CRISPR/Cas System Toward the Development of Next-Generation Recombinant Vaccines: Current Scenario and Future Prospects

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Abstract
The initially developed vaccines were relying mostly on attenuation and inactivation of pathogens. The use of recombinant DNA technology allows the targeting of immune responses focused against a few protective antigens. The conventional recombination methods for generating vaccines are time-consuming, laborious, and less efficient. To overcome these limitations, a new precise CRISPR/Cas9 with high efficacy, specificity, and low-cost properties has solved a lot of current problems of recombinant vaccines that intrigued the inspiration for novel recombinant vaccine development. CRISPR/Cas9 system was discovered as a bacterial adaptive immune system. In the domain of virology, CRISPR/Cas9 is used to engineer the virus genome to understand the fundamentals of viral pathogenesis, gene therapy, and virus–host interactions. One step ahead CRISPR/Cas9 bypassed the vaccine to precisely engineer the B-cells to secrete the specific antibodies against deadly viral pathogens. There is a critical literature review gap especially in the use of CRISPR/Cas9 to generate recombinant vaccines against viral diseases and its prospective application to engineering the B-cells in immunocompromised people. This review height the application of CRISPR/Cas9 compared to conventional approaches for the development of recombinant vaccine vectors, editing the genes of B-cells, and challenges that need to be overcome. The factors affecting CRISPR/Cas9-edited recombinant vaccines and prospects in the context of viral genome editing for the development of vaccines will be discussed.

Keywords CRISPR/Cas9 · Virus · Gene editing · Recombinant · Vaccines · B-cells engineering · Antigens · Antibodies

1 Introduction
Vaccination has prevented hundreds of millions of deaths since 1796 when Edward Jenner injected a boy with cowpox to inhibit smallpox. The development of the vaccination method has a key role to create immunity against various infectious diseases in humans and animals. Our world has to get rid of smallpox and rinderpest fatal diseases with help of vaccination and immunization. The traditional vaccination methods, inoculation of the live attenuated pathogen which is capable of eliciting the immune response, may offer potential risks, such as virulence in susceptible hosts and potential reversal of attenuation. As an alternative, the inoculation of vaccines made through recombinant DNA technology has become an important and innovative strategies due to better antibody response to fight against viral disease. Compared with conventional vaccines made by using attenuated pathogens, recombinant vaccines have various benefits that include improved stimulation of immune responses, limited side effects due to the correct use of immunogenic subunits, long-term persistence of immunogenicity, and cheap large-scale production [1]. The conventional recombination methods for generating the recombinant vaccines such as bacterial artificial chromosomes (BAC), Cosmid, and gene cloning with restriction endonucleases were time-consuming, laborious, and less efficient, the in-site gene insertion/replacement was random, the restriction endonuclease fails to generate the recombinant vaccines if the genome size is more than 30 KB [2]. The latest developed RNA-guided gene editing technology Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR/Cas9) is currently being used to overcome the limitations of conventional recombination methods. CRISPR/Cas9 is low cost and more efficient [3] and has...
changed the whole scenario of the production of fast recombinant vaccines. CRISPR/Cas9 is a highly precise, simple, and efficient genome editing technology compared to conventional recombination methods; CRISPR can efficiently insert the larger gene fragments even more than one antigen gene simultaneously to generate multivalent vaccines with long-lasting antigen expression. Despite the decades of research still no vaccines against viruses that kill tens of millions of people every year: HIV, respiratory syncytial virus, and the cancer-causing Epstein–Barr virus. One of the most important reasons is hard to identify the antigen which can trigger the immune cells to produce effective antibodies. And the other issue is that vaccines do not work against immunocompromised people such as people under treatment for cancers and organ-transplanted patients [4]. CRISPR is to develop a long-time persistent immunity through the development of multivalent recombinant vaccines and direct engineering of the B cells to produce specific antibodies against current and future viral diseases and pandemics. CRISPR was first time testified in human and mammalian cells in 2013 [5], and now it has become one of the most effective, diverse, and less costly novel strategy for genome editing, especially for the generation of recombinant vaccines.

