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Revolutionary world of genome editing through CRISPR/Cas technology: Review

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Abstract
CRISPR/ Cas (Clustered Regularly Interspaced Short Palindromic Repeats– CRISPR associated system) is an adaptive immune system that offers immunity to most prokaryotic organisms by targeting foreign DNA from invading viruses and plasmids. Because of its simplicity, the Cas9 protein from *Streptococcus pyogenes* was first employed, most popular and widely acknowledged genome-editing tool till date. Cas9 single-protein nucleases can be reprogrammed to target other DNA locations by altering a portion of their guide RNA (gRNA). It entails designing a Cas9/sgRNA complex to target the DNA of interest and delivering it to the cell. Once a genomic target that is sufficiently complementary is identified, the complex splits the DNA into its two strands, causing DSBs (Double Stranded Breaks) which can be repaired by non-homologous end joining, introducing indels (sequence specific deletion/insertion), and leading to frame shift mutations. CRISPR-based technologies increase the speed and accuracy of viral and bacterial nucleic acid detection. Using recombinase polymerase amplification, viral or bacterial sequences can be amplified from clinical samples in the first stage (RPA). CRISPR/Cas9 could help accelerate vaccine development in response to rising infectious diseases. The human angiotensin-converting enzyme II (hACE2) was utilized to study the transmission and pathogenesis of SARS-CoV-2 and it was a useful tool for evaluating COVID-19 vaccines and therapeutic chemicals. The researchers used the Cas9 knock-in technique to produce mice models expressing hACE2.

Keywords: CRISPR/ Cas, genome editing, DNA, antiviral, COVID-19

Introduction
CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats- CRISPR associated system) is an adaptive immune system that provides immunity to the majority of prokaryotic organisms by focusing on foreign DNA from plasmids and viruses that are invading their cells. 90% of archaea and 50% of bacteria are responsible for its encoding. From microorganisms to mammals, CRISPR has been employed to alter the genomes of a variety of animals.

The discovery of CRISPR/Cas system goes back to the time in 1987, when Yoshizumi Ishino and his associates at Osaka University unintentionally cloned a collection of interrupted clustered repetitive sequences while studying the iap gene (isozyme conversion of alkaline phosphatase) from the Escherichia coli genome (Ishino *et al.*, 2018) [26]. However, the purpose of these arrays could not be determined at the time due to a lack of DNA sequence data. CRISPRs were named after Francisco Mojica and Ruud Jansen, who uncovered similar sequences in a variety of bacterial and archaeal genomes (Morange *et al* 2015) [37]. Researchers from three distinct groups, Pourcel *et al.*, 2005 [40], Mojica *et al.*, 2005 [36], and Bolotin *et al.*, 2005 [8], confirmed that CRISPR spacers were created from viral and extra-chromosomal origins. Alexander Bolotin discovered the PAM and Cas9 genes in *Streptococcus themophillus*, and Moineau *et al.*, 2010 [58] revealed that CRISPR-Cas9 caused double stranded breaks (DSBs) in target DNA at precise locations. In 2011, Emmanuelle used small RNA sequencing on Streptococcus pyogenes and revealed that there is a second small RNA termed tracrRNA that exists in addition to cr RNA. It creates a duplex with crRNA, which directs Cas9 to its destination. Mali *et al.*, and Cong *et al.*, modified type II *Streptococcus pyogenes* Cas9 (SpCas9) in mammalian cells from genome editing in 2013.

The CRISPR Cas9 system holds great promise for the creation of novel diagnostics and treatments for a variety of viral illnesses (Bayat *et al*., 2018) [7]. An innovative antiviral drug for HIV patients has been created using CRISPR-Cas technology (Huang *et al*., 2017) [25]. The medicine was deemed safe despite the fact that the patient did not experience a cure right away because no side effects were reported during the 19-month follow-up period (Xu *et al*., 2019) [52].

Additionally, the CRISPR-Cas system was employed to treat blindness and cancer (Lin *et al*., 2019; Akram *et al*., 2020) [32, 3]. This technique was being applied to offer a rapid and reliable diagnosis for SARS-CoV-2 identification in clinical samples. CRISPR technology has been used to diagnose illnesses and find microbes, as well as to detect RNA viruses such the Vesicular Stomatitis virus, Influenza A virus, and Lymphocytic Choriomeningitis virus (Chertow, 2018; Freije *et al*., 2019; Quan *et al*., 2019) [14, 19, 41]. In order to save the lives of millions of people who are at risk of contracting COVID-19, this review article focuses on the effects and potential applications of CRISPR-Cas biology in the creation of accurate, specific, and sensitive COVID-19 diagnostics as well as a potentially safe, secure, and potent antiviral therapy. However, in addition to its prospective uses, clinical applications also need to be determined after regulatory authorization.

CRISPR Cas System components

The CRISPR/Cas system consists of a CRISPR array and cas genes that are arranged in an operon and it is illustrated in Fig 1. A palindromic repeat sequence with 21-48 nucleotides is followed by a variable spacer sequence with 26-73 nucleotides connected to an A-T rich ladder sequence in a CRISPR array (Doudna *et al*., 2014) [15]. The usual length of the spacers in CRISPR arrays is 32 to 38 bp (Barrangou *et al*., 2014) [6]. The short DNA sequence known as the protospacer adjacent motif (PAM) domain, situated near to an RNA-binding site, has been identified as a crucial element in Cas9's capacity to discriminate between self and non-self DNA. Guide RNA (gRNA) is a pre-designed RNA sequence that aids Cas9 genes in precise DNA sequence cutting. Tractor RNA (tracrRNA) is a guide for processing pre-cr-RNA and is essential for directing Cas9 to target locations and changing Cas9 from an inhibited to an active state (Tu *et al*., 2015) [49].

CRISPR-Cas System Classification

CRISPR-Cas System can be classified into two classes i.e. Class I and Class II depending upon the difference in their effector molecules (Table 1)

The presence of effector molecules with several subunits distinguishes the Class I CRISPR-Cas system. It is found in 90% of archaea and bacteria, and depending on the type, it can target both DNA and RNA. It is further classified into three categories based on the presence of Cas endonucleases in the system (Type I, III, IV). Multiple cas genes are encoded by one or more operons in the Type I system. Six proteins (E.coli-Cse1, Cse2, Cas7, Cas5, Cas6e) are included, as well as the Cas3 protein (a large protein that typically consists of fused helicase and HD nuclease domains). The mature crRNA is combined with these proteins to create CASCADE (CRISPR associated complex for anti-viral defence), which binds to invading foreign DNA and encourages pairing of the crRNA with the complementary strand of exogenous DNA to form a R loop. It also recruits Cas3 to cleave both complementary and non-complementary DNA strands. Similar to the type I system, the type III system has Cas 10 as a hallmark protein with RNAse activity and CASADE. It can detect cleavage of both DNA and RNA. Type IV is a hypothetical class about which little is known. It does not have any cleavage or spacer-insertion domains.

