	*F9*	Gene correction	[134]
HD	*HTT*	Deletion/NHEJ	[135]
Cystic fibrosis	*CFTR*	Gene correction	[136]
Duchenne musculardystrophy	*Dystrophin*	Gene correction/deletion	[137-140]
Diabetes mellitus type 1	*DMPKexon 15*	Gene editing	[141]
Cancer	*PD-1*	Gene knockout	[142]
HIV	*U3 LTR region*	Gene knockout	[143]
HIV	*CCR5*	Gene knockout	[144-146]
HPV	*E6 and E7*	Gene knockout	[147,148]
HBV	*cccDNA*	Gene knockout	[149-151]
Phlebotomus papatasi	*rel*	Gene knockout	[152]
Malaria	*FREP1*	Gene knockout	[153]
Zika virus	*RNA*	RNA editing	[154]

gene therapy, which relies on the transmission of the normal HBB via lentiviral vectors, is a promising approach for treating thalassemia and SCD[122-124]. The first cell-based gene therapy entitled Zynteglo (betibeglogene autotemcel), an autologous CD34$^+$ cells encoding globin gene for the treatment of individuals with transfusions dependent β-thalassemia, was approved on August 17, 2022 by the US Food and Drug Administration (FDA)[125,126]. The discovery of CRISPR-Cas9 in 2012, which revolutionized the field of genetic manipulation, has emerged as a promising player in the gene therapy approach. Multiple research groups have used CRISPR-Cas9 in patient-derived HSPCs and patient-derived iPSCs to repair β-thalassemia and SCD mutations in the HBB[124,126,127,130,154-158] Moreover, recent therapeutic strategies are based on the use of CRISPR- Cas9 to reactivate HbF expression. Interestingly, the results of studies have shown that increased HbF can mitigate the clinical symptoms of these diseases[128,129-131,159,160]. BCL11A is a master regulator of γ-globin gene that silences and inhibits HbF expression[162]. In previous studies, it has been demonstrated that targeted disruption of the BCL11A erythroid-specific enhancer using CRISPR-Cas9 in the second intron, increases the production of HbF, thereby ameliorating the severity of β-thalassemia and SCD, which can be considered a potential curative therapeutic strategy for β-hemoglobinopathies[161-164]. CTX001 is an ex vivo CRISPR-Cas9 gene-edited therapy for reactivating HbF in HSCs from patients suffering from SCD and transfusion-dependent β-thalassemia[165]. Several clinical trials are underway to assess the safety and efficacy of CRISPR system in β-hemoglobinopathies and other diseases (Table 5).

Duchenne muscular dystrophy

DMD is a rare neuromuscular and X-linked recessive disease in children caused by mutations in the dystrophin gene and characterized by muscle weakness, loss of movement, and early death. DMD occurs about 1 in 3,500 to 5,000 newborn males worldwide. The most

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

Table 5. Current clinical trials in hematology using the CRISPR/Cas9 system

Diseases	Clinical trial no.	Method	Type of edit	Target gene	Phase	Delivery method	Sponsor
β-thalassemia	NCT03655678	Cas9	Gene disruption	*BCL11A*	II/III	Electroporation (ex vivo)	Vertex Pharmaceuticals
HIV	NCT05144386	Cas9	Gene disruption	Three undisclosed genomic sites in the HIV DNA	II	AAV9 (in vivo)	Excision BioTherapeutics
Sickle cell disease	NCT03745287	Cas9	Gene disruption	*BCL11A*	II/III	Electroporation (ex vivo)	Vertex Pharmaceuticals
Acute lymphoblastic leukemia, T-ALL	NCT04984356	Cas9	Gene knock-out	*CD7*, *TRAC*	II	Electroporation (ex vivo)	Wugen inc.
AML	NCT05066165	Cas9	Gene knock-out, gene knock-in	WT1-specific TCR (knock-in), TRAC (knock-out), *TRBC* (knock-out)	II	Undisclosed (ex vivo)	Intellia Therapeutics
Metastatic gastrointestinal epithelial cancer, GI,	NCT04426669	Cas9	Gene knock-out	*CISH*	II	Ex vivo	Intima Bioscience, Inc.
Multiple Myeloma, MM,	NCT04244656	Cas9	Gene knock-out, gene insertion	BCMA	I	Undisclosed (ex vivo)	CRISPR Therapeutics AG
Non-small cell lung cancer	NCT05566223	Cas9	Gene knock-out	*CISH*	II	Ex vivo	Intima Bioscience, Inc.
Blindness, Leber Congenital Amaurosis,	NCT03872479	Cas9	Gene correction	*CEP290*	II	Adeno-associated virus (AAV5) (in vivo)	Editas Medicine, Inc.
Type 1 diabetes	NCT05565248	Cas9	Disruption, insertion	-	II	Ex vivo	CRISPR Therapeutics AG

CISH, cytokine inducible SH2 containing protein; BCMA, b-cell maturation antigen

common type of mutation in the dystrophin gene involves the deletion of one or more exons, leading to frameshift or nonsense mutations, resulting in the absence of functional dystrophin protein[166-168]. In 2014, Long et al.[136] used the CRISPR-Cas9 system for the first time in mouse zygotes to correct mutations and restore the expression of dystrophin. In 2017, Lattanzi et al.[137] reported successful CRISPR-Cas9-mediated restoration of dystrophin expression in DMD myoblasts by removing the duplication of exon 2 and intron 2 in the dystrophin gene. In 2017, El Refaey et al.[139] used the CRISPR-Cas9 technique for deletion of exon 23 in dystrophic mice in which the restoration of dystrophin protein was confirmed. In 2019, Min and Lee[138] used adeno-associated virus serotype 9-mediated Cas9 and sgRNA to restore the exon 50 deletion and exclude exon 44 mutations in cardiomyocytes obtained from patient-derived iPSCs, as well as in a mouse model. The results showed the capability of CRISPR to remove mutations that result in DMD.

Cystic fibrosis

CF is the most common autosomal recessive and monogenic lung disease that occurs due to mutations in the *CFTR* gene[169]. The CFTR protein is a chloride channel located on the surface membrane of epithelial cells and transports small ions through the membrane of multiple organs, including lung, intestine, and pancreas[170]. CRISPR-Cas system has been used to correct CFTR mutations in iPSCs from CF patients, as well as cultured intestinal stem cells. Researchers have utilized the CRISPR-Cas system to precisely repair CFTR-bearing homozygous F508 deletions (F508del) in exon 10[171,172].

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

Huntington's disease

HD is a genetic disorder caused by a mutation in the *HTT* gene. The mutation leads to the production of an abnormal protein that accumulates in the brain, causing damage to nerve cells and leading to progressive neurological symptoms such as involuntary movements, cognitive decline, and psychiatric disturbances[173]. Currently, there is no cure for HD, and the available treatments only alleviate symptoms. CRISPR-Cas9 technology offers a promising approach for treating HD by correcting the underlying genetic mutation. CRISPR technology can be used to remove or replace the mutated section of the HTT gene with a healthy version. In one study, researchers used CRISPR-Cas9 to selectively target and edit the mutant HTT gene in mouse models of HD. The treatment resulted in significant improvements in motor function and reduced accumulation of mutant HTT protein in the brain[174]. Another study used CRISPR-Cas9 to correct the HTT gene mutation in human embryonic stem cells derived from patients with HD. The corrected cells were then differentiated into neurons, which showed normal levels of HTT protein expression and function[175]. While these studies show promise for using CRISPR-Cas9 as a treatment for HD, there are still challenges that are required to be addressed before it can be applied clinically. One major concern is ensuring that CRISPR-Cas9 targets only the mutant HTT gene and no other genes with similar sequences. Additionally, delivery methods are needed to be optimized to ensure the efficient delivery of CRISPR-Cas9 into target cells without causing off-target effects or immune reactions. While there are still challenges to overcome, continued research in this area could lead to a cure for this devastating disease.

Blindness

CRISPR technology has the potential to treat various genetic disorders, including inherited forms of blindness. IRDs are a group of genetic disorders that affect the retina, leading to vision loss and blindness. These diseases are caused by mutations in genes that are essential for the function and survival of photoreceptor cells in the retina[142]. CRISPR-Cas9 can be used to correct these mutations by targeting and editing the DNA sequence of the affected gene. Several studies have demonstrated the potential of CRISPR-Cas9 in treating IRDs. In 2017, researchers used CRISPR-Cas9 to correct a mutation in a gene called CEP290, which is associated with Leber congenital amaurosis, a severe form of childhood blindness. The study showed that CRISPR-Cas9 could effectively correct the mutation in human cells and restore normal protein function[176]. Researchers have used CRISPR-Cas9 to correct mutations in genes associated with retinitis pigmentosa, another form of inherited blindness that impairs vision due to the loss of photoreceptor cells, which can lead to irreversible blindness[177,178]. Recently, Tsai et al.[179] used CRISPR/Cas9 to remove and replace Rho. The authors tested this method on two mouse samples against the P23H and p.Asp190Asn mutations harboring the CRISPR/Cas system using the dual vector system AAV2/8 and the exogenous *RHO* gene. Both models showed an increase in outer nuclear layer thickness of up to 35% and a significantly improved ERG response after removal and replacement, in contrast to the addition of only one gene. The study showed that CRISPR-Cas9 could effectively correct these mutations in patient-derived cells and restore normal protein function. While these studies show promising results, there are still challenges needed to be addressed before CRISPR-Cas9 is used as a clinical treatment for IRDs. These include improving delivery methods for carrying CRISPR components to target cells within the retina and ensuring safety and efficacy in clinical trials.

Infectious diseases

Human immunodeficiency virus

AIDS, caused by HIV-1, is still a severe health problem worldwide. According to the World Health Organization (http://www.who.int/hiv/en/), 38.4 million people in the world were living with HIV in 2021, HIV-1 is an enveloped retrovirus comprising two copies of a 9.8 kb positive-sense RNA genome flanked by two LTR sequences. The HIV genome encodes proteins required for its life cycle, including gag, env, pol, vif, vpr, tat, vpu, rev, nef, and the antisense protein[180]. HIV-1 enters host cells by initial binding of its gp120 Env protein to the CD4 receptor on the surface of target cells, including $CD4^+$ T helper lymphocytes, macrophages, and microglial cells. This interaction induces subsequent binding with the chemokine receptor CCR5 or CXCR4 as a co-receptor on the membrane of the target cell, which is essential for fusion between the virus Env and the cell membrane[181,182]. Highly active antiretroviral therapy is the primary method for controlling viral load by suppressing virus replication but fails to eradicate latent viral reservoirs in patients. In recent years, the CRISPR-Cas system has also provided new hope to cure HIV-1/AIDS. In 2014, Hu et al.[142] used the CRISPR-Cas system to target LTR regions at both ends of viral genes in HIV-1 latent-affected myeloid lineage, promonocytic, and T cells with significant loss of LTR expression. In addition, blocking CCR5, an essential co-receptor for HIV-1 entry into the host cell, is considered the most potential therapeutic strategy for AIDS. In 2013, Cho et al.[183] utilized the SpCas9 to induce mutations with a frequency ranging from 5% to 33% in

[DOI: 10.61186/ibj.3898] [DOR: 20.1001.1.1028852.2023.27.5.1.7] [Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

human cells within the *CCR5* gene. In 2014, Mandal et al.[145] used the CRISPR-Cas9 genome editing tool to delete the *CCR5* gene in CD34+ HSPCs. Their results showed 42% biallelic inactivation frequency for *CCR5* in CD34+ HSPCs with low off-target mutagenesis. Another essential co-receptor that mediates HIV-1 entry into the cells is CXCR4. In 2015, Hou et al.[184] utilized the CRISPR-Cas9 genome editing system to disrupt the CXCR4 gene in human primary CD4+ T cells. The edited cells showed resistance to X4-type HIV-1 infection, as well as 40% mutagenesis with low off-target effects. Additionally, some research groups applied the CRISPR-Cas9 tool to investigate the simultaneous manipulation of *CXCR4* and CCR5 genes in various cell lines. The results showed that the manipulated cells were significantly protected from HIV-1 infection without off-target mutagenesis or cytotoxicity effect[145,144].

Human papillomaviruses

HPVs are double-stranded DNA viruses with a genome size of about 8000 bp. More than 200 types of HPVs have already been identified. HPV is the major cause of cervical cancer in women, as well as anogenital and head and neck cancers in men. More than 50% of HPV-positive cervical cancers are associated with HPV16, and about 12% with HPV18, which are both considered high-risk HPVs[185-188]. HPV E6 and E7 are the vital oncogenes responsible for accelerating HPV-induced carcinogenesis[188]. CRISPR-Cas9 has been used to target the HPV16 and HPV18 E6 and E7 in HeLa cells, as well as cervical carcinoma cell lines, which results in the induction of p53 or Rb, leading to cell cycle arrest and cancer cell death[189]. In 2014, Zhen et al.[190] targeted the promoter of HPV16 E6 and E7 using the CRISPR-Cas9 in SiHa cells and tumor animal models. Their results showed the induction of p53 and p21 proteins, which resulted in the inhibition of tumorigenesis.

Hepatitis B virus

Hepatitis B is a contagious liver infection caused by the HBV, a major global public health problem. Many antiviral agents have been used to cure HBV infection; however, they are not effective in eradicating HBV due to their inability to remove cccDNA, a template used by HBV for replication. cccDNA completely persists during therapy and cannot be eliminated by current antiviral therapies[191]. Thus, new treatments are required to effectively eliminate the cccDNA. Many research groups have used CRISPR-Cas9 to target the HBV core and surface proteins in animal models and hepatoma cell lines such as, HepG2, Huh-7, HepG2-H1, and HepG2.2.15. The results showed that the elimination of episomal cccDNA led to the inhibition of HBV replication and expression both in vitro and in vivo[148-150,192].