Reference [6] CRISPR-Cas9 has a wide range of applications in medicines [7–9]; currently, it is being expanded to research on various recombinant vaccines targeting bacteria [10], viruses [11], yeast [12], animal cells [13] and plants [14]. There is a lot of research undergoing on CRISPR application to develop recombinant vaccines, but there is a critical literature review gap especially in the use of CRISPR/Cas9 to generate the recombinant vaccines against viral diseases and its prospective application to engineer the B-cells in immunocompromised people. In this review article (Fig. 1), we will focus on CRISPR/Cas9 working principles, its applications for the development of recombinant vaccines vectors, engineering B-cells for antibodies production, and we will also highlight the challenges that need to be overcome for further streamlining their usage in the prevention and control of viral pathogens.

2 Working Principle of CRISPR/Cas9 System

CRISPR was first identified as a prokaryotic immune system that prevents the cells by selectively targeting and destroying foreign DNA such as plasmids or viruses [5]. This gene-editing strategy can knock in (KI), knock out (KO), and knock down (KD) the expression of any gene of any species with high efficiency and specificity. The functional system of CRISPR/Cas9 consists of guide RNA (gRNA) and Cas9 nuclease that cuts the DNA. The gRNA instructs the Cas9 nuclease to attach to the targeted sequence upstream of the Protospacer adjacent motif (PAM), 5′-NGG-3′, and then activate the double-strand break (DSB) activity of the Cas9 nuclease. The sequence of PAM varies according to Cas nuclease enzyme variants (Table 1). During the working mechanism (Fig. 2), the gRNAs determine the specificity of the target DNA through sequence complementary, and the Cas9 generates the double-strand break (DSB) at 3 base pair upstream of PAM. The DSB triggers the cellular DNA repair mechanisms, non-homolog end joining (NHEJ) that leads
Table 1 Cas nucleases with their PAM sequences

| Cas nucleases | PAM (5′–3′)      | References |
|---------------|------------------|------------|
| Cas9          | NGG              | [15]       |
| SaCas9        | NNGRRT,NNNRRT    | [16, 17]   |
| xCas9         | NG,GAA,GAT       | [18]       |
| Sniper-Cas9   | NGG              | [19]       |
| HypaCas9      | NGG              | [20]       |
| StCas9        | NNAGAAW          | [21]       |
| evoCas9       | NGG              | [22]       |

to gene disruption of gene knock-out (KO) or homology-directed repair (HDR) that leads to gene-knock-In(KI). When a foreign sequence (donor DNA template) is introduced into the first cell stage with Cas9 and gRNA, the HDR pathway activates and repairs the DSB by recruiting donor DNA as a template that leads to the correction of gene mutations [23]. When DSB occurs in the DNA of cells, the repair of DSB is usually carried by NHEJ which is a robust, error-prone, predominant, and fast DNA repair pathway. This pathway is stable throughout the cell cycle. Another parallel pathway is HDR, which is only active in the S and G2 phases of the cell cycle. Both NHEJ and HDR pathways are involved in the “core complexes” that recognize the broken regions of DNA and their “unique processing factors” that heal the DSB [24]. In NHEJ, the Ku protein recognizes the broken region or blunt ends of DNA and recruits the DNA-dependent protein kinases (DNA-PKs) at DSB sites that initiate the processing factors, Artemis, that remove overhang at the DSB sites, DNA polymerase and ligases sequentially to fill and seal the gaps [25, 26]. The HDR pathway is a faithful DNA repair pathway as compared to NHEJ. That requires the sister chromatids as a template in the S or G2 phases to repair the DNA. The core complex proteins are Ataxia telangiectasia-mutated (ATM) phosphorylate the H2AX. The DNA damage checkpoint 1 protein (MDC1) binds with the gamma H2AX at the DSB sites or DNA broken regions. The MRX types of protein stabilize the DSB sites by preventing breakage. After getting the stabilization, the 5′ exonuclease activity is carried out by C-terminal binding protein (CtIP) that triggers the Rad51 which works with BRCA1 and BRCA2 to look for template or sister chromatids to repair the DSB. In most cases, both NHEJ and HDR remain active during the cell cycle. The HDR pathway is less efficient compared to NHEJ [24]. In recent studies, the researcher identified that the addition of an exogenous insertion template directly activates the HDR pathways that increase the possibility of homologous recombination to reverse the point mutations or insertions of larger DNA fragments. In recent studies, researchers have enhanced the HDR pathways selection in DNA repairs through the addition of homologous recombination proteins such as recombination protein a (Rad1, Fanconi anemia core complex (fa), and tumor-suppressor p53 or by inhibition of NEHEJ factors by chemicals or genetic Ku mutations and shRNA-mediating silencing [27–29].