The presence of a single effector molecule distinguishes the Class II CRISPR-Cas system. Unlike class I, it is only found in bacteria and can target both DNA and RNA. It is further classified into three categories based on the presence of Cas endonucleases in the system (Type II, V, VI). Type II CRISPR-Cas is the most studied, simple, and compact CRISPR-Cas system. Cas9, as well as the auxiliary proteins cas1 and cas2, and RNAse III, play a role in crRNA processing, as well as identifying and cleaving target DNA in the CRISPR–cas system. Six domains make up this protein: REC I (largest domain), REC II, Bridge Helix (arginine-rich bridge), PAM Interacting domain (conveys PAM specificity and binding to target DNA), HNH and RuvC (nuclease domains responsible for cleaving complementary and non-complementary strands of R-loop, respectively), and PAM Interacting domain (Nishimasu *et al*., 2014, Anders *et al*., 2014; Jinek *et al*., 2014; Sternberg *et al*., 2014) [38, 4, 27, 47]. Cas12 is the endonuclease in Type V systems, and it targets DNA for editing. Type V systems, like type II systems, need a tracrRNA to function. Type VI class II system targets RNA for editing using Cas13 endonuclease.

The CRISPR-Cas immune system

Integration of a short fragment of foreign DNA into the CRISPR locus of the host genome is the initial step in the CRISPR-Cas system's cascade of events that leads to protection against the invader (K. S. Makarova *et al*., 2006) [34].

Adaptation phase, also known as spacer acquisition, is the first step in the CRISPR–cas system's general defense mechanism. This is followed by the Expression phase (crRNA processing) and finally the Interference phase (Fig 2) (Rath *et al*., 2015) [43].

The selection and production of a proto spacer, followed by integration of the spacer into the CRISPR array and synthesis of a new repeat, make up the two phases of the adaptation process. Cas1 and Cas2 nucleases are crucial for spacer integration because they get short pieces of DNA termed proto-spacers from invading plasmids or viruses and integrate them into the CRISPR array, where the newly obtained piece of DNA acts as a memory for future attacks (Rath *et al*., 2015) [43]. In contrast to the Type I-B system, which needs Cas4, the Type II-A system needs Cas9, Csn2, and tracrRNA for spacer acquisition. In type I, type II, and type V CRISPR-Cas systems, a short region known as the protospacer adjacent motif (PAM) is located precisely next to the protospacer and is essential for acquisition and interference (Fig 2).

The expression step begins with the transcription of the CRISPR array into a protracted precursor crRNA (pre cr-RNA), which is then transformed into mature guide crRNA containing the sequences of encroaching particles (Haurwitz *et al*., 2010) [23]. With the exception of type I-C, where Cas5d takes the place of Cas6, members of the Cas6 family are involved in the processing of crRNA in type I and type III systems. In the Type II system, spacer sequences act as templates to produce short crRNAs that assemble with tracrRNA and direct the Cas9 nuclease to any complementary invading DNA. TracrRNA is therefore necessary for the processing of pre-crRNA. Cpf1 assists in the processing of premature crRNAs and the usage of the processed crRNA to cleave the target DNA in the type V-A CRISPR-Cas system (Fonfara *et al* 2016) [17]. The CRISPR Cas system's interference phase, where mature crRNAs are used to interfere with the nucleic acids of invasive viruses or

plasmids, is the last stage of immunity. In the Class I system, complexes similar to CASADE (CRISPR-associated complex for antiviral defense) are utilized to achieve target degradation, whereas in the Class II system, target interference can be achieved with just one effector protein (Fig 2).

Cas3 nuclease/helicase is recruited by CASADE in the type I system to target and destroy foreign DNA. However, both Type I and Type II systems feature protospacer-crRNA complementarity in the seed region, which prompts "non-self-activation," to prevent the systems from attacking their own CRISPR locus (Semenova *et al.,* 2011, Sternberg *et al.,* 2014) [45, 47].

Cas9 for interference and a dual-crRNA:tracrRNA complex (single-guide RNA or sgRNA) for binding and cutting DNA targets to introduce double-strand breaks (DSBs) are required in the Type II system (Jinek *et al.,* 2012) [28]. Similarly, type V CRISPR-Cas systems require an RNA duplex comprised of tracrRNA and crRNA to cleave the target, whereas type V-A systems only use crRNA to degrade the target (Fonfara *et al.,* 2016, Zetsche *et al.,* 2015) [17, 55]. The target DNA is thereby cleaved, and the infection is thwarted.

The Type III system is composed of the dual-targeting complexes Cas10-Csm (III-A and III-D) and Cas10-Cmr (III-B and III-C). Cas 10 cleaves DNA in type III-A and type III-B CRISPR Cas systems, while Csm3 and Cmr4 cleave transcribed mRNA (Shmakov *et al*., 2015) [46].

Applications of CRISPR Cas9

The Cas9 protein from *Streptococcus pyogenes* was the first genome editing tool used because of its simplicity, and it continues to be the most liked and commonly accepted genome editing tool today. Cas9 single-protein nucleases can be re-programmed to target other DNA locations by altering a portion of their guide RNA (gRNA) (Jinek *et al.,* 2014, Mali *et al.,* 2013) [27, 35]. It is turning into a promising tool for diagnosing various diseases and developing drugs against them. Gene editing, genome imaging, gene regulation, and epigenetic alteration are all possible using the CRISPR Cas9 system (Fig 3).

Gene editing

In order to target the desired DNA, a Cas9/sgRNA complex must be created and delivered to the cell. Once a genomic target that is sufficiently complementary is identified, the complex will split the DNA into its two strands, causing DSBs (Double Stranded Breaks) that can be repaired by non-homologous end joining, which introduces indels (sequence specific deletion/insertion), leading to frame shift mutations. Homologous directed repair is another type of DNA repair that involves the precise insertion or replacement of desired DNA sequences as well as the knock-in of desired genes. (Doudna and colleagues, 2014) [15]

Methods of delivering Cas9-sgRNA complex to eukaryotic cells include Plasmid Transfection, Viral Transfection/Transduction, Protein Transfection, Micro injection, Liposome (Cationic lipids have been used to transfect DNA into cells) and Electroporation (Ran *et al.,* 2013) [42]

Gene regulation by crispr/dcas9 technology

Gene function can be studied by turning gene expression on or off at the transcription level. It can be accomplished in two ways i.e. fusion of dCas9 with the transcription repressor (KRAB), which controls gene transcription, resulting in down regulation (repression/CRISPR interference) and recruiting transcription activators like VP64 to the CRISPR complex, which recruits the RNA polymerase and increases gene expression, thus allows for upregulation (CRISPR activation) (Xiong *et al.,* 2016) [51].