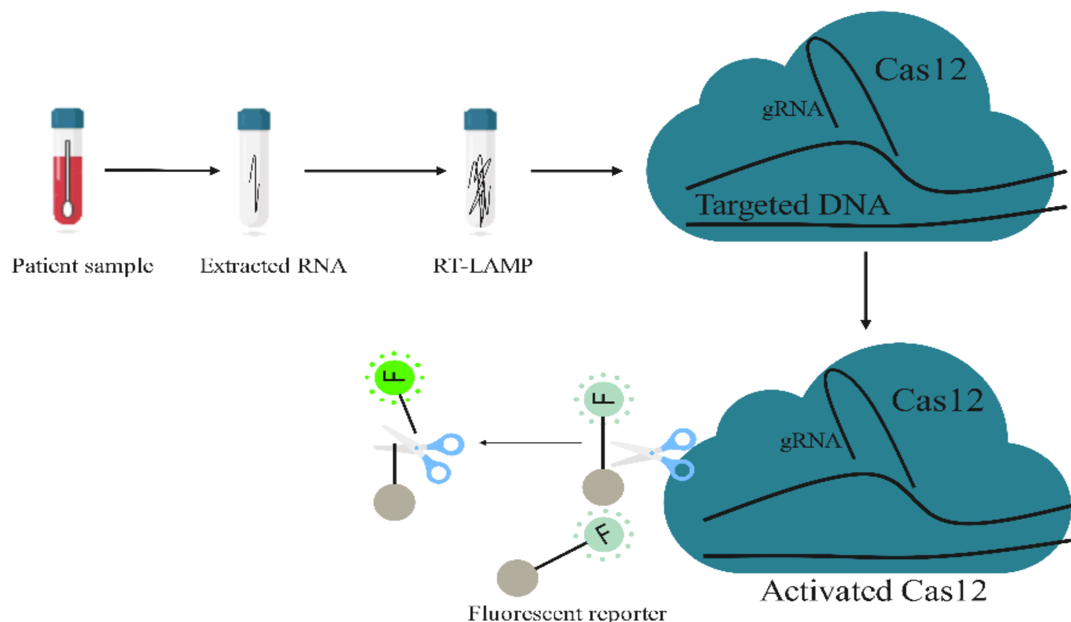

Fig. 3. The general principle of nucleic acid detection by the DETECTR based on CRISPR/Cas 12. Nucleic acid is extracted from clinical samples, and the target sites are amplified by RT-RPA. Detection is performed in a reaction mixture containing T7 RNA polymerase, Cas12a, a target-specific gRNA, and an ssDNA probe that fluorescence when cleaved. Gray circles indicate quenchers that are connected by a short oligonucleotide. F, FAM

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

[DOR: 20.1001.1.1028852.2023.27.5.1.7]

[DOI: 10.61186/ibj.3898]

Severe Acute Respiratory Syndrome Coronavirus 2

In December 2019, a novel beta coronavirus, namely SARS-CoV-2, was identified in Wuhan, China. SARS-CoV-2 causes a respiratory illness called COVID-19. The SARS-CoV-2 virus quickly spread worldwide and led to a pandemic[193,194]. A low-cost, rapid, simple, and sensitive point-of-care testing for the early detection of viruses in pandemics such as COVID-19 was required. In this regard, the CRISPR genome editing system can be applied for nucleic acid detection, owing to its exclusive features. For this purpose, researchers have utilized different types of CRISPR for the design and development of CRISPR-based diagnostic systems. Broughton and colleagues[195] used the called DETECTR. In CRISPR-Cas12a to develop an accurate, simple, and rapid (<40 min) diagnostic method for SARS-CoV2 this method, gRNAs were designed to detect SARS-CoV-2, bat SARS-like coronavirus, and SARS-CoV in the E gene and specifically detects only SARS-CoV-2 in the N gene (Fig. 3). Ding et al.[196] enveloped an all-in-one dual CRISPR-Cas12a diagnostic method rapidly targeted nucleic acids. In this method, all components for CRISPR-mediated detection and nucleic acid amplification are merged into a single reaction. CREST was introduced by Rauch et al.[197] for SARS-CoV-2 detection. The CREST assay was developed to address the main barriers of virus detection, including high cost, limited access to equipment and materials, and lack of highly trained operators. Azhar et al.[198] applied a Cas9 ortholog from FnCas9 to develop FELUDA. In the FELUDA-based lateral flow assay, gRNAs were designed to target the NSP8 and nucleocapsid phosphoprotein of SARS-CoV-2 with rapid, cost-effective, and machine-independent detection of a small amount of SARS-CoV-2 RNA within one hour. Recently, Yoshimi et al.[199] have developed a CRISPR/Cas3-based detection method called CONAN. The CANON assay combines isothermal amplification methods for a fast, sensitive, and tool-free diagnosis of SARS-CoV-2.

Parasites

Parasites are biological organisms that live and feed on another organism, known as a host. These organisms can cause severe health issues in humans and animals, making the development of new treatments highly necessary. In principle, parasitic diseases are the main reason for morbidity and economic constraints. CRISPR gene editing technology has emerged as a powerful tool in the fight against parasites. With CRISPR, genetic sequences can be specifically targeted and edited to make precise modifications. CRISPR/Cas9-mediated gene editing, for instance, could be used to disrupt the reproduction of parasites or selectively edit their virulence genes, which would lead to the reduced parasite transmission and virulence, ultimately aiding in the development of novel methods for controlling parasitic infections. Moreover, CRISPR has been used to study the functional genomics of parasites, offering new insights into their biology and identifying potential drug targets. A study demonstrated the use of CRISPR/Cas9-mediated gene editing to investigate new targets for drug development against the protozoan parasite Trypanosoma brucei, which causes African trypanosomiasis. The study identified several proteins that play an essential role in the parasite's life cycle, which could be targeted with new therapeutics[200]. Additionally, CRISPR technology could potentially be used to develop vaccines against parasites by enabling the manipulation of antigens to enhance host’s immunity. CRISPR technology also offers the possibility of creating genetically modified parasites that can serve as live vaccines[201]. Hence, the design and development of proper detection methods and efficient treatments are essential. CRISPR-Cas9 has been used for genome editing of *Leishmania*, *Plasmodium* spp., *Toxoplasma gondii*, and *Trypanosoma cruzi*. It has also been employed in the modification of medically important vectors such as different species of mosquitos and water fleas. Understanding diverse aspects of parasite biology has greatly benefited from the development of CRISPR technology. Malaria is a life-threatening and severe disease caused by the *Plasmodium* parasites species, which is transmitted to humans through the bite of infected female mosquitoes of the Anopheles genus. Malaria remains a public health threat worldwide, causing the death of more than 400,000 people annually[202,203]. Genome editing technology, especially the CRISPR/Cas9 system, enables researchers to investigate genetically engineered mosquitoes to eradicate malaria. In 2014, Ghorbal et al.[204] successfully used the CRISPR/Cas system to edit the genome of *P. falciparum* through gene disruption and single-nucleotide gene editing. In addition, they generated an artemisinin-resistant strain by introducing a mutation (C580Y) in the PF3D7_1343700 K13-propeller. Artemisinin-based combination therapy is a primary treatment for *P. falciparum*, and mutations in K13-propeller domains are associated with the artemisinin phenotype. The sexual phase of malaria parasites, which results in the formation of infectious sporozoites, is necessary to be completed by transmission to a mosquito vector. Inhibiting the fertility of mosquitoes through chemical spraying or genetically modifying the population to render them infertile is one of the most effective strategies to combat malaria. Transgenic mosquitoes do not transmit the malaria parasite because they express

inhibitors of Plasmodium growth. Gantz et al.[205] used the CRISPR/Cas9 system to integrate antiparasite effector genes against the *P. falciparum* ookinete proteins, such as single-chain antibodies m1C3 to target chitinase 1 and m2A10 to target CSP, to prevent malaria transmission. In 2018, Dong et al.[152] used the CRISPR/Cas9 system to eliminate the *FREP1* gene, which encodes an immune protein, from the genome of *Anopheles gambiae* mosquitoes, to generate mosquitoes that are highly resistant to malaria parasites. *FREP1* acts as a receptor for plasmodium on the midgut of the Anopheles mosquito. Consequently, the knockout of the *FREP1* mosquitoes makes them resistant to the parasite and significantly reduces the likelihood of transmitting the malaria parasite to the host. The CRISPR system has widely been used to knock out target genes, replace nucleotides, and tag endogenous loci with epitope tags in order to permanently alter the target genes. The CRISPR tool, however, has only been sporadically used to control gene expression through epigenetic regulation. A dCas9 was successfully used in a recent study by Xiao et al.[206] to epigenetically regulate the transcription of genes related to parasite invasion, including reticulocyte binding protein homology 4 (RH4) and erythrocyte binding antigen 175. In order to activate the expression of RH4, the GCN5, as a histone acetyl transferase, was fused to the dCas9 (dCas9GCN5). The recombinant dCas9GCN5 was specifically directed to the promoter region of the *rh4* gene by the RH4sgRNA. The findings showed a significant overexpression of the *rh4* gene in *P. falciparum* using the CRISPR/dCas9GCN5 system. Moreover, PfSir2a, as a histone deacetylase, was fused to dCas9 (dCas9Sir2a) to repress the expression of the *eba-175* gene in *P. falciparum*. The *P. falciparum eba-175* gene was disrupted using CRISPR/dCas9Sir2a, which inhibits parasites from invading erythrocytes treated with chymotrypsin. Furthermore, the gene PfSET1 of *P. falciparum*, which is essential for the growth of its *P. falciparum* asexual stage, was suppressed using the CRISPR/dCas9Sir2a system. The phenotypic changes in parasite growth were caused by the precise inactivation of the PfSET1 gene. In addition to the asexual blood stages of the malaria parasite life cycle, the CRISPR tool has been applied to identify the functional role of genes in other steps of the parasite's life cycle. Both eukaryotic organisms and malaria parasites use many of the same transcription factors. Unexpectedly, a family of genes encoding Apetala2 (ApiAP2) proteins, which are essential for parasite development in Plasmodium, has been discovered in many plants. In a study performed by Zhang et al.[207], 12 out of 26 *Plasmodium yoelii ApiAP2* genes were successfully knocked out using the CRISPR/Cas9 system, and the findings revealed the crucial role of some of the genes in the development of gametocytes, oocysts, and sporozoites. The CRISPR/Cas9 system is also used to identify antimalarial compounds and elucidate the mechanisms underlying their actions. Benzoxaboroles have demonstrated strong activity against a variety of infectious pathogens. Using the CRISPR/Cas9 system, Sonoiki et al.[208] demonstrated the potent anti-malarial activity of AN3661 (a benzoxaborole) against cultured *P. falciparum* asexual blood stage parasites. They made mutations to the active site of the cleavage and polyadenylation specificity factor subunit 3 (CPSF3) gene where AN3661 should bind. In addition to malaria, CRISPR technology could be used to create genetically engineered mosquitoes that are resistant to infection by the Zika virus. The Zika virus is a flavivirus spread by the bite of infected mosquitoes of the *Aedes aegypti* species, which causes fever, rash, red eyes, muscle pain, headache, and birth defects and is linked to the Guillain-Barré syndrome. There is no specific treatment and vaccine for the Zika virus[209]. In 2022, Chen et al.[153] used CRISPR-Cas13b to develop an anti-Zika system in 293T cell lines. The study demonstrated the efficacy of CRISPR-Cas13b as an anti-RNA virus therapy by degrading targeted viral RNA with Cas13. Leishmaniasis is a neglected tropical disease caused by protozoan parasites from over 20 *Leishmania* species. This vector-borne disease is transmitted by about 30 species of infected phlebotomine sandflies and is widespread in 97 countries throughout the world. In spite of comprehensive research on various aspects of this infectious disease, there is currently no effective or preventive strategy against leishmaniasis[210]. In 2015, Sollelis et al.[211] applied the CRISPR-Cas9 system to knock out the paraflagellar rod-2 genes (a tandemly repeated gene family) in the *Leishmania major* parasite. As a result, they obtained null mutants in a single round of transfection, as well as the absence of off-target editions. In 2015, Zhang et al.[212] developed two loss-of-function Indel mutations in *Leishmania donovani* through the precise deletion of the 3-kb from *LdMT* gene using CRISPR-Cas9. Furthermore, they identified a novel single-point mutation created by CRISPR-Cas9 in the *LdMT* (M381T) gene that led to miltefosine resistance, a concern for the only available oral antileishmanial drugs. Several research groups have successfully removed genes, such as Cysteine peptidases, LeishIF4E-3, LeishIF4E1, ubiquitin, RAD51-related genes, flagellar motility factor Pf16, adenine phosphoribosyltransferase, casein kinase 1.1, and HSP23, along with HSP100, from various Leishmania species, including *Leishmania mexicana*, *L. major*, *Leishmania tarentolae*, *L. donovani*, and

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

[DOR: 20.1001.1.1028852.2023.27.5.1.7]

[DOI: 10.61186/ibj.3898]

Leishmania braziliensis[213-218]. Chagas disease is an inflammatory and infectious disease caused by *Trypanosoma cruzi*. Peng et al.[219] used CRISPR-Cas9 in *T. cruzi* to knock down expression of β-galactofuranosyltransferase enzyme, which plays a role in *T. cruzi* pathogenesis. The results showed a significant downregulation in enzyme production without evident off-target mutations. In another study, Lander et al.[220] used CRISPR-Cas9 to improve the analysis of the functionality of the *T. cruzi* genome. In this regard, sgRNAs were designed to disrupt the expression of GP72-related genes and paraflagellar rod proteins 1 (PFR1) and 2 (PFR2). The results showed that the PFR1, PFR2, and GP72 proteins contribute to the attachment of flagellar to the cell body and also to the motility of the parasites. Scientists are also utilizing the CRISPR-Cas9 system for gene editing in mosquitoes to modify their genes, with the aim of inhibiting the parasite's life cycle and reducing their ability to spread pathogens[221,222]. CRISPR can be used to modify a wide range of parasites, including Leishmania, Toxoplasma, Trypanosoma, and Plasmodium. For instance, the Cas9/T7-RNAP construct can be used to induce gene deletion or disruption mutants in Leishmania species. This technique can also be utilized to develop gene drive strategies in insect vectors that transmit parasites from one host to another, which could potentially help reduce the transmission of diseases caused by these parasites. In addition, active RNA interference machinery has been developed for use in parasitology research, which can be employed to knockdown or knock-in target gene into various parasite systems, such as Trypanosoma and Plasmodium. This technology enables gene deletions or insertions with high transgene copy numbers and compensatory adaptations that enable the parasite to survive and replicate even after the targeted gene is deleted or disrupted. CRISPR has also been explored as a potential tool for creating gene drive strategies in other systems, such as Trypanosoma. Such strategies could potentially increase transgene copy numbers while reducing vector populations through increased mortality rates of the infected individuals or reduced ability of the infected individuals to transmit the disease agent[205].