With controlled, precise, and variable targeting gRNA regions, the CRISPR/Cas9 gene editing technology has revolutionized the genome editing applications in mammalian, vertebrate, plant, and viral cells for multiple purposes.

Fig. 2 Working mechanism of CRISPR/Cas9 technology. The figure has been created with BioRender.com
3 Engineering the Viruses Genome Through CRISPR/Cas9

Since the inception of CRISPR/Cas9, it used to target the viral genome for multiple purposes, to determine the virulence factors, virus–host interaction, antiviral therapy, and vaccine vector developments. The CRISPR/Cas9 system edited and modulated the genome of the human immunodeficiency virus (HIV) to stop its replication in the host cells. The HIV-1 long terminal repeats serve as a promoter to drive the replication and integration of viral genome to host genomes. Editing the LTR regions provides a therapeutic strategy to reduce the replication of HIV in the host cells. In 2013, the CRISPR/Cas9 tool was used for the first time to edit or knock out (KO) the LTR regions to suppress the HIV replication process in the Jurkat cells, immortalized line of human T lymphocytes cells. The gRNA expression vector was designed under the control of the U6 promoter. Two target sites T5 and T6 selected to edit in LTR regions of the HIV-1 virus. This strategy significantly reduced the expression and replication of HIV-1 in the host cell lines, and it also removed and blocked the expression of latently integrated HIV-1 provirus from the host genomes. The researchers used modified Cas nuclease, Staphylococcus aureus (SaCas9), with 8 tested gRNAs to target replicative factors of HIV-1 in latent infected Jurkat C11 cell lines that showed a significant reduction in the replication and production of HIV in the host cell lines with no any off-target effects [30]. The TRIM5α is a ring-type protein secreted by the TRIM5 gene in the cytoplasm and cytosol of human cells that act as retrovirus restriction factors. It is observed and studied that TRIM5α in humans has limited efficacy against HIV-1 as compared to TRIM5α of the rhesus macaque. As a comparative single-nucleotide polymorphism (SNP) basis, researchers observed that there is only one alternation from arginine to proline at the 332 points of protein in TRIM5α of the rhesus macaque. Based on this, a theoretical model was proposed and published to stop HIV-1 in human cells (Fig. 3). Theoretically, they hypothetically proposed that through substitution of point mutation (arginine to proline) in the TRIM5α of humans by saCas9/base editor could change the function of TRIM5α like rhesus TRIM5α to make it highly protective/restrictive against HIV-1 [31].

Hepatitis is inflammation of the liver caused by hepatitis viruses A, B, and C. Hepatitis virus A is a short-term infection compared to B and C, which causes a long-term chronic infection. The vaccine is available commercially against Hepatitis B but not C due to its high genetic heterogeneity and variability. The detail is explained in [22]. Recently, CRISPR has been employed to edit and restrict both types of B and C. In the first study, the humanized liver in mice was generated through the intrasplenic human hepatocyte transplant method followed by HBV infection (Fig. 4). The saCas9 was selected due to the smallest size to deliver the gRNA and Cas9 in a single vector, AAV. Two specific and highly efficient gRNA were selected to target the plus and minus strands of consensus sequences of HBV. This AAV-SaCas9-based delivery showed a higher editing efficiency in the HBV+ human hepatocytes as compared to controlled, without editing hepatocytes. The AAV-SaCas9 editing efficiency has increased the survival of human hepatocytes as compared to the control [32].

A new Cas nuclease variant, Cas13a, targets the RNA used to knock down (KD) the expression of HCV. The seven gRNA selected specifically target the IRES-conserved regions. The Cas13a significantly decreased the expression of HCV in vitro [33], which could be used in future as a better therapeutic strategy against HBV and HCV viruses.
3.1 Targeting the RNA

Since the COVID-19 pandemic, the CRISPR/Cas9 technology was used to target RNA viruses to knock down (KD) their replication in host cells. Multiple types of Cas nucleases have been identified that target the single-strand RNAs (ssRNAs) and even double-strand RNAs viruses [34]. The Cas9 endonucleases from *Francisella novicida* reported they can edit both + ssRNA and –ssRNA with low off-target editing [35]. Cas13 nucleases can target a wide range of ssRNA. The bioinformatics analyses showed more than 300 Cas13 variants in the mammalian genome. Cas13 has a potential activity to target the viruses such as influenza A virus and COVID-19 virus. In the PAC-MAN (prophylactic antiviral CRISPR in human cells) (Fig. 5), the antiviral strategy has been developed based on Cas13d which inhibited the COVID-19 viral replication in the human lung epithelial cells. For Cas13, single CRISPR RNA (crRNA) is required to target the host RNA. They discovered that six crRNAs can circumvent higher than 90% of all coronaviruses in the human epithelial cells [36].