Epigenetic modification

It involves introducing specific changes to genes, such as DNA methylation (gene silencing) and histone acetylation, to modulate gene expression. It works by changing an epigenetic mark at a particular locus to heal hereditary disorders e.g. curing cystic fibrosis (CF) in cultivated intestinal stem cells by addressing the CFTR (cystic fibrosis transmembrane conductance regulator) locus (Schwank G *et al.,* 2013) [44].

Genome imaging

When paired with FISH, CRISPR allows for target-specific, rapid, and convenient genome imaging. In mammalian cells, genome imaging is used to picture non-repeated genomic elements and to capture the dynamics of repetitive genomic loci (telomeres) during the cell cycle. The dCas9 protein is fused with green fluorescent protein in this technique (GFP). It marks the complementary DNA sequence to the sgRNA, resulting in the production of fluorescent signals that may be seen on a screen, allowing for imaging of a specific genomic area targeted by sgRNA. (Xiong *et al.,* 2016) [51]

CRISPR-based immunoprecipitation approach: This technique can be used to investigate DNA-binding proteins and evaluate protein-DNA interactions at certain genomic loci. The dCas9 protein is fused with affinity protein tags, and when the tagged dCas9 is immune-precipitated, proteins bound to a particular genomic region targeted by gRNA are extracted (Foss *et al*., 2019) [18].

Applications in veterinary and biomedical sciences

CRISPR- Cas based diagnostics

A novel diagnostic strategy called SHERLOCK (specific high-sensitivity enzymatic reporter unlocking platform) combines Cas13a cleavage with reverse transcription (RT)-RPA or isothermal recombinase polymerase amplification (RPA) (Fig 4). A crRNA-Cas13a combination binds and cleaves the target nucleic acid in combination with the fluorescent reporter with high specificity, producing a fluorescence signal for pathogen detection (Gootenberg *et al*., 2018) [20]. A similar strategy, DETECTOR (DNA endonuclease-targeted CRISPR trans reporter), combines isothermal RPA with Cas12a enzymatic activity. In this test, the crRNA-Cas12a combination attaches to target DNA and cleaves ssDNA coupled to a fluorescent reporter arbitrarily (Chen *et al*., 2018) [12] (Fig 4).

CRISPR-Cas based therapeutics

Wiskott Aldrich syndrome, sickle cell disease (BCL11a locus), -thalassemia, and HIV infection are only a few of the blood and immune disorders that can be treated with the CRISPR Cas system. Examples of chronic viral infections include the human immunodeficiency virus (HIV), Herpes simplex virus type 1 (HSV I), Epstein-Barr virus (EBV), which increases the risk of developing certain lymphomas and nasopharyngeal cancers, and human cytomegalovirus (HCMV) (CMV). In order to destroy harmful bacteria,

artificial CRISPR arrays have been developed that specifically target genes involved in antibiotic resistance or virulence. This makes CRISPR-Cas a direct antibacterial weapon (BLP). Targeting harmful bacterial strains while allowing non-pathogenic variations to thrive is a clever strategy. Lysogenic phages are used to target antibiotic resistance genes and develop the CRISPR Cas system in *E. coli* (Foss *et al*., 2019) [18].

Specific takeaways for Veterinarians

1) CRISPR can be used to correct genetic flaws and improve certain qualities in animals, particularly companion animals.
2) CRISPR gene editing is a more user-friendly method for producing crops and farm animals with higher disease resistance and productivity as well as improved nutritional qualities.
3) The development of novel vaccines could be sped up by using CRISPR technology to make vaccinations with fewer allergic side effects when prepared in eggs.

CRISPER/CAS9 and emerging diseases

CRISPR-based technologies could improve the speed and precision of detecting bacterial and viral nucleic acids (Yuen *et al*., 2018) [54]. The diagnostic tools like DETECTR (Yang *et al*., 2015) [53] and SHERLOCK (Chen *et al*., 2018) [12] for detecting RNA and DNA sequences, respectively, are comparable. In the first step, viral or bacterial sequences from clinical samples are amplified using recombinase polymerase (RPA). The CRISPR/Cas system is then added to the DNA, allowing it to recognize and cut the desired sequence. The Cas13a for DNA (SHERLOCK) and Cas12a for RNA (DETECTR) are illustrated in the figure (Yang *et al*., 2015) [53]. (2017) (Niu *et al*.). After cleaving their target sites, Cas12a and Cas13a starts cleaving other DNAs or RNAs in the solution. When a quenched reporter sequence is introduced, Cas12a or Cas13a will cleave the reporter sequence, producing a fluorescence signal. If the target sequence is absent from the solution, Cas12a and Cas13a will not be activated, and the reporter sequence will remain quenched. Single copies of viruses can be found by SHERLOCK and DETECTR due to their extreme sensitivity. Furthermore, the 37 degrees Celsius operating temperature of the enzymatic reactions eliminates the need for costly heat cyclers. They are very rapid, finishing in just one to two hours. SHERLOCK and DETECTR are therefore being used as field diagnostic tools for viruses like Ebola, Zika, and Dengue Fever, and there is a lot of interest in doing so (Chertow 2018) [14].