CRISPR-based diagnostic methods for detection of parasites

The CRISPR system has been applied for parasite detection. In recent years, accurate parasite diagnosis has been made possible by combining parasitological and CRISPR/Cas systems. In 2022, Dueñas et al.[223] developed a CRISPR-based diagnostic tool using the CRISPR-Cas12a system to detect *Leishmania* spp. Two gRNAs were designed to target the 18S rDNA (18S ribosomal RNA gene), a highly conserved region across Leishmania species, and kDNA minicircles that are conserved in the *Leishmania* (*Viannia*) subgenus. They could identify 5×10^0 (18S rDNA) and 5×10^2 (kDNA) parasites/reactions using this system[224]. CRISPR-based malaria screening has been emerged as a promising method for detecting the presence of malaria parasites in blood samples. This method utilizes the CRISPR/Cas system to target and cleave specific sequences of the parasite genome present in a blood sample, which can then be detected using various molecular methods, such as PCR or fluorescence assays. One of the main advantages of using CRISPR-based malaria screening is its high specificity and sensitivity. Additionally, it has the potential to greatly improve diagnostic accuracy and speed, compared to the traditional methods such as microscopy. Furthermore, CRISPR-based malaria screening can also be used to detect drug-resistant strains of the parasite by targeting specific mutations in the genome associated with resistance. This technology has immense potential in preventing and controlling the spread of malaria, especially in regions with limited access to the advanced diagnostic resources. In a study, researchers utilized the CRISPR/Cpf1 (Cas12a), a SHERLOCK-based diagnostic platform, to establish an ultrasensitive, one-pot, lyophilized, and free nucleic acid extraction method for detecting and distinguishing *P. falciparum* (Pfr364 gene), *Plasmodium vivax* (18s rRNA), *Plasmodium ovale* (18s rRNA), and *Plasmodium malariae* (18s rRNA). The results indicated an ultrasensitive and specific assay for differentiating *P. falciparum* and *P. vivax* in clinical samples and were able to detect fewer than two parasites per microliter of blood[225]. Overall, CRISPR-based malaria screening represents a promising approach for improving malaria diagnosis and management, with potential applications in both clinical settings and field studies. In the future, further research will be necessary to optimize CRISPR-based malaria screening technology and expand its potential applications.

Ethical implications of fighting malaria with genome editing

CRISPR-Cas9 technology has shown promising results in the development of novel strategies for combating parasitic infections and controlling mosquito populations, which are important vectors for many parasitic diseases. CRISPR has been used to genetically modify mosquitoes so that they are unable to transmit diseases, such as malaria and dengue fever. CRISPR has also been utilized to study the function of genes in parasitic organisms and understand their biology, which can lead to the development of new drugs and therapies for parasitic infections affecting millions of people

worldwide[226]. While the potential use of genome editing technology to combat malaria is promising, it raises ethical concerns that need to be addressed. Some of the concerns in using genome editing technology to combat malaria include safety-related issues, equity, and the unintended consequences of altering the mosquito population along with potential environmental impacts. When considering the implementation of genome editing technology for malaria control, it is of most importance to thoroughly evaluate and weigh the potential risks and benefits. This evaluation should not only include ethical concerns but also take into account the views of local communities and stakeholders who would be impacted by such interventions. Overall, the use of CRISPR technology in parasitology and mosquito control holds great promise for improving global public health and reducing the burden of parasitic infections and mosquito-borne diseases. Continued research and development in this field will be critical to fully realize the potential of CRISPR technology in addressing some of the most pressing public health challenges worldwide. It is important for scientists, policymakers, and stakeholders to engage in thoughtful discussions and collaborations to ensure that the use of this technology is safe, ethical, and effective. Furthermore, it will be crucial to ensure that these advances are accessible and affordable for those who live in low-resource countries where parasitic infections and mosquito-borne diseases are highly prevalent. In conclusion, while there are still challenges and uncertainties to be addressed using CRISPR technology for parasitology and mosquito control, it has the potential to revolutionize our approach to global health issues and improve the lives of millions of people around the world.

CRISPR protein tagging

CRISPR protein tagging is a revolutionary technique that allows for precise labeling and visualization of proteins in living cells. This method involves using CRISPR-Cas9 to introduce a DNA construct that contains the protein of interest fused with a fluorescent tag or affinity tag. The tagged protein can then be tracked and studied in real-time, enabling researchers to gain a better understanding of its function within the cell. This technique has the potential to revolutionize our understanding of protein function and localization, providing more precise analysis and manipulation of cellular processes. In the field of parasitology, CRISPR protein tagging has proven to be particularly useful in studying the complex lifecycle and host interactions of parasites. The precise tagging of proteins within parasites has been a challenging task for many years. This technology has already led to important discoveries about the mechanisms underlying malaria transmission and infection and has the potential to uncover new targets for drug development. Additionally, CRISPR protein tagging could be used to explore the diversity of parasitic species and their adaptations to different environments, providing insight into the evolutionary processes that have shaped these organisms over time. Overall, this cutting-edge technique is poised to significantly advance our understanding of parasitic diseases and improve our ability to combat them. One of the key advantages of CRISPR protein tagging is its versatility. This technique can be applied to a wide range of parasitic organisms, from single-celled protozoa to complex multicellular worms. It also allows researchers to study different stages in the life cycle of parasites, such as the transmission from vector to host or the development within specific organs. By labeling different proteins within these stages, scientists can gain a better understanding of how parasites interact with their environment and host cells. Furthermore, CRISPR protein tagging can be used in combination with other techniques, such as transcriptomics and proteomics, to generate comprehensive datasets that shed light on various aspects of parasite biology. As this technology continues to improve and become more widely adopted, we can anticipate even more exciting discoveries about parasitic diseases in the years ahead. Another advantage of CRISPR protein tagging is its potential for drug discovery. By identifying key proteins involved in virulence and drug resistance, researchers can develop targeted therapies that disrupt the parasite's ability to survive within the host. This approach has been proven to be successful in treating malaria. Clinical trials of drugs targeting specific enzymes have shown promises. CRISPR protein tagging can be used to screen large libraries of compounds for their ability to inhibit or enhance specific protein functions, potentially leading to the discovery of new drugs with novel mechanisms of action. Overall, this technology has the potential to significantly improve our ability to treat parasitic diseases and reduce their impact on global health[227,228]. Researchers have utilized CRISPR protein tagging to study PlsoT1, a protein found in the malaria parasite that is responsible for the invasion of host red blood cells. PlsoT1 is a surface protein that plays a crucial role in the initial stages of erythrocyte invasion by the malaria parasite. The ability to track PlsoT1 using CRISPR protein tagging has led to a better understanding of its localization and function during the invasion process. In addition, CRISPR protein tagging has been used to study other important proteins involved in malaria pathogenesis, such as PfEMP1 and CSP. With continued advancements in this technology, we can expect even more breakthroughs in our understanding of parasitic

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

diseases and the development of new treatments. PfEMP1 protein is essential for the cytoadherence of *P. falciparum*-infected erythrocytes within the host, and its expression is associated with severe malaria. CSP protein is critical for the development of *P. falciparum* sporozoites, which are responsible for transmitting malaria from mosquitoes to humans. By using CRISPR protein tagging to track the location and activity of PfEMP1 and CSP proteins, researchers have gained a better understanding of how these proteins contribute to pathogenesis and sporozoite development and identify potential targets for drug development. Moreover, this technique has allowed for visualization of PfEMP1 and CSP trafficking within the infected cells, providing insights into the cellular mechanisms underlying cytoadherence and sporozoite formation[227]. CRISPR/Cas9-mediated endogenous C-terminal tagging has been utilized to study *T. cruzi* genes, specifically the IP3R. By tagging the IP3R protein and observing its localization within the cell, researchers have gained a better understanding of its role in acidocalcisome function. Acidocalcisomes are acidic organelles that store calcium and other essential ions in *T. cruzi*, making them an attractive target for drug development. The use of CRISPR/Cas9 technology has provided a more precise and efficient tagging proteins in *T. cruzi*, enabling investigators to evaluate the localization and function of proteins with greater accuracy. This technique has opened up new avenues for research on *T. cruzi* biology and potential therapies for Chagas disease[228]. CRISPR protein tagging has been used to investigate the role of DNA polymerases in *Trypanosoma brucei*, the parasite responsible for African sleeping sickness. By labeling these enzymes with fluorescent tags, scientists could track their movement and activity during different stages of the cell cycle, which provides valuable insights into the regulation of DNA replication in these parasites and leads to the discovery of potential targets for drug development. Similarly, CRISPR protein tagging has been employed to study RNA polymerases in *P. falciparum*. By understanding how these enzymes function within the context of parasite biology, we can develop new strategies to disrupt their activity and combat parasitic diseases. However, there are also some challenges associated with CRISPR protein tagging in parasitic organisms. One of the main challenges is the delivery of the CRISPR system into these organisms, which can be difficult due to their complex life cycles and cellular structures. In addition, off-target effects can occur when using this technique, potentially leading to unintended changes in gene expression or function. To mitigate these issues, researchers are required to carefully design and optimize their experiments to minimize off-target effects and ensure accurate labeling of target proteins.

Future perspective and considerations

Until date, the CRISPR-Cas technology is the most potent gene editing tool and has been applied in various fields because of its simplicity and efficient gene-editing capability compared to previous gene editing tools. Although the CRISPR-Cas system has potential advantages in gene manipulation, concerns remain regarding off-target effects in which editing events happen at the untargeted regions within the genome. Therefore, investigations are underway to improve CRISPR-Cas activity and reduce off-target effects by optimizing the design of gRNA and applying different types of Cas or Cas9 variants. Despite the fact that off-target mutations on the genome could have unexpected effects on the parasite phenotype, this might not be a significant issue since apicomplexans have small genomes with less potential for off-target mutation. No off-target mutations introduced by Cas9 was reported in both *P. falciparum* and *P. yoelii*[207], indicating that this system is extremely specific in these parasites that lack NHEJ. However, it is advised to be further investigated in other apicomplexan parasites, such as *T. gondii*, which has a functioning NHEJ system and may also repair DSBs in off-target locations. Another issue related to the CRISPR-Cas system is immunogenicity, in which the immune system of the host cell will respond to Cas protein. A study recently underlined the question of whether immunological responses to Cas9 negatively impact its clinical use[229]. Anti-Cas9 responses have been detected in healthy human adults. The presence of anti-Cas9 antibodies does not necessarily indicate an immune response against Cas9-mediated gene editing; however, anti-Cas9 T cells are detected in the blood of test subjects. These T cells can react to Cas9 in circulation, as they are effectively presented Cas9 through major histocompatibility complex molecules. The CRISPR-Cas9 system may become ineffective if the immune system destroys the corrected cells. To minimize the risk of developing anti-Cas9 CTLs in CRISPR-Cas9 gene editing, known strategies should be employed. The potential of gene editing is promising, but we must be cautious in our approach. Gene therapy offers valuable insights, as it has found ways to overcome anti-capsid and anti-transgene CTL responses. This goal was achieved by carefully considering factors such as the vector, dose, target tissue, administration route, promoter, and immune suppression. By taking these factors into account, the gene-editing field can proceed with more confidence and ensure that it is being conducted safely and effectively. CRISPR-Cas9 platforms, leading to short-

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

term expression of Cas9 should be developed for CRISPR-Cas9 platforms. Gene therapy has shown potential difficulties due to immune responses. Studies involving AAV-CRISPR-Cas9, which silences T cells, impairs product-specific $CD8^+$ T cells and uses immune evasion by muscle-specific gene expression by muscle-specific gene expression, demonstrate potential difficulties. Results suggest that immune response may impact the efficacy of gene therapy and lead to the development of antibodies that can interfere with the treatment. Moreover, CRISPR-Cas9 can induce a p53-mediated DNA damage response, affecting the safety of gene therapy[190]. It is important to evaluate immune responses in gene editing in order to determine the most appropriate treatment approach and minimize the potential risks. There are several ways to address the issue of immune recognition. Some possible strategies consist of modifying the structure of Cas9 proteins to hide immunogenic epitopes, utilizing Cas9 orthologs from nonpathogenic bacteria, inducing immune tolerance or immune suppression, or focusing on immune-privileged organs, such as the eye. Additionally, altering antigen presentation of Cas9 epitopes is a promising approach derived from Epstein-Barr Virus, which interferes with proteasomal degradation and the subsequent antigen presentation[230]. While there is evidence suggesting that CRISPR-Cas systems can be immunogenic, further research is necessary to fully comprehend the potential risks and develop effective mitigation strategies. As the use of the CRISPR system continues to expand in various fields, it is crucial to consider ethical issues tha may arise carefully. Multiple improvements are necessary to address all current challenges related to the application of CRISPR in order to maximize on-target efficiency and minimize untargeted editing.