4 CRISPR/Cas9, A New Tool for the Development of Recombinant Vaccines

4.1 CRISPR-Cas Gene Editing to Develop the Recombinant Vaccine Vectors

Herpesvirus of turkey (HVT) is commercially used as a vaccine vector against multiple viral diseases by introducing a particular antigen due to its high growth capacity in the cell lines. Marek disease is a common viral disease of chickens caused by herpes viruses. For 40 years, the a-virulence strain, herpesvirus of turkey (HVT) is used as a live vector as a virus vaccine against Marek disease. The live vector was limited by the low immunogenic response [37]. Conventional recombination methods such as Cosmid and bacterial artificial chromosome (BAC) clones have been used to develop HVT vector-based recombinant vaccines against multiple viral diseases mentioned. In 2016, CRISPR/Cas9 was used to create the targeted mutation in the genes (gB, gI, gE) of HVT. That targeted mutation enhanced its immunogenic property as a vaccine vector. The CRISPR/Cas9 was used to generate the HVT vector-based recombinant vaccine against infectious bursal disease (IBD) by knock-in of VP2 gene cassette at UL45/UL-56 locus of HVT vector genome. The red fluorescence protein (RFP) is used as a selection marker. Results demonstrated that the CRISPR/Cas9 strategy is more convenient and efficient to generate the recombinant HVT vector vaccine compared to conventional techniques with no off-target effects [38]. The same group extended their research by inserting triple genes with CRISPR/Cas9 knock-in strategy and generated the multivalent recombinant vaccine against multiple avian pathogens. The gDgI and H9N2 expression cassettes were introduced sequentially into the already HVT-VP2 generated vectors that resulted in multivariate recombinant HVT vector vaccine, HVT-VP2-gDgI-HA9N2 against major avian viral poultry disease. Both inserted cassettes were stable after 15 passages and were confirmed by both immunostaining and PCR [39]. Further, the researcher employed the HDR-CRISPR/Cas9 method to introduce the H9N2-HA gene at the UL45/UL46 locus of the HVT-BAC genome and generated the bivalent vaccine vector as a vaccine against avian influenza viruses (Fig. 6). In this study, they used the green fluorescence protein (GFP)-fused cassette as a selection marker for the mutants. To optimize the screening, erythrocytes binding selection was done. The result suggested that the rHVT-H9-edited vaccine recombinant vector provides long-term protection against the avian virus in chicken [40].

Infectious laryngotracheitis virus (ILTv) is a widely adopted vaccine vector due to its low pathogenicity. The CRISPR/Cas9 combined with the Cre-lox system employed for simultaneous deletion of the virulence factors such as thymidine kinase (TK) and unique short 4(US4) genes and inserted the fusion (F) gene of Newcastle diseases virus...
to generate the ILTV vector-based recombinant multivalent vaccine. This strategy was highly successful against Newcastle diseases virus (NDV) with no off-target effects [42]. Conventional gene recombination methods have been employed to generate the recombinant duck enteritis virus (DEV)-based vector vaccine against the influenza virus. The CRISPR/Cas9 with Cre-lox system was used to develop the recombinant bivalent DEV-based vaccine vector by inserting the hemagglutinin (HA) antigen gene of Avian influenza virus at UL26/UL27 locus of DEV genome with stable expression. They proved that CRISPR/Cas9 with Cre-lox is an effective technique for prompt development of DEV-AIV recombinant vaccine [44]. Another group has generated the multivalent DEV vaccine vector against avian influenza virus and duck Tembusu virus (DTV) infections by sequential insertion of antigen genes, hemagglutinin (HA) for avian influenza, pre-membrane proteins (PrM), and glycoprotein (E) for duck Tembusu virus. That strategy resulted in the generation of DEV-HA/PrM-E, a trivalent DEV-recombinant
vaccine vector that showed long-lasting antigen expression [45].