Recently, SARS-CoV-2 detection using DETECTR was published with a 45-minute turnaround time, a 95% positive prediction agreement, and a 100% negative prediction agreement with PCR techniques (Broughton *et al*., 2020) [10]. The sensitivity of Next-Generation Sequencing can also be increased using CRISPR techniques, making it simpler to find low-frequency sequences in clinical samples, such as genes for antibiotic resistance (NGS). In a pre-processing stage, the Depletion of Abundant Sequences to Hybridization (DASH) method uses CRISPR/Cas9 to cleave high-frequency targets that lower NGS sensitivity, like human mitochondrial ribosomal RNA genes (Gu *et al*., 2016) [22]. Once these high-frequency targets have been eliminated, the desired pathogenic sequences can subsequently be amplified quickly. The sensitivity of fungal and parasite sequences retrieved from cerebral fluid samples was greatly increased using this technique (Gu *et al*., 2016) [22]. Another CRISPR-based technique called FLASH (Finding Low Abundance Sequences by Hybridization) employs alkaline phosphatase to block every sequence in the sample. The sequence of interest is then ligated to universal sequencing adapters and partially digested by a CRISPR/Cas system, with low amplification of other sequences in the sample (Quan *et al*., 2019) [41]. Patients with *Staphylococcus aureus* pneumonia's tracheal aspirates were examined for antibiotic resistance genes using FLASH-NGS (Quan *et al*., 2019) [41]. The method produced a 5000-fold enrichment of the target genes over NGS alone. Additionally, it has been used to successfully find genes associated with malaria resistance in dried blood spot samples (Quan *et al*., 2019) [41]. Again, FLASH-NGS had a significantly greater detection sensitivity than NGS alone. It is anticipated that these ground-breaking techniques will be used right away in clinical laboratories. They make a desirable mix because they are not only very sensitive but also swift and reasonably priced (Ai *et al*., 2019) [2]. In contrast to CRISPR therapies, there are also no significant patient risks that need to be considered.

Antiviral therapy

Current antiviral drugs usually fail to combat emerging infectious diseases including SARS, MERS, and COVID-19. On the other hand, creating an effective antiviral medication is a time-consuming process. Therefore, the majority of clinical therapy is supportive and preventative, even if this strategy can have a higher case fatality rate in the absence of specific antivirals. It might have significant effects on early illness prevention and control if scientists could develop methods to eliminate viruses more quickly and precisely. In recent years, professionals have used CRISPR/Cas9 gene-editing technology to treat a range of clinically refractory illnesses (Fig 5). For starters, by focusing on virus-specific areas, the researchers used CRISPR/Cas9 gene editing to specifically destroy the virus to treat the illness. For instance, Edward M. Kennedy used Cas9 to specifically target the HPV E6E7 gene in a cell-free system experiment to treat cervical cancer and eradicate HPV infection (Kennedy *et al*., 2014). Zhen's findings in animal experiments show that Cas9-targeted eradication of covalently closed circular DNA (cccDNA) is effective in the treatment of hepatitis B. (Zhen *et al*., 2015). Additionally, researchers effectively treated Epstein-Barr virus and Cytomegalovirus infections *in vitro* with Cas9 (Ebina *et al*., 2013; Hu *et al*., 2014). Additionally, some researchers have used Cas9 to create T cells that could indirectly eliminate viral infections by treating specific illnesses (Zhao *et al*., 2018). For instance, Cas9 was developed to enhance the CAR-T (Chimeric Antigen Receptor T-Cell Immunotherapy) therapy used to treat cancers that are incurable, such as B-cell leukemias and lymphomas (Labanieh *et al*., 2018). Additionally, numerous *in vivo* studies demonstrate that Cas9 gene editing changes CD4+ T cells to be effective in treating HIV infection, indicating that this could be a unique approach to treating infectious diseases that are resistant to treatment (Ebina *et al*., 2013; Liu *et al*., 2017; Xiao *et al*., 2019). One of the most effective treatments for SARSCoV-2 and other difficult-to-treat emerging infectious diseases is expected to be CRISPR/Cas9 gene editing in the future (Grifoni *et al*., 2020).

Compared to Cas9, gene editing using Cas13-targeted RNA may offer a fresh approach to RNA virus therapy (Freije *et al*., 2019). By precisely focusing on HPV E6E7mRNA, *in vivo* experiments demonstrated that the LshCas13a system prevented the development of human cervical cancer (Chen Y. *et al*., 2020). In addition, Hao Li used the Cas13a Dengue virus NS3 gene cleavage to successfully stop viral replication *in vivo* (Li H. *et al*., 2020). In order to create the PAC-MAN platform (prophylactic antiviral CRISPR in human cells) for viral suppression in human lung epithelial cells, Timothy R. Abbott used Cas13d. This platform is capable of successfully degrading RNA from SARSCoV-2 sequences and live influenza A virus (IAV). This method offers a novel treatment option for the SARS-CoV-2 and influenza viruses, among other things (Abbott *et al*., 2020).

Vaccine development

In addition to the COVID, other developing infectious diseases, causing severe outbreaks and epidemics, call for immunizations in addition to specific antiviral medications for prevention and control. One of the most successful practical and cost-effective public health measure for the prevention and control of infectious diseases is vaccination. On the other hand, creating a vaccine is a time-consuming, difficult, and expensive process. Easy and quick development of specialized vaccine is a challenging process. In response to the escalating rate of infectious diseases, CRISPR/Cas9 could hasten the creation of vaccines. For instance, Mustafa Ozan Atasoy created and used a highly effective and quick NHEJ-CRISPR/Cas9 and Cre-Lox-mediated genome editing approach for the contagious laryngotracheitis virus to simultaneously delete virulence factors and insert antigens to create recombinant, multivalent, and safer vaccine vectors (Atasoy *et al*., 2019) [5]. Homology-directed repair (HDR) CRISPR/Cas9 and erythrocyte binding should be used for the quick synthesis of recombinant turkey herpesvirus-vectored avian influenza virus vaccines (Chang *et al*., 2019). Manuel has used the CRISPR/Cas9 gene-editing method to produce recombinant African swine fever viruses (ASFVs) (Borca *et al*., 2018). Additionally, the creation of a live attenuated vaccination depends on the recombinant ASFV.

The scientists developed mouse models expressing human angiotensin-converting enzyme II (hACE2) using the Cas9 knock-in method, which they utilised to investigate the transmission and pathophysiology of SARS-CoV-2 and give a useful tool for evaluating COVID-19 vaccines and therapy substances (Sun *et al*., 2020).

Limitations of CRISPR/Cas system

Lack of specificity in the CRISPR-Cas9 system: Although the gRNA is designed to cleave a specific sequence in the genome, it may also cut partially homologous sequences.

Off-target effects are possible: sgRNA contains 20 nucleotides, yet DNA binding can still take place when the target sequence is only five nucleotides different.

The development of mosaic animals (cells with two or more genotypes): Only one of the two alleles get edited during MI in zygotes due to a delayed mRNA expression of CRISPR-Cas9. Animal mosaics may not successfully pass on the desired trait.

How to overcome Limitations of CRISPR/Cas system

Increase specificity: A double strand break won't happen until both complimentary strands are identified and cleaved by the Cas9 protein, which has double nickase activity rather than nuclease activity.

Reduce off target effects: When truncated sgRNAs with a length of 17 or 18 nucleotides are used, their specificity will be higher and their off-target effects will be reduced.