In conclusion, the application of CRISPR technology in manipulating Leishmania and *T. gondii* genomes offers a promising approach to develop new treatments for these diseases. The use of CRISPR has enabled researchers to identify essential genes that are required for survival of the parasite, which can be targeted for drug development. Moreover, CRISPR can be used to study the biology of the parasite and provides insights into the mechanisms of pathogenesis and drug resistance. The potential of CRISPR in the field of parasitology is vast, and it is exciting to see how this technology will continue to revolutionize the field of genetic engineering in the future.

DECLARATIONS

Ethical statement

Not applicable.

Data availability

Data supporting this article are included within the article.

Author contributions

SE: conceptualization of research, design of study, analysis of data and interpretation, and preparation of manuscript; MAK: design of study, analysis of data and interpretation, and preparation of manuscript; AR: preparation of manuscript; MK: design of study, review and editing of the final manuscript, and validation and visualization; PP: design of study and validation and visualization. All authors reviewed and approved the final version for publication.

Conflict of interest

None declared.

Funding/support

This study presents partial outcomes of Ph.D. thesis written by Sahar Ebrahimi and granted by the Pasteur Institute of Iran (Tehran). The funders had no role in the study design, data collection and analysis, decision to publish or preparation of the manuscript.

REFERENCES

1. Maeder ML, Gersbach CA. Genome-editing technologies for gene and cell therapy. *Molecular therapy* 2016; **24**(3): 430-446.
2. Carroll D. Genome engineering with zinc-finger nucleases. *Genetics* 2011; **188**(4): 773-782.
3. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nature biotechnology* 2014; **32**(4): 347-355.
4. Guptt P, Smih F, Jasin M. Introduction of double-strand breaks into the genome of mouse cells by expression of a rare-cutting endonuclease. *Molecular and cellular biology* 1994; **14**(12): 8096-8106.
5. Segal DJ, Meckler JF. Genome engineering at the dawn of thegolden age. *Annual review of genomics and human genetics* 2013; **14**: 135-158.
6. Tadepally HD, Aubry M. Evolution of C2H2-zinc finger genes and subfamilies in mammals: Species-specific duplication and loss of clusters, genes and effector domains. *BMC evolutionary biology* 2008; **8**: 176.
7. Gaj T, Gersbach CA, Barbas CF. ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends in Biotechnology* 2013; **31**(7): 397-405.
8. Landgraf A, Kay S, Schornack S, Bonas U, Nickstadt A, Lahaye T, et al. Breaking the code of DNA binding specificity of TAL-type III effectors. *Science* (80-). 2009; **326**(5959): 1509-1512.
9. Gupta RM, Musunuru K. Expanding the genetic editing tool kit: ZFNs, TALENs, and CRISPR-Cas9. *Journal of clinical investigation* 2014; **124**(10): 4154-4161.

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

10. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, Charpentier E. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* 2012; **337**(6096): 816-821.
11. Lim JM, Kim HH. Basic principles and clinical applications of CRISPR-based genome editing. *Yonsei medical Journal* 2022; **63**(2): 105-113.
12. Makarova KS, Wolf YI, Alkhnbashi OS, CostaF, Shah SA, Saunders SJ, BarrangouR, Stan JJ, Brouns SJJ, Charpentier E, HaftDH, Horvath P, Moineau S, Mojica FJM, Terns RM, Terns MP White MF, Yakunin AF Garrett RA, Oost JVD, Backofen R, Koonin EV. An updated evolutionary classification of CRISPR–Cas systems. *Nature reviews microbiology* 2015; **13**(11): 722-736.
13. Bishop TF, van Eenennaam AL. Genome editing approaches to augment livestock breeding programs. *Journal of exprimental biology* 2020; **223**(Pt Suppl 1): jeb207159.
14. Li H, Yang Y, Hong W, Huang M, Wu M, Zhao X. Applications of genome editing technology in the targeted therapy of human diseases: mechanisms, advances and prospects. *Signal transduction and targeted therapy* 2020; **5**(1): 1.
15. Kozovska Z, Rajcaniova S, Munteanu P, Dzacovska S, Demkova L. CRISPR: History and perspectives to the future. *Biomedicine and pharmacotherapy* 2021; **141**: 111917.
16. Ishino Y, Shinagawa H, Makino K, Amemura M, Nakatura A. Nucleotide sequence of the iap gene, responsible for alkaline phosphatase isoenzyme conversion in Escherichia coli, and identification of the gene product. *Journal of bacteriology* 1987; **169**(12): 5429-5433.
17. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, Hermans P, Martin C, McAdam R, Shinnick TM. Strain identification of Mycobacterium tuberculosis by DNA fingerprinting:recommendations for a standardized methodology. *Journal of clinical microbiology* 1993; **31**(2): 406-409.
18. Mojica FJ, Díez-Villaseñor C, García-Martínez J, Soria E. Intervening sequences of regularly spaced prokaryotic repeats derive from foreign genetic elements. Journal of molecular evolution 2005; **60**: 174-182.
19. Garneau JE, Dupuis MÈ, Villion M, Romero DA, Barrangou R, Boyaval P, Fremaux C, Horvath P, Magadán AH, Moineau S. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. *Nature* 2010; **468**(7320): 67-71.
20. Deltcheva E, Chylinski K, Sharma CM, Gonzales K, Chao Y, Pirzada ZA, Eckert MR, Vogel J, Charpentier E. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature* 2011; **471**(7340): 602-607.
21. Rodríguez-Rodríguez DR, Ramírez-Solís R, Garza-Elizondo MA, Garza-Rodríguez MDL, Barrera-Saldaña1 HA. Genome editing: A perspective on the application of CRISPR/Cas9 to study human diseases. *International journal of molecular sciences* 2019; **43**(4): 1559-1574.
22. Mali P, Yang L, Esvelt KM, Aach J, Guell M, DiCarlo JE, Norville JE, Church GM. RNA-guided human genome engineering via Cas9. *Science* 2013; **339**(6121): 823-826.
23. Cong L, Ran FA, Cox D, Lin S, Barretto R, Habib N, Hsu PD, Wu X, Jiang W, Luciano Marraffini LA, Zhang F. Multiplex genome engineering using CRISPR/Cas systems. *Science* 2013; **339**(6121): 819-823.
24. Cornu TI, Mussolino C, Cathomen T. Refining strategies to translate genome editing to the clinic. *Nature medicine* 2017; **23**(4): 415-423.
25. Cyranoski D. First trial of CRISPR in people: Chinese team approved to test gene-edited cells in people with lung cancer. *Nature* 2016; **535**(7613): 476-477.
26. Cyranoski D. CRISPR gene-editing tested in a person for the first time. *Nature* 2016 ; **539**(7630): 479.
27. Mengstie MA, Wondimu BZ. Mechanism and applications of crispr/ cas-9-mediated genome editing. *Biologics: targets and therapy* 2021; **15**: 353-361.
28. Makarova KS, Wolf YI, Iranzo J, Shmakov SA, Alkhnbashi OS, Brouns SJJ, Charpentier E, Cheng D, Haft DH, Horvath P, Moineau S, Mojica FJM, Scott D, Shah SA, Siksnys V, Terns MP, Venclovas, White MF, Yakunin AF, Yan W, Zhang F, Garrett RA, Backofen R, Oost JVD, Barrangou R, Koonin EV. Evolutionary classification of CRISPR–Cas systems: a burst of class 2 and derived variants. *Nature review microbiology* 2020; **18**(2): 67-83.
29. Jiang F, Doudna JA. The Structural biology of CRISPR-Cas systems. *Current opinion of structural biology* 2015; **30**: 100-111.
30. Tang H, Yuan H, Du W, Li G, Xue D, Huang Q. Active-site models of streptococcus pyogenes Cas9 in DNA cleavage state. *Frontires in molecular bioscience* 2021; **8**: 235.
31. Nishimasu H, Ran FA, Hsu PD, Konermann S, Shehata SI, Dohmae N, Ishitani R, Zhang F, Nureki O. Crystal structure of Cas9 in complex with guide RNA and target DNA. *Cell* 2014; **156**(5): 935-949.
32. Liu Z, Dong H, Cui Y, Cong L, Zhang D. Application of different types of CRISPR/Cas-based systems in bacteria. *Microbial cell factories* 2020; **19**(1): 172.
33. Hille F, Charpentier E. CRISPR-cas: Biology, mechanisms and relevance. *Philosofical transactions of the royal society B* 2016; **371**(1707): 20150496.
34. Gleditzsch D, Pausch P, Müller-Esparza H, Özcan A, Guo X, Bange G, Randaua L. PAM identification by CRISPR-Cas effector complexes: diversified mechanisms and structures. *RNA biology* 2018; **16**(4): 504-517.
35. Newsom S, Parameshwaran HP, Martin L, Rajan R. The CRISPR-Cas mechanism for adaptive immunity and alternate bacterialfunctions fuels diverse biotechnologies. *Frontiers in cellular and infection microbiology* 2021; **10**: 898.
36. Karvelis T, Gasiunas G, Miksys A, Barrangou R, Horvath P, Siksnys V. crRNA and tracrRNA guide Cas9-mediated DNA interference in Streptococcus thermophilus. *RNA biology* 2013; **10**(5): 841-851.

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

37. Gasiunas G, Barrangou R, Horvath P, Siksnys V. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proceedings of the national academy of sciences of the united states of america* 2012 ; **109**(39): E2579-E2586.
38. Friedland AE, Baral R, Singhal P, Loveluck K, Shen S, Sanchez M, Marco E, Gotta GM, Maeder ML, Kennedy EM, Kornepati AVR, Sousa A, Collins MA, Jayaram H, Cullen BR, Bumcrot D. Characterization of staphylococcus aureus Cas9: a smaller Cas9 for all-in-one adeno-associated virus delivery and paired nickase applications. *Genome biology* 2015; **16**: 257.
39. Ran FA, Cong L, Yan WX, Scott DA, Gootenberg JS, Kriz AJ, , Zetsche B, Shalem O, Wu X, Makarova KS, Koonin EV, Sharp PA, Zhang F. In vivo genome editing using Staphylococcus aureus Cas9. *Nature* 2015; **520**: 186-191.
40. Hou Z, Zhang Y, Propson NE, Howden SE, Chu LF, Sontheimer EJ, Thomson JA. Efficient genome engineering in human pluripotent stem cells using Cas9 from Neisseria meningitidis. *Proceedings of the national academy of sciences of the united states of america* 2013; **110**(39): 15644-15649.
41. Chen F, Ding X, Feng Y, Seebeck T, Jiang Y, Davis GD. Targeted activation of diverse CRISPR-Cas systems for mammalian genome editing via proximal CRISPR targeting. *Nature communication* 2017; **8**: 14958.
42. Wiedenheft B, Sternberg SH, Doudna JA. RNA-guided genetic silencing systems in bacteria and archaea. *Nature* 2012; **482**(7385): 331-338.
43. Semenova E, Jore MM, Datsenko KA, Semenova A, Westra ER, Wanner B, Oost JVD, Brouns SJJ, Severinov K. Interference by clustered regularly interspaced short palindromic repeat (CRISPR) RNA is governed by a seed sequence. *Proceedings of the national academy of sciences of the united states of america* 2011; **108**(25): 10098-10103.
44. Manghwar H, Lindsey K, Zhang X, Jin S. CRISPR/Cas system: recent advances and future prospects for genome editing. *Trends in plant science* 2019; **24**(12): 1102-1125.
45. Chiang TWW, Le Sage C, Larrieu D, Demir M, Jackson SP. CRISPR-Cas9D10A nickase-based genotypic and phenotypic screening to enhance genome editing. *Scientific reports* 2016; **6**: 24356.
46. Li PP and Margolis RL. Use of single guided Cas9 nickase to facilitate precise and efficient genome editing in human iPSCs. *Scientific reports* 2021; 11: article number 9865.
47. Fauser F, Schiml S, Puchta H. Both CRISPR/Cas-based nucleases and nickases can be used efficiently for genome engineering in Arabidopsis thaliana. *Plant journal* 2014; **79**(2): 348-359.
48. Trevino AE, Zhang F. Genome editing using Cas9 nickases. *Methods enzymol* 2014; **546**:161-174.
49. Brocken DJW, Tark-Dame M, Dame RT. dCas9: A versatile tool for epigenome editing. *Current issues in molecular biology* 2018; **26**: 15-32.
50. Karlson CKS, Mohd-noor SN, Nolte N, Tan BC. Crispr/dcas9-based systems: mechanisms and applications in plant sciences. *Plants* 2021; **10**(10): 2055.
51. Brezgin S, Kostyusheva A, Kostyushev D, Chulanov V. Dead cas systems: types, principles, and applications. *International journal of molecular sciences* 2019; **20**(23): 6041.
52. Li Y, Zhou LQ. dCas9 techniques for transcriptional repression in mammalian cells: Progress, applications and challenges. *Bio essays* 2021; **43**(9): e2100086
53. Bikard D, Jiang W, Samai P, Hochschild A, Zhang F, Marraffini LA. Programmable repression and activation of bacterial gene expression using an engineered CRISPR-Cas system. *Nucleic acids research* 2013; **41**(15): 7429-37.
54. Qi LS, Larson MH, Gilbert LA, Doudna JA, Weissman JS, Arkin AP, Lim WA. Repurposing CRISPR as an RNA-guided platform for sequence-specific control of gene expression. *Cell* 2013; **152**(5): 1173-1183.
55. Alerasool N, Segal D, Lee H, Taipale M. An efficient KRAB domain for CRISPRi applications in human cells. *Nature methods* 2020; **17**(11): 1093-1096
56. Liu Y, Wan X, Wang B. Engineered CRISPRa enables programmable eukaryote-like gene activation in bacteria. *Nature communication* 2019; **10**(1): 3693.
57. Chavez A, Tuttle M, Pruitt BW, Ewen-Campen B, Chari R, Ter-Ovanesyan D, Haque SJ , Cecchi RJ, Kowal EJK , Buchthal J , Housden BE, Perrimon N, Collins JJ, Church G. Comparison of Cas9 activators in multiple species. *Nature methods* 2016; **13**(7): 563-567.
58. Konermann S, Brigham MD, Trevino AE, Joung J, Abudayyeh OO, Barcena C, Hsu PD, Habib N, Gootenberg JS, Nishimasu H, Nureki O, Zhang F. Genome-scale transcriptional activation by an engineered CRISPR-Cas9 complex. *Nature* 2015; **517**(7536): 583-588.
59. Perez-Pinera P, Kocak DD, Vockley CM, Adler AF, Kabadi AM, Polstein LR, Thakore PI, Glass KA, Ousterout DG, Leong KW, Guilak F, Crawford GE, Reddy TE, Gersbach CA. RNA-guided gene activation by CRISPR-Cas9–based transcription factors. *Nature methods* 2013; **10**(10): 973-976.
60. Pandelakis M, Delgado E, Ebrahimkhani MR. CRISPR-Based synthetic transcription factors in vivo: the future of therapeutic cellular programming. *Cell systems* 2020; **10**(1): 14.
61. Chavez A, Scheiman J, Vora S, Pruitt BW, Tuttle M, P R Iyer EPR, Lin S , Kiani S, Guzman CD, Wiegand DJ, Ter-Ovanesyan D, Braff JL, Davidsohn N, Housden BE, Perrimon N, Weiss R, Aach J, Collins JJ, Church GM2. Highly efficient Cas9-mediated transcriptional programming. *Nature methods* 2015; **12**(4): 326-328.
62. Zhang Y, Yin C, Zhang T, Li F, Yang W, Kaminski R, Fagan PR, Putatunda R, Young WB, Khalili K, Hu W. CRISPR/gRNA-directed synergistic activation mediator (SAM) induces specific, persistent and robust reactivation of the HIV-1 latent reservoirs. *Scientific reports* 2015; **5**: 1-14.
63. Tanenbaum ME, Gilbert LA, Qi LS, Weissman JS, Vale RD. A protein-tagging system for signal amplification in