Herpes simplex virus type 1 (HSV-1) is a highly epidemic pathogen. In a few studies, editing therapeutics anti-cancerous genes by replacing the non-essential in HSV-1 virus has been demonstrated and under clinical trials [46, 47]. A robust efficient gene editing/replacement developed through CRISPR/Cas9 gene knock-in HDR by inhibition of NHEJ DNA repair system by the addition of inhibitor, SCR7, which enhanced the immunogenicity and showed stable expression of the vaccine vector [48]. The vaccinia virus-based vectors are widely used in cancer immunotherapy and also against many other viral diseases. A single-step CRISPR/Cas9-based new strategy with a simple pipeline has been developed, MAVERICK (marker-free vaccinia virus engineering of recombinants through in vitro CRISPR/Cas9 cleavage) to create the pure recombinant vaccines vectors with zero off-target effects. They have used this pipeline (Fig. 7) to edit, delete, and replace multiple loci of the vaccinia virus. MAVERICK is an ideal choice to generate future recombinant viral vaccines [43, 49]. In the last couple of decades, T4 bacteriophage was employed as a genetic vector to transfer the foreign gene to generate recombinant DNA for multiple purposes. The T4 bacteriophage contains the Soc (small outer capsid proteins) and Hoc (highly integrin outer capsid proteins). The Soc and Hoc sites of T4 bacteriophages were used as excellent adopters to adopt the foreign viral proteins to T4 capsid. The T4 bacteriophage can be employed to generate multivalent vaccines as candidates against emerging pathogens. Recently, researchers generated the recombinant phage containing the multiple vaccines through CRISPR engineering against SARS-CoV2 by insertion of spike, envelope, and nucleocapsid targets genes into the T4 phage through. This alternative vaccine strategy was tested in rats and rabbits. That strategy generated a robust immune response by triggering the T cells that block/neutralize/complete protection against viral infection [50, 51].

4.2 Gene Editing in B Cells to Produce Specific Antibodies

Since the inception of the vaccination and immunization concept, it remains more effective against the deadly viral disease. But still, despite considerable research, the vaccines are not available for some deadly viral pathogens, respiratory syncytial virus (RSV), human immune deficiency virus (HIV), influenza, and Epstein–Barr virus (EBV). These viral diseases are prevented through the infusion of monoclonal antibody-mediated protection. But the critical issue with the antibody-based vaccine is that the antibody gets depleted within a short time. The monthly reinfusion is required to maintain long-term protection against RSV, HIV, and EBV. To overcome reinfusion, the researcher has edited the B cells with a specific antibody gene against SRV, HIV, and influenza in mice and human cell lines. The long-lasting antigenic expression was observed after editing the B cells. The antibodies are generated through the synergistic effect of the heavy chain with VDJ genes and light chain. The emAb cassette (Fig. 8) contains a light chain, and a partially heavy chain (VDJ) inserted into the intronic region of the B cells genome through CRISPR/Cas9. This strategy generates persistent desirable antibodies against SRV, HIV, and influenza. It could adopt for the development of future vaccines against emerging deadly viruses [52]. But editing the B-cells would be limited by different factors such as immune rejection, antigenic drift, and off-target effects. Transplanted edited B-cells usually face immune rejection in the human body to overcome this; the B-cells would have to engineer on an individual basis, but it would highly expensive technique. To overcome the immune rejection, CRISPR/Cas9 editing would have to be done in universal donor B-cells [53, 54]. Such cells would not be rejected by host immune cells. Viruses often mutate very fast, usually leading to antigenic drift like many variants of SARS-CoV-2 emerged; to overcome this, a swarm of different antigen-engineered B cells could produce through a multi-genic CRISPR/Cas9-based knock-in strategy. The last concern would be off-target effects, which can be optimized through the usage of different Cas-nuclease variants [54].