Increase efficiency: Increased standardisation and automatization might make ranging less effective. Additionally, this will result in fewer mosaic animals.

Table 1: Classification (Jolany *et al.*, 2020) [59]

Classes	Types	Subtypes	Cas endonucleases	crRNA processing	Target	tracrRNA requirment
Class I	Type I	A-F3	Cas 3	Cas 6	DNA	No
	Type III	IA-F	Cas 10	Cas 6	DNA/RNA	No
	Type IV*	A-C	-	-	-	-
Class II	Type II	A-C	Cas 9	RNAse III	DNA	Yes
	Type V	A-I,U	Cas 12, Cas 14	-	DNA	Yes
	TypeVI	A-D	Cas 13	-	RNA	No

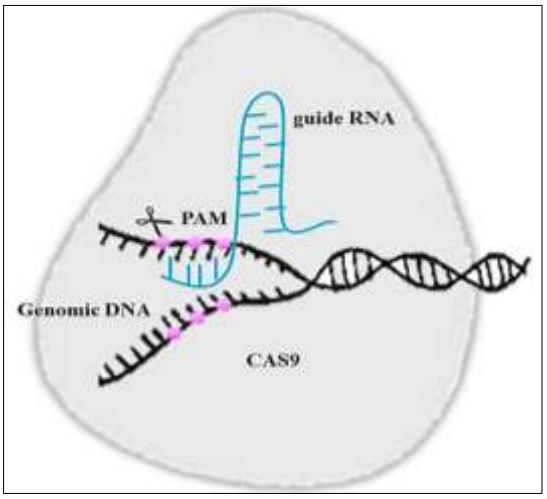

Fig 1: Components CRISPR Cas System

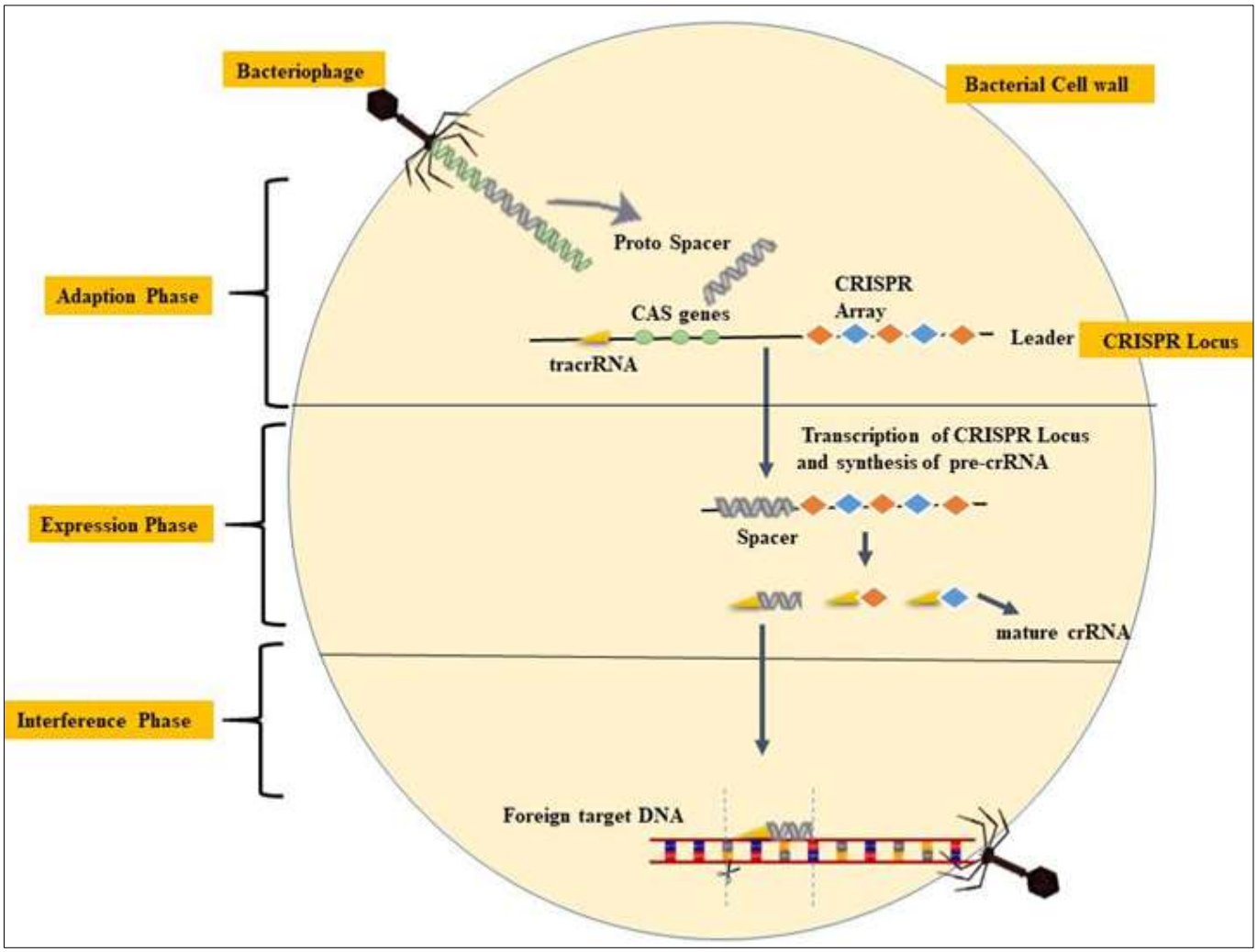

Fig 2: CRISPR–cas system's general defense mechanism

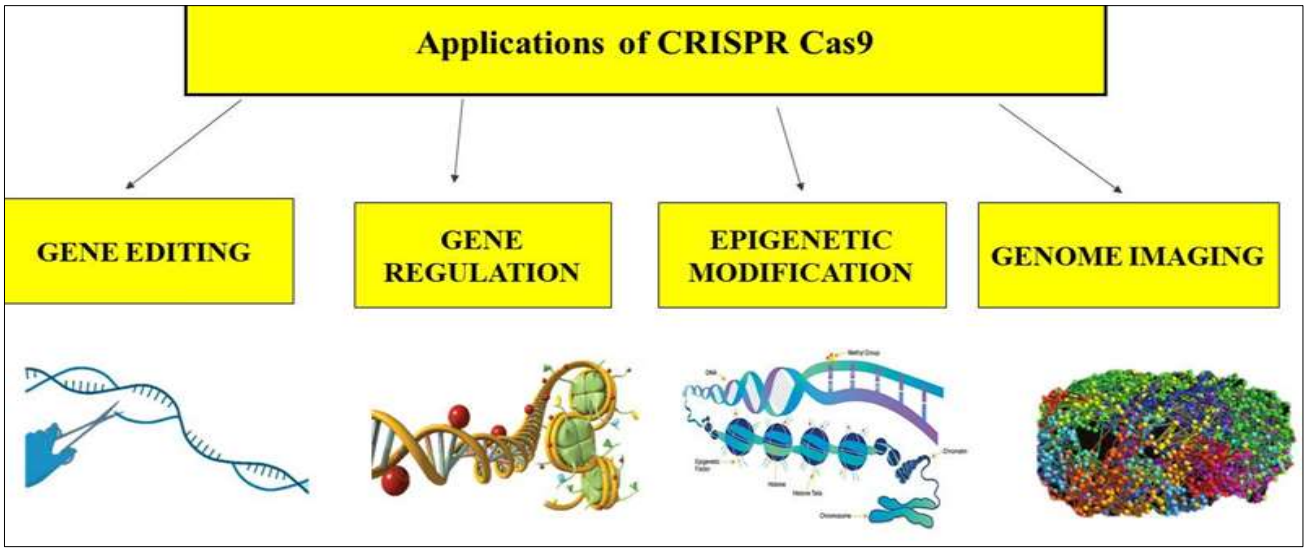

Fig 3: Applications of CRISPR Cas9

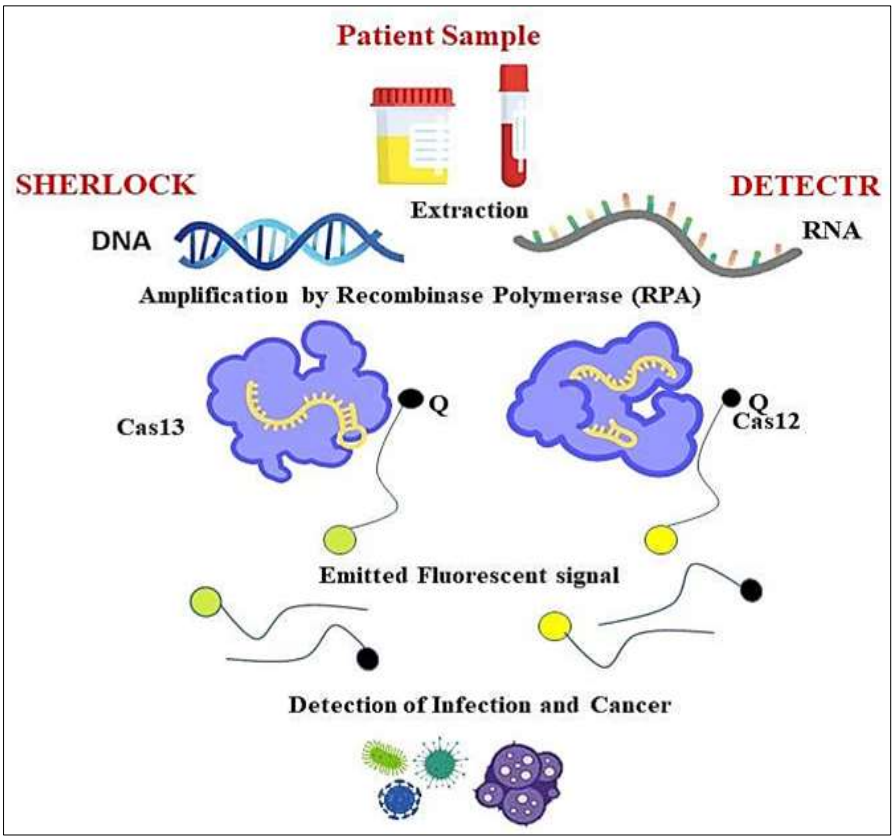

Fig 4: CRISPR- Cas based diagnostics

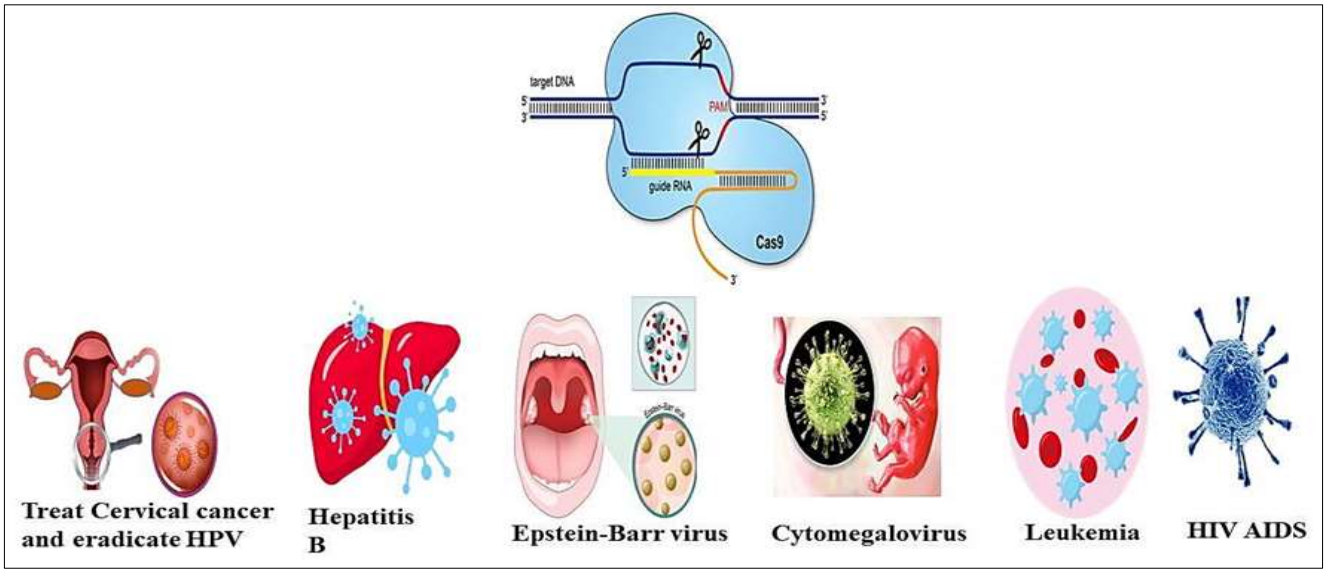

Fig 5: CRISPR/Cas9 gene-editing technology to treat a range of clinically refractory illnesses