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

gene expression and fluorescence imaging. *Cell* 2014; **159**(3): 635-646.
64. Zhou H, Liu J, Zhou C, Gao N, Rao Z, Li H, Hu X, Li C, Yao X, Shen X, Sun Y, Wei Y, Liu F, Ying W, Zhang J, Tang C, Zhang X, Xu H, Shi L, Cheng L, Huang P, Yang H. In vivo simultaneous transcriptional activation of multiple genes in the brain using CRISPR–dCas9-activator transgenic mice. *Nature neuroscience* 2018; **21**(3): 440-446.
65. Hornschuh M, Wirthgen E, Wolfien M, Singh KP, Wolkenhauer O, Däbritz J. The role of epigenetic modifications for the pathogenesis of Crohn's disease. *Clinical epigenetics* 2021; **13**(1): 1-14.
66. Zhang W, Song M, Qu J, Liu GH. Epigenetic modifications in cardiovascular aging and diseases. *Circulation research* 2018; **123**(7): 773-786.
67. Sar P, Dalai S. CRISPR/Cas9 in epigenetics studies of health and disease. *Progress in molecular biology and translational science* 2021; **181**: 309-343.
68. Yamano T, Nishimasu H, Zetsche B, Hirano H, Slaymaker IM, Li Y, Fedorova I, Nakane T, Makarova KS, Koonin EV, Ishitani R, Zhang F, Nureki O. Crystal structure of Cpf1 in complex with Guide RNA and target DNA. *Cell* 2016 2016; **165**(4): 949-962.
69. Paul B, Montoya G. CRISPR-Cas12a: Functional overview and applications. *Biomedical journal* 2020; **43**(1): 8-17.
70. Schindele P, Wolter F, Puchta H. Transforming plant biology and breeding with CRISPR/Cas9, Cas12 and Cas13. *FEBS letters* 2018; **592**(12): 1954-1967.
71. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler P, Volz SE, Joung J, van der Oost J, Regev A, Koonin EV, Zhang F. Cpf1 is a single RNA-Guided endonuclease of a class 2 CRISPR-Cas system. *Cell* 2015; **163**(3): 759-771.
72. Yang Z, Edwards H, Xu P. CRISPR-Cas12a/Cpf1-assisted precise, efficient and multiplexed genome-editing in Yarrowia lipolytica. *Metabolic engineering communications* 2020; **10**: e00112.
73. Bandyopadhyay A, Kancharla N, Javalkote VS, Dasgupta S, Brutnell TP. CRISPR-Cas12a (Cpf1): a versatile tool in the plant genome editing tool box for agricultural advancement. *Frontiers in plant science* 2020; **11**: 1589
74. Chen JS, Ma E, Harrington LB, Da Costa M, Tian X, Palefsky JM, Doudna JA. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. *Science* 2018; **360**(6387): 436-439.
75. Gootenberg JS, Abudayyeh OO, Lee JW, Essletzbichler P, Dy AJ, Joung J, Verdine V, Donghia N, Daringer NM, Freije CA, Myhrvold C, Bhattacharyya RP, Livny J, Regev A, Koonin EV, Hung DT, Sabeti PC, Collins JJ, Zhang F. Nucleic acid detection with CRISPR-Cas13a/C2c2. *Science* 2017; **356**(6336): 438-442.
76. Abudayyeh OO, Gootenberg JS, Konermann S, Joung J, Slaymaker IM, Cox DBT, Shmakov S, Makarova KS, Semenova E, Minakhin L, Severinov K, Regev A, Lander ES, Koonin EV, Zhang F. C2c2 is a single-component programmable RNA-guided RNA-targeting CRISPR effector. *Science* 2016; **353**(6299): aaf5573.
77. Shmakov S, Abudayyeh OO, Makarova KS, Wolf YI, Gootenberg JS, Semenova E, Minakhin L, Joung J, Konermann S, Severinov K, Zhang F, Koonin EV. Discovery and functional characterization of diverse class 2 CRISPR-Cas systems. *Molecular cell* 2015; **60**(3): 385-397.
78. Cox DBT, Gootenberg JS, Abudayyeh OO, Franklin B, Kellner MJ, Joung J, Zhang F. RNA editing with CRISPR-Cas13. *Science* 2017; **358**(6366): 1019-1027.
79. East-Seletsky A, O'Connell MR, Knight SC, Burstein D, Cate JHD, Tjian R, Doudna JA. Two distinct RNase activities of CRISPR-C2c2 enable guide-RNA processing and RNA detection. *Nature* 2016; **538**(7624): 270-273.
80. Watanabe S, Cui B, Kiga K, Aiba Y, Tan XE, Sato'o Y, Kawauchi M, Boonsiri T, Thitiananpakorn K, Taki Y, Li FY, Azam AH, Nakada Y, Sasahara T, Cui L. Composition and diversity of CRISPR-Cas13a systems in the genus Leptotrichia. *Frontiers in microbiology* 2019; **10**: 283.
81. Li H, Bello A, Smith G, Kielich DMS, Strong JE, Pickering BS. Degenerate sequence-based CRISPR diagnostic for Crimean-Congo hemorrhagic fever virus. Forshey BM, editor. *PLoS neglected tropical diseases* 2022; **16**(3):e0010285
82. Liu Y, Xu H, Liu C, Peng L, Khan H, Cui L, Huang R, Wu C, Shen S, Wang S, Liang W, Li Z, Xu B, He N. CRISPR-Cas13a nanomachine based simple technology for avian influenza A (H7N9) virus on-site detection. *Journal of biomedical nanotechnology* 2019; **15**(4): 790-798.
83. Aquino-Jarquin G. CRISPR-Cas14 is now part of the artillery for gene editing and molecular diagnostic. *Nanomedicine* 2019; **18**: 428-431.
84. Harrington LB, Burstein D, Chen JS, Paez-Espino D, Ma E, Witte IP, Cofsky JC, Kyrpides NC, Banfield JF, Doudna JA . Programmed DNA destruction by miniature CRISPR-Cas14 enzymes. *Science* 2018; **362**(6416): 839-342.
85. Liu X, Hussain M, Dai J, Li Y, Zhang L, Yang J, Ali Z, He N, Tang Y. Programmable biosensors based on RNA-Guided CRISPR/Cas endonuclease. *Biological procedures online* 2022; **24**(1): 2.
86. Wang Q, Alariqi M, Wang F, Li B, Ding X, Rui H, Li Y, Xu Z, Qin L, Sun L, Li J, Zou J, Lindsey K, Zhang X, Jin S. The application of a heat-inducible CRISPR/Cas12b (C2c1) genome editing system in tetraploid cotton (G. hirsutum) plants. *Plant biotechnology journal* 2020; **18**(12): 2436-2443.
87. Chen X, Tan Y, Wang S, Wu X, Liu R, Yang X, Wang Y, Tai J, Li S. A CRISPR-Cas12b–based platform for ultrasensitive, rapid, and highly specific detection of hepatitis B Virus genotypes B and C in clinical application. *Frontiers in bioengineering and biotechnology* 2021; **9**: 844.
88. Strecker J, Jones S, Koopal B, Schmid-Burgk J, Zetsche B, Gao L, Makarova KS, Koonin EV, Zhang F.

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

Engineering of CRISPR-Cas12b for human genome editing. *Nature communications* 2019; **10**(1): 212.
89. Teng F, Cui T, Feng G, Guo L, Xu K, Gao Q, Li T, Li J, Zhou Q, Li W. Repurposing CRISPR-Cas12b for mammalian genome engineering. *Cell discovery* 2018; **4**: 63.
90. Kato K, Zhou W, Okazaki S, Isayama Y, Nishizawa T, Gootenberg JS, Abudayyeh OO, Nishimasu H. Structure and engineering of the type III-E CRISPR-Cas7-11 effector complex. *Cell* 2022; **185**(13): 2324-2337.
91. Goswami HN, Rai J, Das A, Li H. Molecular mechanism of active Cas7-11 in processing CRISPR RNA and interfering target RNA. *Elife* 2022; **11**: e81678.
92. Yu G, Wang X, Zhang Y, An Q, Wen Y, Li X, Yin H, Deng Z, Zhang H. Structure and function of a bacterial type III-E CRISPR–Cas7-11 complex. *Nature microbiology* 2022; **7**(12): 2078-2088.
93. Kurihara N, Nakagawa R, Hirano H, Okazaki S, Tomita A, Kobayashi K, Kusakizoko T, Nishizowoa T, Yamashito K. Structure of the type V-C CRISPR-Cas effector enzyme. *Molecular cell* 2022; **82**(10): 1865-1877
94. Slaymaker IM, Mesa P, Kellner MJ, Kannan S, Brignole E, Koob J, Feliciano PR, Stella S, Abudayyeh OO, Gootenberg JS, Strecker J, Montoya G, Zhang F. High-resolution structure of Cas13b and biochemical characterization of RNA targeting and cleavage. *Cell reports* 2019; **26**(13):3741-3751.
95. Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, Kang Y, Zhao X, Si W, Li W, Xiang AP, Zhou J, Guo X, Bi Y, Si C, Hu B, Dong G, Wang H, Zhou Z, Li T, Tan T, Pu X, Wang F, Ji S, Zhou Q, Huang X, Ji W, Sha J. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. *Cell* 2014 ; **156**(4): 836-843.
96. Zhou W, Wan Y, Guo R, Deng M, Deng K, Wang Z, Zhang Y, Wang F. Generation of beta-lactoglobulin knock-out goats using CRISPR/Cas9. *PLoS one* 2017; **12**(10): e0186056
97. Naert T, Vleminckx K. CRISPR/Cas9 disease models in zebrafish and Xenopus: The genetic renaissance of fish and frogs. *Drug discovery today* 2018; **28**: 41-52.
98. Dickinson DJ, Goldstein B. CRISPR-Based methods for caenorhabditis elegans genome engineering. *Genetics* 2016; **202**(3): 885.
99. Xue Z, Ren M, Wu M, Dai J, Rong YS, Gao G. Efficient gene knock-out and knock-in with transgenic Cas9 in Drosophila. *G3 (Bethesdsa)* 2014; **4**(5): 925-929.
100. Tzur YB, Friedland AE, Nadarajan S, Church GM, Calarco JA, Colaiácovo MP. Heritable custom genomic modifications in Caenorhabditis elegans via a CRISPR-Cas9 system. *Genetics* 2013; **195**(3): 1181-1185.
101. Zuckermann M, Hovestadt V, Knobbe-Thomsen CB, Zapatka M, Northcott PA, Schramm K, Belic J, Jones DTW, Tschida B, Moriarity B, Largaespada D, Roussel MF, Korshunov A, Reifenberger G, Pfister SM, Lichter P, Kawauchi D, Gronych J. Somatic CRISPR/Cas9-mediated tumour suppressor disruption enables versatile brain tumour modelling. *Nature communication* 2015; **6**: 7391.
102. Weber J, Öllinger R, Friedrich M, Ehmer U, Barenboim M, Steiger K, Heid I, Muller S, Maresch R, Engleitner T, Gross N, Geumann U, Fu B, Segler A, Yuan D, Lange S, Strong A, de la Rosa J, Esposito I, Liu P, Cadiñanos J, Vassiliou GS, Schmid RM, Schneider G, Unger K, Yang F, Braren R, Heikenwälder M, Varela I, Saur D, Bradley A, Rad R. CRISPR/Cas9 somatic multiplex-mutagenesis for high-throughput functional cancer genomics in mice. *Proceedings of the national academy of sciences of the united states of america* 2015; **112**(45): 13982-13987.
103. Chiou SH, Winters IP, Wang J, Naranjo S, Dudgeon C, Tamburini FB, Brady JJ, Yang D, Grüner BM , Chuang CH, Caswell DR, Zeng H, Chu P, Kim GE, Carpizo DR, Kim SK, Winslow MM. Pancreatic cancer modeling using retrograde viral vector delivery and in vivo CRISPR/Cas9-mediated somatic genome editing. *Genes and development* 2015; **29**(14): 1576-1585.
104. Heckl D, Kowalczyk MS, Yudovich D, Belizaire R, Puram RV, McConkey ME, Thielke A, Aster JC, Regev A, Ebert BL. Generation of mouse models of myeloid malignancy with combinatorial genetic lesions using CRISPR-Cas9 genome editing. *Nature biotechnology* 2014; **32**(9): 941-946.
105. Xue W, Chen S, Yin H, Tammela T, Papagiannakopoulos T, Joshi NS, Cai W, Yang G, Bronson R, Crowley DG, Zhang F, Anderson DG, Sharp PA, Jacks T. CRISPR-mediated direct mutation of cancer genes in the mouse liver. *Nature* 2014; **514**(7522): 380-384.
106. Sanchez-Rivera FJ, Papagiannakopoulos T, Romero R, Tammela T, Bauer MR, Bhutkar A, Joshi NS, Subbaraj L, Bronson RT, Xue W, Jacks T. Rapid modelling of cooperating genetic events in cancer through somatic genome editing. *Nature* 2014; **516**(7531): 428-431.
107. Platt RJ, Chen S, Zhou Y, Yim MJ, Swiech L, Kempton HR, Dahlman JE, Parnas O, Eisenhaure TM, Jovanovic M, Graham DB, Jhunjhunwala S, Heidenreich M, Xavier RJ, Langer R, Anderson DG, Hacohen N, Regev A, Feng G, Sharp PA, Zhang F. CRISPR-Cas9 knockin mice for genome editing and cancer modeling. *Cell* 2014; **159**(2): 440-455.
108. Carroll-KJ, Makarewich CA, McAnally J, Anderson DM, Zentilin L, Liu N, Giacca M, Bassel-Duby R, Olson EN. A mouse model for adult cardiac-specific gene deletion with CRISPR/Cas9. *Proceedings of the national academy of sciences of the united states of america* 2016; **113**(2): 338-343.
109. Tabebordbar M, Zhu K, Cheng JKW, Chew WL, Widrick JJ, Yan WX, Maesner C, Wu EY, Xiao R, Ran FA, Cong L, Zhang F, Vandenberghe LH, Church GM, Wagers AJ. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. *Science* 2016; **351**(6271): 407-411.
110. Yang S, Chang R, Yang H, Zhao T, Hong Y, Kong HE, Sun X, Qin Z, Jin P, Li S, Li XJ. CRISPR/Cas9-mediated gene editing ameliorates neurotoxicity in mouse model of Huntington's disease. *The journal of clinical investigation* 2017; **127**(7): 2719-2724.
111. Mizuno S, Dinh TTH, Kato K, Mizuno-Iijima S,