5 Challenges in the Development of Recombinant Vaccines

The safety issue of replication-competent vaccines compared to the killed or subunit vaccines has become a subject of discussion. The attenuated live vaccines are delivered at relatively lower doses but exert high efficacy. In contrast, viral delivery systems with replication-deficient vectors such as DNA and RNA virus vectors have high transduction efficiency but are generally considered safer; however, replication deficiency poses a major drawback. To overcome this problem, the development of recombinant vaccines was initiated. However, it also poses inherent issues of controllability and stability that are resolved using the CRISPR Cas9 strategy [55]. This technology specifically targets the genome with high-editing efficiency facilitating quick and easy gene targeting and screening of recombinant viruses. The DNA repair induced by CRISPR-Cas9 seems to be quick, effective, and controllable, with recombination stability and fidelity in the case of multisite editing [56].

5.1 Effectiveness

However, several factors have been identified affecting the gene-editing insertion efficiencies at the targeted loci.
Improvement of editing efficiency was achieved by the implementation of dual or multiple gRNAs; however, the CRISPR-Cas9 editing with the expanding repertoire of CRISPR-Cas endonucleases has become the most efficacious system [57]. Using this technology, the cutting efficiency of Cas9 can be increased up to 90%, while the efficiency of homology direct DNA repair pathway could be between 1 and 7%. However, one of the major drawbacks is that a large proportion of the viral genome cannot be efficiently repaired, which might result in the inhibition of viral replication. The CRISPR-Cas9 system potentially inhibits the wild-type virus genome multiplication, which subsequently led to a greater percentage of recombinant progeny viruses [58].

5.2 Off-Target Effects

The unwanted result of CRISPR-Cas9 genome editing has also been the subject of much research. Another major concern with CRISPR-Cas9 technology is potential off-target mutations [59], which can have serious consequences like disruption in the function or regulation of non-targeted genes. Furthermore, relatively larger structural changes in the genome sequence at the intended on-target editing site pose another cause of concern. However, within viral genome editing, low off-target effects can be anticipated because of the smaller genome size of viruses. These studies suggest that the vaccines developed using recombinant technology and CRISPR-Cas9 are efficient and safer than the attenuated or killed vaccine [60, 61].

6 Conclusion and Future Prospectus

Latest advances in CRISPR/Cas9 have enabled targeted gene modification such as insertion, deletions, replacement, and recombination in the virus genome to determine the virus pathogenicity and virus–host interactions at the molecular level. CRISPR/Cas9 gene-editing technology has shown high editing efficiency in many viral genome editing cases compared to conventional techniques. CRISPR/Cas9 can edit multiple sites simultaneously in the viral genome to generate
multivalent recombinant vaccines for particular highly infectious strains. Many bioinformatics tools have been developed already, which could be used to analyze the gRNA hits to eliminate the off-target effects. Safety and predictability of virus attenuation could be further enhanced through multiple knockouts of virulence factors and multiple knock-ins of therapeutic insertions to develop refinement in the vaccine development. The immunogenicity of recombinant viral vector-based vaccines can be optimized by regulating the promoters’ elements of viruses. The CRISPR/Cas9 technology is a long period is required to test recombinant vaccines at the laboratory level; a long period is required to observe the avian cell lines through CRISPR/Cas9 has been approved and funded by the Bill and Melinda Gates foundation. However, a long period is required to observe the editing efficiency and working stability of CRISPR-produced recombinant vaccines. With high specificity and stability, CRISPR/Cas9 is emerging as an innovative method in the progress of both science and technology of vaccine production for future pandemics. Despite the lot of merit of CRISPR/Cas9, there are still rooms for improvement needed in the generation of recombinant vaccines. Still, there is a need to evaluate the CRISPR/Cas9 biosafety implications of off-target effects. In recent studies, the researcher has developed stimulus-based smart nanoparticles for controlled delivery of CRISPR/Cas9 to target specific cell sites [62]. These can be employed to resolve the controllability issues. The stability and off-target effects issues can be resolved through recently discovered Cas variants with fewer off-target effects [60]. Still, there is a need for molecular assays to detect and analyze/quantify the relation between the CRISPR/Cas9 editing speed and virus genome replication discrepancy. Still, the application of CRISPR/Cas9 in recombinant vaccine generation is at the laboratory level; a long period is required to test recombinant vaccines passage stability and efficiency. Certainly, the site-specific CRISPR/Cas9 gene technology is providing endless possibilities and will promote the more rapid development of a recombinant vaccine against mammalian viral diseases, especially against future pandemics. Together with modern technologies, next-generation sequencing, and artificial intelligence the CRISPR/Cas9 will continue to improve its safety and other limitations.

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