References

1. Abbott TR, Dhamdhere G, Liu YX, Lin XQ, Goudy L, Zeng LP, *et al*. Development of CRISPR as an Antiviral Strategy to Combat SARSCov-2 and Influenza. Cell. 2020;181:865-86.
2. Ai J-W, Zhou X, Xu T, Yang M, Chen Y, He GQ, *et al*. CRISPR based rapid and ultra-sensitive diagnostic test for Mycobacterium tuberculosis. Emerging Microbes and Infection. 2019;8:1361–1369.
3. Akram F, Haq I, Ahmed Z, Khan H, Ali MS. CRISPR-Cas9, A promising therapeutic tool for cancer therapy: A review. Protein and Peptide Letters. 2020;27:931-944.
4. Anders C, Niewoehner O, Duerst A, Jinek M. Structural basis of PAM-dependent target DNA recognition by the Cas9 endonuclease. Nature. 2014;513:569-573.
5. Atasoy MO, Rohaim MA, Munir M. Simultaneous Deletion of Virulence Factors and Insertion of Antigens Into the Infectious Laryngotracheitis Virus Using NHEJ-CRISPR/Cas9 and Cre-Lox System for Construction of a Stable Vaccine Vector. Vaccines. 2019;7(4):207.
6. Barrangou R, Marraffini LA. CRISPR-Cas systems: Prokaryotes upgrade to adaptive immunity. Molecular Cell. 2014;54:234-244.
7. Bayat H, Naderi F, Hayat A, Memarnejadin A,

Rahimpour A. The impact of CRISPR-Cas system on antiviral therapy. Advanced Pharmaceutical Bulletin. 2018;8:591-597.
8. Bolotin A, Quinquis B, Sorokin A, Ehrlich SD. Clustered regularly interspaced short palindrome repeats (CRISPRs) have spacers of extra chromosomal origin. Microbiology. 2005;151:2551-2561.
9. Borca MV, Holinka LG, Berggren KA, Gladue DP. CRISPRCas9, a Tool to Efficiently Increase the Development of Recombinant African Swine Fever Viruses. Scientific Reports. 2018;8(1):3154.
10. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, *et al*. CRISPR-Cas12-based detection of SARS-CoV-2. Nature Biotechnology. 2020;38:870-874.
11. Chang PX, Ameen F, Sealy JE, Sadeyen JR, Bhat S, Li YQ, *et al*. Application of HDR-CRISPR/Cas9 and Erythrocyte Binding for Rapid Generation of Recombinant Turkey Herpesvirus-Vectored Avian Influenza Virus Vaccines. Vaccines. 2019;7(4):192.
12. Chen JS, Ma E, Harrington LB, Costa MD, Tian X, Palefsky JM, *et al*. CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity. Science. 2018;360:436-439.
13. Chen Y, Jiang H, Wang T, He D, Tian R, Cui Z, *et al*. *In Vitro* and *in vivo* Growth Inhibition of Human Cervical Cancer Cells Via Human Papillomavirus E6/E7 Mrnas' Cleavage by CRISPR/Cas13a System. Antiviral Research. 2020;178:104794.
14. Chertow DS. Next-generation diagnostics with CRISPR. Science. 2018;360:381-382.
15. Doudna JA, Charpentier E. The new frontier of genome engineering with CRISPR-Cas9. Science. 2014, 346.
16. Ebina H, Misawa N, Kanemura Y. Harnessing the CRISPR/Cas9 System to Disrupt Latent HIV-1 Provirus. Scientific Reports. 2013;3:2510.
17. Fonfara I, Richter H, Bratovic M, Rhun AL, Charpentier E. The CRISPR- associated DNA- cleaving enzyme Cpfl also processes precursor CRISPR RNA. Nature. 2016;532:517-521.
18. Foss DV, Hochstrasser ML, Wilson RC. Clinical applications of CRISPR-based genome editing and diagnostics. Transfusion. 2019. p. 1-11.
19. Freije CA, Myhrvold C, Boehm CK, Lin AE, Welch NL, Carter A, *et al*. Programmable Inhibition and Detection of RNA Viruses Using Cas13. Molecular Cell. 2019;76(5):826-837.
20. Gootenberg JS, Abudayyeh OO, Lee JW, Kellener MJ, Joung J, Collins JJ, *et al*. Multiplex and portable nucleic acids detection platform with Cas13, Cas12a, and Csm6. Science. 2018;360:439-444.
21. Grifoni A, Weiskopf D, Ramirez SI, Mateus J, Dan JM, Moderbacher CR, *et al*. Targets of T Cell Responses to SARS-Cov-2 Coronavirus in Humans With COVID-19 Disease and Unexposed Individuals. Cell. 2020;181(7):1489-1501.
22. Gu W, Crawford ED, O'Donovan BD, Wilson MR, Chow ED, Retallack H, *et al*. Depletion of abundant sequences by hybridization (DASH): using Cas9 to remove unwanted high-abundance species in sequencing libraries and molecular counting applications. Genome Biology. 2016;17:41.
23. Haurwitz R, Jinek M, Wiedenheft B, Zhou K, Doudna J. Sequence- and structure- specific RNA processing by a CRISPR endonuclease. Science. 2010;329:1355-1358.
24. Hu W, Kaminski R, Yang F, Zhang Y, Cosentino L, Li F, *et al*. RNADirected 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;111:11461-11466.
25. Huang Z, Nair M. A CRISPR/Cas9 guidance RNA screen platform for HIV and provirus disruption and HIV/AIDS gene therapy in astrocytes. Scientific Reports, 2017.
26. Ishino Y, Krupovic M, Forterrae P. History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. Journal of Bacteriology. 2018;200:7.
27. Jinek M, Jiang F, Taylor DW, Sternberg SH, Hauer M, Kaya E, *et al*. Structures of Cas9 endonucleases reveal RNA- mediated conformational activation. Science. 2014;343:1247997.
28. Jinek M, Chylinski K, Fonfara I, Hauer M, Doudna JA, charpentier E. A programmable Dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science; c2012. p. 816-821.
29. Kennedy EM, Kornepati AVR, Goldstein M, Bogerd HP, Poling BC, Whisnant AW, *et al*. 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;88:11965-11972.
30. Labanieh L, Majzner RG, Mackall CL. Programming CAR-T Cells to Kill Cancer. National Biomedical Engineering. 2018;2;377-391.
31. Li H, Wang S, Dong X, Li Q, Li M, Li J, *et al*. CRISPR-Cas13a Cleavage of Dengue Virus NS3 Gene Efficiently Inhibits Viral Replication. Molecular Therapy Nucleic Acids. 2020;19:1460-1469.
32. Lin H, Deng Q, Li L, Shi L. Application and development of CRISPR/ Cas9 technology in pig research; c2019.
33. Liu Z, Chen S, Jin X, Wang Q, Yang K, Li C, *et al*. Genome Editing of the HIV Co-Receptors CCR5 and CXCR4 by CRISPR-Cas9 Protects CD4 (+) T Cells From HIV- Infection. Cell and Bioscience. 2017;7:47.
34. Makarova KS, Grishin NV, Shabalina SA, Wolf YI, Koonin EV. A putative RNA-interference-based immune system in prokaryotes: computational analysis of the predicted enzymatic machinery, functional analogies with eukaryotic RNAi, and hypothetical mechanisms of action. Biology Direct. 2006;1:7.
35. Mali P, Yang L, Esvelt KM, Aach J, Dicarlo JE, Norville JE, *et al*. RNA- guided human genome engineering via Cas9. Science. 2013;339:823-826.
36. Mojica FJM, Villasenor CD, Martinez JG, Soria E. Intervening Sequences of Regularly Spaced prokaryotic repeats derive from foreign genetic elements. Journal of Molecular Evolution. 2005;60:174-182.
37. Morange M. What history tells us XXXVII. CRISPR-Cas: The discovery of an immune system in prokaryotes. Journal of Biosciences. 2015;40:221-223.
38. Nishimasu H, Ran FA, Hsu PD, Ishitani R, Zhang F, Nureki O. Crystal structure of Cas9 in complexwith guide RNA and target DNA. Cell. 2014;156:935-949.
39. Niu D, Wei HJ, Lin L, George H, Wang T, Zhao HY, *et al*. Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9. Science; c2017.