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

Tanimoto Y, Daitoku Y, Hoshino Y, Ikawa M, Takahashi S, Sugiyama F, Yagami K. Simple generation of albino C57BL/6J mice with G291T mutation in the tyrosinase gene by the CRISPR/Cas9 system. *Mammalian* 2014; **25**(7-8): 327-334.

112. Matharu N, Rattanasopha S, Tamura S, Maliskova L, Wang Y, Bernard A, Hardin A, Eckalbar WL, Vaisse C, Ahituv N. CRISPR-mediated activation of a promoter or enhancer rescues obesity caused by haploinsufficiency. *Science* 2019; **363**(6424): eaau0629.
113. Wang L, Yang Y, Breton CA, White J, Zhang J, Che Y, Saveliev A, McMenamin D, He Z, Latshaw C, Li M, Wilson JM. CRISPR/Cas9-mediated in vivo gene targeting corrects hemostasis in newborn and adult factor IX–knockout mice. *Blood* 2019; **133**(26): 2745-2752.
114. Matano M, Date S, Shimokawa M, Takano A, Fujii M, Ohta Y, Watanabe T, Kanai T, Sato T. Modeling colorectal cancer using CRISPR-Cas9-mediated engineering of human intestinal organoids. *Nature medicine* 2015; **21**(3): 256-262.
115. Chang CY, Ting HC, Su HL, Jeng JR. Combining induced pluripotent stem cells and genome editing technologies for clinical applications. *Cell transplantation* 2018; **27**(3): 379-392.
116. Hotta A, Yamanaka S. From genomics to genetherapy: induced pluripotent stem cells meet genome editing. *Annual review of genetics* 2015; **49**: 47-70.
117. Bjursell M, Porritt MJ, Ericson E, Taheri-Ghahfarokhi A, Clausen M, Magnusson L, Admyre T, Nitsch R, Mayr L, Aasehaug L, Seeliger F, Maresca M, Bohlooly M, Wiseman J. Therapeutic genome editing with CRISPR/Cas9 in a humanized mouse model ameliorates α1-antitrypsin deficiency phenotype. *eBioMedicine* 2018; **29**: 104-111.
118. Rabaan AA, AlSaihati H, Bukhamsin R, Bakhrebah MA, Nassar MS, Alsaleh AA, Alhashem YN, Bukhamseen AY, Al-Ruhimy K, Mohammed Alotaibi, Alsubki RA, Alahmed HE, Al-Abdulhadi S , Alhashem FA, Alqatari AA, Alsayyah A, Farahat RA, Abdulal RH, Al-Ahmed AH, Imran M, Mohapatra RK. Application of CRISPR/Cas9 technology in cancer treatment: a future direction. *Current oncology* 2023; **30**(2): 1954-1976.
119. Wang SW, Gao C, Zheng YM, Yi L, Lu JC, Huang XY, Cai JB, Zhang PF, Cui YH, Ke AW. Current applications and future perspective of CRISPR/Cas9 gene editing in cancer. *Molecular cancer* 2022; **2**1(1): 57.
120. Thein SL. Molecular basis of β thalassemia and potential therapeutic targets. *Blood cells, molecules and diseases* 2018; **70**: 54-65.
121. Weatherall DJ, Clegg JB. Inherited haemoglobin disorders: An increasing global health problem. Bull *World health organization* 2001; **79**(8): 704-712.
122. Cavazzana M, Antoniani C, Miccio A. Gene therapy for β-hemoglobinopathies. *Molecular therapy* 2017; **25**(5): 1142-1154.
123. Cavazzana-Calvo M, Payen E, Negre O, Wang G, Hehir K, Fusil F, Down J, Denaro M, Brady T, Westerman K, Cavallesco R, Gillet-Legrand B, Caccavelli L, Sgarra R, Maouche-Chretien L, Bernaudin F, Girot R, Dorazio R, Mulder GJ, Polack A, Bank A, Soulier J, Larghero J, Kabbara N, Dalle B, Gourmel B, Socie G, Chretien S, Cartier N, Aubourg P, Fischer A, Cornetta K, Galacteros F, Beuzard Y, Gluckman E, Bushman F, Hacein-Bey-Abina S, Leboulch P. Transfusion independence and HMGA2 activation after gene therapy of human β-thalassaemia. *Nature* 2010; **467**(7313): 318-322.
124. Abbasalipour M, Khosravi MA, Zeinali S, Khanahmad H, Azadmanesh K, Karimipoor M. parkcontaining beta-globin gene for beta thalassemia gene therapy. *Gene reports* 2022; **27**: 101615.
125. Kanter J, Walters MC, Matthew M, Krishnamurti L, Kwiatkowski J, Kamble RT, Von Kalle C, Kuypers FA, Cavazzana M, Leboulch P, Joseney-Antoine M, Asmal M, Thompson AA, Tisdale JF. Interim results from a phase 1/2 clinical study of lentiglobin gene therapy for severe sickle cell disease. *Blood* 2016; **28**(22): 1176.
126. Thompson AA, Kwiatkowski J, Rasko J, Hongeng S, Schiller GJ, Anurathapan U, Cavazzana M, Ho J, von Kalle C, Kletzel M, Leboulch P, Vichinsky E, Petrusich A, Asmal M, Walters MC. Lentiglobin gene therapy for transfusion-dependent β-thalassemia: Update from the northstar Hgb-204 phase 1/2 clinical study. *Blood* 2016; **128**(22): 1175.
127. Liu Y, Yang Y, Kang X, Lin B, Yu Q, Song B, GaoG 2, Chen Y, Sun X, Li X, Bu L, Fan1 Y. One-step biallelic and scarless correction of a β-Thalassemia mutation in patient-specific iPSCs without drug selection. *Molecular therapy nucleic acids* 2017; **6**: 57-67.
128. Khosravi MA, Abbasalipour M, Concordet JP, Berg J Vom, Zeinali S, Arashkia A, Azadmanesh K, Buch T, Karimipoor M. Targeted deletion of BCL11A gene by CRISPR-Cas9 system for fetal hemoglobin reactivation: A promising approach for gene therapy of beta thalassemia disease. *European journal of pharmacology* 2019; **854**: 398-405.
129. Khosravi MA, Abbasalipour M, Concordet JP, Berg JV, Zeinali S, Arashkia A, Buch T, Karimipoor M. Expression analysis data of BCL11A and γ-globin genes in KU812 and KG-1 cell lines after CRISPR/Cas9-mediated BCL11A enhancer deletion. *Data in brief* 2019; **28**: 104974.
130. Dever DP, Bak RO, Reinisch A, Camarena J, Washington G, Nicolas CE, Pavel-Dinu M, Saxena N, Wilkens AB, Mantri S, Uchida N, Hendel A, Narla A, Majeti R, Weinberg KI, Porteus MH. CRISPR/Cas9 β-globin gene targeting in human haematopoietic stem cells. *Nature* 2016; **539**(7629): 384-389.
131. Canver MC, Smith EC, Sher F, Pinello L, Sanjana NE, Shalem O, Chen DD, Schupp PG, Vinjamur DS, Garcia SP, Luc S, Kurita R, Nakamura Y, Fujiwara Y, Maeda T, Yuan GC, Zhang F, Orkin SH, Bauer DE. BCL11A enhancer dissection by Cas9-mediated in situ saturating mutagenesis. *Nature* 2015; **527**(7577): 192-197.
132. Park CY, Kim DH, Son JS, Sung JJ, Lee J, Bae S, Kim JH, Kim DW, Kim JS. Functional correction of large factor VIII gene chromosomal inversions in hemophilia a patient-derived iPSCs using CRISPR-Cas9. *Cell stem cell* 2015; **17**(2): 213-220.