40. Pourcel C, Salvignol G, Vergnaud G. CRISPR elements in Yersinia pestis acquire new repeats by preferential uptake of bacteriophage DNA, and provide additional tools for evolutionary studies. Microbiology. 2005;151:653-663.
41. Quan J, Langelier C, Kuchta A, Batson J, Teyssier N, Lyden A, *et al*. FLASH: a next-generation CRISPR diagnostic for multiplexed detection of antimicrobial resistance sequences. Nucleic Acids Research. 2019;47:e83.
42. Ran FA, Hsu P, Wright J, Agarwala V, Scott D, Zhang F. Genome engineering using the CRISPR- Cas9 system. Nature Protocols; c2013. p. 2281-2308.
43. Rath D, Amlinger L, Rath A, Lundgren M. The CRISPR-Cas immune system: biology, mechanisms and applications. Biochemistry. 2015;28:117-119.
44. Schwank G, Koo BK, Sasselli V, Dekkers JF, Boymnas S, Cuppen E, *et al*. Functional repair of CFTR by CRISPR/Cas9 in intestinal stem cell organoids of cystic fibrosis patients. Cell Stem Cell; c2013. p. 653-658.
45. Semenova E, Jore MM, Datsenko KA, Westra ER, Wanner B, Oost JVD, *et al*. 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;108:10098-10103.
46. Shamkov S, Abudayyeh OO, Makarova KS, Joung J, Minakin L, Konermann S, *et al*. Discovery and functional characterization of diverse class 2 CRISPR- Cas systems. Molecular Cell. 2015;60:385-397.
47. Sternberg SH, Doudana JA. Expanding the Biologist's toolkit with CRISPR-Cas9. Molecular Cell; c2014. p. 568-574.
48. Sun SH, Chen Q, Gu HJ, Yang G, Wang YX, Huang XY, *et al*. A Mouse Model of SARS-Cov-2 Infection and Pathogenesis. Cell Host Microbe. 2020;28(1):124-133.
49. Tu Z, Yan S, Guo X, Li XJ. CRISPR/Cas9: a powerful genetic engineering tool for establishing large animal models of neurodegenerative diseases. Molecular Neurodegeneration ; c2015.
50. Xiao Q, Chen S, Wang Q, Liu Z, Liu S, Deng H, *et al*. CCR5 Editing by Staphylococcus Aureus Cas9 in Human Primary CD4 (+) T Cells and Hematopoietic Stem/Progenitor Cells Promotes HIV-1 Resistance and CD4 (+) T Cell Enrichment in Humanized Mice. Retrovirology. 2019;16:15.
51. Xiong X, Chen M, Lim WA, Zhao D, Qi LS. CRISPR/Cas9 for Human Genome Engineering and Disease Research. Annual Review of Genomics and Human Genetics. 2016;17:131-154.
52. Xu L, Wang J, Xie L, Liu Y, Liu T, Wamng T, *et al*. CRISPR-Edited Stem cells in a patient with HIV and Acute Lymphocytic Leukemia. The New England Journal of Medicine. 2019;381:1240-1247.
53. Yang L, Güell M, Niu D, George H, Lesha E, Grishin D, *et al*. Genome-wide inactivation of porcine endogenous retroviruses (PERVs). Science. 2015;350:1101-1104.
54. Yuen KS, Wang ZM, Wong NHM, Zhang ZQ, Cheng TF, Lui WY, *et al*. Suppression of Epstein-Barr virus DNA load in latently infected nasopharyngeal carcinoma cells by CRISPR/Cas9. Virus Research. 2018;244:296-303.
55. Zetsche B, Gootenberg JS, Abudayyeh OO, Slaymaker IM, Makarova KS, Essletzbichler Volz S, *et al*. Cpf1 is a single RNA- guided endonucleases of a class 2 CRISPR-Cas system. Cell. 2015;163:759-771.
56. Zhao JJ, Lin QD, Song YP, Liu DL. Universal Cars, Universal T Cells, and Universal CAR T Cells. J. Of Hematology and Oncology. 2018;11(1):132.
57. Zhen S, Hua L, Liu YH, Gao LC, Fu J, Wan DY, *et al*. Harnessing the Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)/ CRISPR-Associated Cas9 System to Disrupt the Hepatitis B Virus. Gene Therapy. 2015;22:404-412.
58. Garneau JE, Dupuis MÈ, Villion M, Romero DA, Barrangou R, Moineau S. The CRISPR/Cas bacterial immune system cleaves bacteriophage and plasmid DNA. Nature. 2010 Nov 4;468(7320):67-71.
59. Jolany Vangah S, Katalani C, Boone HA, Hajizade A, Sijercic A, Ahmadian G. CRISPR-Based Diagnosis of Infectious and Noninfectious Diseases. Biol. Proced; c2020. Online. 22, 22. doi:10.1186/s12575-020-00135-3