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

133. Morishige S, Mizuno S, Ozawa H, Nakamura T, Mazahery A, Nomura K, Seki R, Mouri F, Osaki K, Yamamura K, Okamura T, Nagafuji K . CRISPR/Cas9-mediated gene correction in hemophilia B patient-derived iPSCs. *International journal of hematology* 2022; **111**(2): 225-233.
134. Monteys AM, Ebanks SA, Keiser MS, Davidson BL. CRISPR/Cas9 editing of the mutant huntingtin allele in vitro and in vivo. *Molecular therapy* 2017; **25**(1): 12-23.
135. Schwank G, Koo BK, Sasselli V, Dekkers JF, Heo I, Demircan T, Sasaki N, Boymans S, Cuppen E, van der Ent CK, Nieuwenhuis EES, Beekman JM, Clevers H. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. *Cell Stem Cell* 2013; **13**(6): 653-658.
136. Long C, McAnally JR, Shelton JM, Mireault AA, Bassel-Duby R, Olson EN. Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA. *Science* 2014; **345**(6201): 1184-1188.
137. Lattanzi A, Duguez S, Moiani A, Izmiryan A, Barbon E, Martin S, Mamchaoui K, Vincent M, Bernardi F, Mavilio F, Bovolenta M. Correction of the exon 2 duplication in DMD myoblasts by a single CRISPR/Cas9 system. *Molecular therapy. Nucleic acids* 2017; **7**: 11-19.
138. Min YL, Li H, Rodriguez-Caycedo C, Mireault AA, Huang J, Shelton JM, McAnally JR, Amoasii L, Mammen PPA, Bassel-Duby R, Olson EN. CRISPR-Cas9 corrects duchenne muscular dystrophy exon 44 deletion mutations in mice and human cells. *Science advances* 2019; **5**(3): eaav4324.
139. El Refaey M, Xu L, Gao Y, Canan BD, Ayodele Adesanya TM, Warner SC, et al. In vivo genome editing restores dystrophin expression and cardiac function in dystrophic mice. *Circulation research* 2017; **121**(8): 923-929.
140. van Agtmaal EL, André LM, Willemse M, Cumming SA, van Kessel IDG, van den Broek WJAA, Gourdon G, Furling D, Mouly V, Monckton DG, Wansink DG, Wieringa B. CRISPR/Cas9-induced (CTG·CAG)n repeat instability in the myotonic dystrophy type 1 locus: implications for therapeutic genome editing. *Molecular therapy* 2017; **25**(1): 24-43.
141. Chamberlain CA, Bennett EP, Kverneland AH, Svane IM, Donia M, Met Ö. Highly efficient PD-1-targeted CRISPR-Cas9 for tumor-infiltrating lymphocyte-based adoptive T cell therapy. *Molecular therapy oncolytics* 2022; **24**: 417-428.
142. Hu W, Kaminski R, Yang F, Zhang Y, Cosentino L, Li F, Luo B, Alvarez-Carbonell D, Garcia-Mesa Y, Karn J, Mo X, Khalili K. RNA-directed gene editing specifically eradicates latent and prevents new HIV-1 infection. *Proceedings of the national academy of sciences of the united states of america* 2014; **111**(31): 11461-11466.
143. Liu Z, Chen S, Jin X, Wang Q, Yang K, Li C, Qiaoqiao X, Hou P, Liu S, Wu S, Hou W, Xiong Y, Kong C, Zhao X, Wu L, Li C, Sun G, Guo D. Genome editing of the HIV co-receptors CCR5 and CXCR4 by CRISPR-Cas9 protects CD4+ T cells from HIV-1 infection. *Cell and bioscience* 2017; **7**(1): 1-15
144. Yu S, Yao Y, Xiao H, Li J, Liu Q, Yang Y, Adeh D, Lu J, Zhao S, Qin L, Chen X. Simultaneous knockout of CXCR4 and CCR5 genes in $CD4^+$ T cells via CRISPR/Cas9 confers resistance to both X4- and R5-tropic human immunodeficiency virus type 1 infection. *Human gene therapy* 2018; **29**(1): 51-67.
145. Mandal PK, Ferreira LMR, Collins R, Meissner TB, Boutwell CL, Friesen M, Vrbanac V, Garrison BS, Stortchevoi A, Bryder D, Musunuru K, Brand H, Tager AM, Allen TM, Talkowski ME, Rossi DJ, Cowan CA. Efficient ablation of genes in human hematopoietic stem and effector cells using CRISPR/Cas9. *Cell stem cell* 2014; **15**(5):643-652.
146. Zhao J, Zhan Q, Guo J, Liu M, Ruan Y, Zhu T, Han L, Li F. Phylogeny and polymorphism in the E6 and E7 of human papillomavirus: Alpha-9 (HPV16, 31, 33, 52, 58), alpha-5 (HPV51), alpha-6 (HPV53, 66), alpha-7 (HPV18, 39, 59, 68) and alpha-10 (HPV6, 44) in women from Shanghai. *Infectious agents and cancer* 2019; **14**: 38.
147. Kennedy EM, Kornepati AVR, Goldstein M, Bogerd HP, Poling BC, Whisnant AW, Kastan MB, Cullen BR. 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* 2014; **88**(20): 11965-11972.
148. Karimova M, Beschorner N, Dammermann W, Chemnitz J, Indenbirken D, Bockmann JH, Grundhoff A, Lüth S, Buchholz F, zur Wiesch JS, Hauber J. CRISPR/Cas9 nickase-mediated disruption of hepatitis B virus open reading frame S and X. *Scientific reports* 2015; **5** 5: 13734.
149. Sakuma T, Masaki K, Abe-Chayama H, Mochida K, Yamamoto T, Chayama K. Highly multiplexed CRISPR-Cas9-nuclease and Cas9-nickase vectors for inactivation of hepatitis B virus. *Genes to cells* 2016; **21**(11): 1253-1262.
150. Lin SR, Yang HC, Kuo YT, Liu CJ, Yang TY, Sung KC, Lin YY, Wang HY, Wang CC, Shen YC, Wu FY, Kao JH, Chen DS, Chen PJ. The CRISPR/Cas9 system facilitates clearance of the intrahepatic HBV templates in vivo. *Molecular therapy nucleic acids* 2014; **3**: e186.
151. Louradour I, Ghosh K, Inbar E, Sacks DL. CRISPR/Cas9 mutagenesis in Phlebotomus papatasi: The immune deficiency pathway impacts vector competence for Leishmania major. *MBio* 2019; **10**(4): e01941-01919.
152. Dong Y, Simões ML, Marois E, Dimopoulos G. CRISPR/Cas9-mediated gene knockout of Anopheles gambiae FREP1 suppresses malaria parasite infection. *PLoS pathogenes* 2018; **14**(3): e1006898.
153. Chen P, Chen M, Chen Y, Jing X, Zhang N, Zhou X, Li X, Long G, Hao P. Targeted inhibition of Zika virus infection in human cells by CRISPR-Cas13b. *Virus research* 2022; **312**: 198707.
154. Park SH, Lee CM, Dever DP, Davis TH, Camarena J, Srifa W, Zhang Y, Paikari A, Chang AK, Porteus MH, Sheehan VA, Bao G. Highly efficient editing of the β-

globin gene in patient-derived hematopoietic stem and progenitor cells to treat sickle cell disease. *Nucleic acids research* 2019; **47**(15): 7955-7972.
155. Song B, Fan Y, He W, Zhu D, Niu X, Wang D, Ou Z, Lou M, Sun X. Improved hematopoietic differentiation efficiency of gene-corrected beta-thalassemia induced pluripotent stem cells by CRISPR/Cas9 System. *Stem cells and development* 2015; **24**(9): 1053-1065.
156. Huang X, Wang Y, Yan W, Smith C, Ye Z, Wang J, Gao Y, Mendelsohn L, Cheng L. Production of gene-corrected adult beta globin protein in human erythrocytes differentiated from patient iPSCs after genome editing of the sickle point mutation. *Stem cells* 2015; **33**(5): 1470-1479.
157. Xie F, Ye L, Chang JC, Beyer AI, Wang J, Muench MO, Kan YW. Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac. *Genome research* 2014; **24**(9): 1526-1533.
158. Lai X, Liu L, Zhang Z, Shi L, Yang G, Wu M, Huang R, Liu R, Lai Y, Li Q. Hepatic veno-occlusive disease/sinusoidal obstruction syndrome after hematopoietic stem cell transplantation for thalassemia major: incidence, management, and outcome. *Bone marrow transplantation* 2021; **56**: 1635-1641.
159. Bauer DE, Kamran SC, Lessard S, Xu J, Fujiwara Y, Lin C, Shao Z, Canver MC, Smith EC, Pinello L, Sabo PJ, Vierstra J, Voit RA, Yuan GC, Porteus MH, Stamatoyannopoulos JA, Lettre G, Orkin SH. An erythroid enhancer of BCL11A subject to genetic variation determines fetal hemoglobin level. *Science* 2013; **342**(6155): 253-257.
160. Vierstra J, Reik A, Chang K, Stehling-Sun S, Zhou Y, Hinklley S, Paschon DE, Zhang L, Psatha N, Bendann YR, O'Neil C, Song AH, Mich AK, Liu PQ, Lee G, Bauer DE, Holmes MC, Orkin SH, Papayannoppulou T, Stamatoyannopoulos G, Rebar EJ, Gregory PD, Urnov FD, Stamatoyannopoulos JA. Functional footprinting of regulatory DNA. *Nature methods* 2015; **12**(10): 927-930.
161. Sankaran V, Xu J, Ragoczy T, Ippolito GC, Walkley CR, Maika SD, Fujiwara Y, Ito M, Groudine M, Bender MA, Tucker PW, Orkin SH. Developmental and species-divergent globin switching are driven by BCL11A. *Nature* 2009; **460**(7259): 1093-1097.
162. Uda M, Galanello R, Sanna S, Lettre G, Sankaran VG, Chen W, Usala G, Busonero F, Maschio A, Albai G, Piras MG, Sestu N, Lai S, Dei M, Mulas A, Crisponi L, Naitza S, Asunis I, Deiana M, Nagaraja R, Perseu L, Satta S, Cipollina MD, Sollaino C, Moi P, Hirschhorn JN, Orkin SH, Abecasis GR, Schlessinger D, Cao A. Genome-wide association study shows BCL11A associated with persistent fetal hemoglobin and amelioration of the phenotype of beta-thalassemia. *Proceedings of the National Academy of Sciences of the United States of America* 2008; **5**(105): 1620-1625.
163. Xu J, Peng C, Sankaran VG, Shao Z, Esrick EB, Chong BG, Ippolito GC, Fujiwara Y, Ebert BL, Tucker PW, Orkin SH. orrection of sickle cell disease in adult mice by interference with fetal hemoglobin silencing. *Science* 2011; **334**(6058): 993-996.
164. Bjurström CF, Mojadidi M, Phillips J, Kuo C, Lai S, Lill GR, Cooper A, Kaufman M, Urbinati F, Wang X, Hollis RP, Kohn DB. Reactivating fetal hemoglobin expression in human adult erythroblasts through BCL11A knockdown using targeted endonucleases. *Molecular therapy-nucleic acids* 2016; **5**(8): e351.
165. Frangoul H, Bobruff Y, Cappellini MD, Corbacioglu S, Marie C, de la Fuente FJ, Grupp S, Handgretinger R, Ho TW, Imren S, Kattamis A, Lekstrom-Himes J, Locatelli F, Lu Y, Mapara M, Mulcahey S, de Montalembert M, Rondelli D, Shanbhag N, Sheth S, Soni S. Safety and efficacy of CTX001 in patients with transfusion-dependent b-thalassemia or sickle cell disease: Early results from the CLIMB THAL-111 and CLIMB SCD-121 studies of autologous CRISPR-CAS9-modified $CD34^+$ hematopoietic stem and progenitor cells. 62nd Annual American Society of Hematology Meeting December 6, 2020.
166. Wicki J, Seto JT, Chamberlain JS. Duchenne muscular dystrophy. In: Brenner's Encyclopedia of Genetics: Second Edition. Elsevier; 2013. P. 421-424.
167. Wu SS, Li QC, Yin CQ, Xue W, Song CQ. Advances in CRISPR/Cas-based gene therapy in human genetic diseases. *Theranostics* 2020; **10**(10): 4374-4378.
168. Choi E, Koo T. CRISPR technologies for the treatment of Duchenne muscular dystrophy. *Molecular therapy* 2021; **29**(1): P3179-P31914.
169. Rey MM, Bonk MP, Hadjiliadis D. Cystic fibrosis: emerging understanding and therapies. *Annual reviews* 2019; **70**:197-210.
170. Hanssens LS, Duchateau J, Casimir GJ. CFTR protein: not just a chloride channel? *Cells* 2021; **10**(11): 2844.
171. Firth AL, Menon T, Parker GS, Qualls SJ, Lewis BM, Ke E, Dargitz CT, Wright R, Khanna A, Gage FH, Verma IM. Functional gene correction for cystic fibrosis in lung epithelial cells generated from patient iPSCs. *Cell reports* 2015; **12**(9): 1385-1390.
172. Ruan J, Hirai H, Yang D, Ma L, Hou X, Jiang H, Wei H, Rajagopalan C, Mou H, Wang G, Zhang J, Li K, Chen YE, Sun F, Xu J. Efficient gene editing at major CFTR mutation loci. *Molecular therapy nucleic acids* 2019; **16**: 73-81.
173. Parsons MP, Raymond LA. Huntington disease. *Neurobiology of brain disorders* 2015; **2015**: 303-320.
174. Ekman FK, Ojala DS, Adil MM, Lopez PA, Schaffer DV, Gaj T. CRISPR-Cas9-mediated genome editing increases lifespan and improves motor deficits in a huntington's disease mouse model. *Molecular therapy nucleic acids* 2019; **17**: 829-839.
175. Shin JW, Kim KH, Chao MJ, Atwal RS, Gillis T, MacDonald ME, Gusella JF, Lee JM. Permanent inactivation of Huntington's disease mutation by personalized allele-specific CRISPR/Cas9. *Human molecular genetics* 2016; **25**(20): 4566-4576.
176. Ruan GX, Barry E, Yu D, Lukason M, Cheng SH, Scaria A. Scaria. CRISPR/Cas9-mediated genome editing as a

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[DOI: 10.61186/ibj.3898]

[DOI: 10.61186/ibj.3898]
[DOR: 20.1001.1.1028852.2023.27.5.1.7]
[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

therapeutic approach for leber congenital amaurosis 10. *Molecular therapy* 2017; **25**(2): 331-341.
177. Li P, Kleinstiver BP, Leon MY, Prew MS, Navarro-Gomez D, Greenwald SH, Pierce EA, Joung JK, Liu Q. Allele-specific CRISPR-Cas9 genome editing of the single-base P23H mutation for rhodopsin-associated dominant retinitis pigmentosa. *The CRISPR journal* 2018; **1**(1): 55-64.
178. Buskin A, Zhu L, Chichagova V, Basu B, Mozaffari-Jovin S, Dolan D, Droop A, Collin J, Bronstein R, Mehrotra S, Farkas M, Hilgen G, White K, Pan KT, Treumann A, Hallam D, Bialas K, Chung G, Mellough C, Ding Y, Krasnogor N, Przyborski S, Zwolinski S, Al-Aama J, Alharthi S, Xu Y, Wheway G, Szymanska K, McKibbin M, Inglehearn CF, Elliott DJ, Lindsay S, Ali RR, Steel DH, Armstrong L, Sernagor E, Urlaub H, Pierce E, Lührmann R, Grellscheid SN, Johnson CA, Lako M. Disrupted alternative splicing for genes implicated in splicing and ciliogenesis causes PRPF31 retinitis pigmentosa. *Nature communications* 2018; **9**(1): 4234.
179. Tsai YT, Wu WH, Lee TT, Wu WP, Xu CH, Park KS, Cui X, Justus S, Lin CS, Jauregui R, Su PY, Tsang SH. Clustered regularly interspaced short palindromic repeats-based genome surgery for the treatment of autosomal dominant retinitis pigmentosa. *Ophthalmology* 2018; **125**(9): 1421-1430.
180. Liu Z, Torresilla C, Xiao Y, Nguyen PT, Caté C, Barbosa K, Rassart E, Cen S, Bouragault S, Barbeau B. HIV-1 antisense protein of different clades induces autophagy and associates with the autophagy factor p62. *Journal of virology* 2019; **93**(2): e01757-e01757.
181. Chen B. Molecular mechanism of HIV-1 entry. *Trends in microbiology* 2019; **27**(10): 878-891.
182. Klasse PJ. The molecular basis of HIV entry. *Cell microbiology* 2012; **14**(8): 1183-1192.
183. Cho SW, Kim S, Kim JM, Kim JS. Targeted genome engineering in human cells with the Cas9 RNA-guided endonuclease. *Nature biotechnology* 2013; **31**(3): 230-232.
184. Hou P, Chen S, Wang S, Yu X, Chen Y, Jiang M, Zhuang K, Ho W, Hou W, Huang J, Guo D. Genome editing of CXCR4 by CRISPR/cas9 confers cells resistant to HIV-1 infection. *Scientific reports* 2015; **5**: 15577.
185. Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders JF, Meijer CJLM, International Agency of Research on Cancer Multicancer Cervical Cancer Study Group. Epidemiologic classification of human papillomavirus types associated with cervical cancer. *The new England journal of medicine* 2003; **348**(6): 518-527.
186. Zur Hausen H. Papillomaviruses in the causation of human cancers - a brief historical account. *Virology* 2009; **384**(2): 260-265.
187. Münger K, Baldwin A, Edwards KM, Hayakawa H, Nguyen CL, Owens M, Grace M, Huh K. Mhumans-induced oncogenesis. *Journal of virology* 2004; **78**(21): 11451-11460.
188. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Human Papillomaviruses. Lyon (FR): International Agency for Research on Cancer; 2007. (IARC Monographs on the Evaluation of Carcinogenic Risks to Humans, No. 90.) Available from: https://www.ncbi.nlm.nih.gov/books/NBK321760/.
189. Inturi R, Jemth P. CRISPR/Cas9-based inactivation of human papillomavirus oncogenes E6 or E7 induces senescence in cervical cancer cells. *Virology* 2021; **562**: 92-102.
190. Zhen S, Hua L, Takahashi Y, Narita S, Liu YH, Li Y. In vitro and block of human papillomavirus 16-positive cervical cancer cells by CRISPR/Cas9. B*iochemical and biophysical research communications* 2014; **450**(4): 1422-1426.
191. Block TM, Rawat S, Brosgart CL. Chronic hepatitis B: A wave of new therapies on the horizon. *Antiviral research* 2015; **121**: 69-81.
192. Zhen S, Hua L, Liu YH, Gao LC, Fu J, Wan DY, Dong LH, Song HF, Gao X. Harnessing the clustered regularly interspaced short palindromic repeat (CRISPR)/ CRISPR-associated Cas9 system to disrupt the hepatitis B virus. *Gene therapy* 2015; **22**(5): 404-412.
193. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, Zhao X, Huang B, Shi W, Lu R, Niu P, Zhan F, Ma X, Wang D, Xu W, Wu G, Gao G, Tan W, China Novel Coronavirus Investigating and Research Team. A novel coronavirus from patients with pneumonia in China, 2019. New journal of medicine 2020; **382**(8): 727-733.
194. Feng W, Zong W, Wang F, Ju S. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2): A review. *Molecular cancer* 2020; **19**(1): 1001.
195. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, Miao X, Streithorst JA, Granados A, Sotomayor-Gonzalez A, Zorn K, Gopez A, Hsu E, Gu W, Miller S, Pan CY, Guevara H, Wadford DA, Chen JS, Chiu CY. CRISPR-Cas12-based detection of SARS-CoV-2. *Nature biotechnology* 2022; **38**(7): 870-874.
196. Ding X, Yin K, Li Z, Liu C. All-in-one dual CRISPR-Cas12a (AIOD-CRISPR) assay: a case for rapid, ultrasensitive and visual detection of novel coronavirus SARS-CoV-2 and HIV virus. *BioRxiv* 2020; 21: 2020.03.19.998724.
197. Rauch JN, Valois E, Solley SC, Braig F, Lach RS, Audouard M, Ponce-Rojas JC, Costello MS, Baxter N, Kosik KS, Arias C, Acosta-Alvear D, Wilson MZ. A scalable, Eeasy-to-deploy protocol for Cas13-based detection of SARS-CoV-2 genetic material. *Journal of clinical microbiology* 2021; **59**(4): e02402-e024020.
198. Azhar M, Phutela R, Kumar M, Ansari AH, Rauthan R, Gulari S, Sharma N, Sinha D, Sharma S, Singh S, Acharya S, Sarkar S, Paul D, Kathpalia P, Aich M, Sehgal P, Singhal K, Lad H, Patra PK, Makharia G, Chandak GR, Pesala B, Chakraborty D, Maiti S. Rapid, field-deployable nucleobase detection and identification using FnCas9. *Biosens Bioelectron* 2021; **183**:113207.
199. Yoshimi K, Takeshita K, Yamayoshi S, Shibumura S, Yamauchi Y, Yamamoto M, Yotsuyanagi H, Kawaoka Y, Nashimo T. Rapid and accurate detection of novel

[DOI: 10.61186/ibj.3898] [DOR: 20.1001.1.1028852.2023.27.5.1.7] [Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

coronavirus SARS-CoV-2 using CRISPR-Cas3. *MedRxiv* 2020; doi: 0.1016/j.isci.2022.103830.

200. Briggs EM, Rojas F, McCulloch R, Matthews KR, Otto TD. Single-cell transcriptomic analysis of bloodstream Trypanosoma brucei reconstructs cell cycle progression and developmental quorum sensing. *Nature communication* 2021; **12**(1): 5268.
201. Zhang WW, Karmakar S, Gannavaram S, Dey R, Lypaczewski P, Ismail N, Siddiqui A, Simonyan V, Oliveira F, Coutinho-Abreu IV, DeSouza-Vieira T, Meneses C, Oristian J, Serafim TD, Musa A, Nakamura R, Saljoughian N, Volpedo G, Satoskar M, Satoskar S, Dagur PK, McCoy JP, Kamhawi S, Valenzuela JG, Hamano S, Satoskar AR, Matlashewski G, Nakhasi HL. A second generation leishmanization vaccine with a markerless attenuated Leishmania major strain using CRISPR gene editing. *Nature communication* 2020; **11**(1): 3461.
202. Chomicz L, Conn DB, Szaflik JP, Szostakowska B. Newly emerging parasitic threats for human health: national and international trends. *Biomed research international* 2016; **2016**: 4283270.
203. Talapko J, Škrlec I, Alebić T, Jukić M, Včev A. Malaria: the past and the present. *Microorganisms* 2019; **7**(6): 179.
204. Ghorbal M, Gorman M, MacPherson CR, Martins RM, Scherf A, Lopez-Rubio JJ. Genome editing in the human malaria parasite Plasmodium falciparum using the CRISPR-Cas9 system. *Nature biotechnology* 2014; **32**(8): 819-821.
205. Gantz VM, Jasinskiene N, Tatarenkova O, Fazekas A, Macias VM, Bier E, James AA. Highly efficient Cas9-mediated gene drive for population modification of the malaria vector mosquito Anopheles stephensi. *Proceedings of the National Academy of Sciences of the United States of America* 2015; **112**(49): E6736-E6743.
206. Xiao B, Yin S, Hu Y, Sun M, Wei J, Huang Z, Wen Y, Dai X, Chen H, Mu J, Cui L, Jiang L. Epigenetic editing by CRISPR/dCas9 in Plasmodium falciparum. *Proceedings of the National Academy of Sciences of the United States of America* 2019; **116**(1): 255-260.
207. Zhang C, Li Z, Cui H, Jiang Y, Yang Z, Wang X, Gao H, Liu C, Zhang S, Su XZ, Yuan J. Systematic CRISPR-Cas9-mediated modifications of Plasmodium yoelii ApiAP2 genes reveal functional insights into parasite development. *MBio* 2017; **8**(6): e01986-17.
208. Sonoiki E, Ng CL, Lee MCS, Guo D, Zhang YK, Zhou Y, Alley MRK, Ahyong V, Sanz LM, Lafuente-Monasterio MJ, Dong C, Schupp PG, Gut J, Legac J, Cooper RA, Gamo FJ, DeRisi J, Freund YR, Fidock DA, Rosenthal P. A potent antimalarial benzoxaborole targets a Plasmodium falciparum cleavage and polyadenylation specificity factor homologue. *Nature communication* 2017; **8**: 14574.
209. Dukhovny A, Lamkiewicz K, Chen Q, Fricke M, Jabrane-Ferrat N, Marz M, Jung JU, Sklan EH. A CRISPR activation screen identifies genes that protect against zika virus infection. *Journal of virology* 2019; **93**(16): e00211-e00219.
210. Volpedo G, Pacheco-Fernandez T, Holcomb EA, Zhang WW, Lypaczewski P, Cox B, Fultz R, Mishan C, Verma C, Huston RH, Wharton AR, Dey R, Karmakar S, Oghumu S, Hamano S, Gannavaram S, Nakhasi HL, Matlashewski G, Satoskar AR. Centrin-deficient Leishmania mexicana confers protection against New World cutaneous leishmaniasis. *NPJ vaccines* 2022; **7**(1): 32.
211. Sollelis L, Ghorbal M, Macpherson CR, Martins RM, Kuk N, Crobu L, Bastien P, Scherf A, Lopez-Rubio JJ, Sterkers Y. First efficient CRISPR-Cas9-mediated genome editing in Leishmania parasites. *Cellular microbiology* 2015; **17**(10): 1405-1412.
212. Zhang WW, Matlashewski G. CRISPR-Cas9-mediated genome editing in Leishmania donovani. *MBio* 2015; 6(4): e00861.
213. Beneke T, Madden R, Makin L, Valli J, Sunter J, Gluenz E. A CRISPR Cas9 high-throughput genome editing toolkit for kinetoplastids. *Royal society open science* 2017; **4**(5): 170095.
214. Grewal J, Catta-Preta C, Brown E, Anand J, Mottram JC. Evaluation of clan CD C11 peptidase PNT1 and other Leishmania mexicana cysteine peptidases as potential drug targets. *Biochimie* 2019; **166**: 150-160.
215. Damasceno JD, Reis-Cunha J, Crouch K, Beraldi D, Lapsley C, Tosi LRO, Bartholomeu D, McCulloch R. Conditional knockout of RAD51-related genes in Leishmania major reveals a critical role for homologous recombination during genome replication. *PLoS genetics* 2020; **16**(7): e1008828.
216. Turra G, Schneider L, Liedgens L; Deponte M. Testing the CRISPR-Cas9 and glmS ribozyme systems in Leishmania tarentolae. *Molecular and Biochemical parasitology* 2021; **241**: 111336.
217. Martel D, Beneke T, Gluenz E, Späth GF, Rachidi N. Characterisation of casein kinase 1.1 in Leishmania donovani using the CRISPR Cas9 toolkit. *Biomed research international* 2017; **2017**: 4635605.
218. Adaui V, Kröber-Boncardo C, Brinker C, Zirpel H, Sellau J, Arévalo J, Dujardin JC, Clos J. Application of crispr/cas9-based reverse genetics in leishmania braziliensis: Conserved roles for hsp100 and hsp23. *Genes (Basel)* 2020; **11**(10): 1159.
219. Peng D, Kurup SP, Yao PY, Minning TA, Tarleton RL. CRISPR-Cas9-mediated single-gene and gene family disruption in Trypanosoma cruzi. *MBio* 2014; **6**(1): e02097-14.
220. Lander N, Li ZH, Niyogi S, Docampo R. CRISPR/Cas9-induced disruption of paraflagellar rod protein 1 and 2 genes in Trypanosoma cruzi reveals their role in flagellar attachment. *MBio* 2015; **6**(4): e01012.
221. Dong S, Lin J, Held NL, Clem RJ, Passarelli AL, Franz AWE. Heritable CRISPR/Cas9-mediated genome editing in the yellow fever mosquito, Aedes aegypti. *PLoS one* 2015; **10**(3): e0122353.
222. Kistler KE, Vosshall LB, Matthews BJ. Genome engineering with CRISPR-Cas9 in the mosquito Aedes aegypti. *Cell reports* 2015; **11**(1): 51-60.

223. Dueñas E, Nakamoto JA, Cabrera-Sosa L, Huaihua P, Cruz M, Arévalo J, Milón P, Adaui V. Novel CRISPR-based detection of Leishmania species. *Frontiers in microbiology* 2022; **13**: 958693.
224. Lee RA, De Puig H, Nguyen PQ, Angenent-Mari NM, Donghia NM, McGee JP, Dvorin JD, Klapperich CM, Pollock NR, Collins JJ. Ultrasensitive CRISPR-based diagnostic for field-applicable detection of Plasmodium species in symptomatic and asymptomatic malaria. *Proceedings of the National Academy of Sciences of the United States of America* 2020; **117**(41): 25722-25731.
225. Kudyba HM, Cobb DW, Florentin A, Krakowiak M, Muralidharan V. CRISPR/Cas9 gene editing to make conditional mutants of human malaria parasite P. falciparum. *Journal of visualized experiments*; **18**(139): 57747.
226. Kuang D, Qiao J, Li Z, Wang W, Xia H, Jiang L, Dai J, Fang Q, Dai X. Tagging to endogenous genes of Plasmodium falciparum using CRISPR/Cas9. *Parasites and vectors* 2017; **10**(1): 595
227. Zhang X, Deitsch KW, Dzikowski R. CRISPR-Cas9 editing of the Plasmodium falciparum genome: special applications. *Methods in molecular biology* 2022; **2470**: 241-253.
228. Lander N, Chiurillo MA, Storey M, Vercesi AE, Docampo R. CRISPR/Cas9-mediated endogenous C-terminal tagging of Trypanosoma cruzi genes reveals the acidocalcisome localization of the inositol 1,4,5-trisphosphate receptor. *Journal of biological chemistry*; **291**(49): 25505-25515.
229. Mehta A, Merkel OM. Immunogenicity of Cas9 protein. *Journal of pharmaceutical sciences* 2020; **109**(1): 62-67.
230. Crudele JM, Chamberlain JS. Cas9 immunity creates challenges for CRISPR gene editing therapies. *Nature communication* 2018; **9**(1): 3497.

[Downloaded from ibj.pasteur.ac.ir on 2026-05-18]

[DOR: 20.1001.1.1028852.2023.27.5.1.7]

[DOI: 10.61186/ibj.3898]