Review Article

CRISPR-Cas9: A cutting-edge genome-editing technology with many potential applications

ABSTRACT

Unregulated human activities are responsible for climate change which is a major factor for the emergence of new infectious (bacterial, viral, fungus, and parasitic) and non-infectious diseases (genetic disorders, cancers, etc.). To tackle this situation, we must have better therapeutics, diagnostics, and vaccines for the treatment and prevention of emerging diseases in humans and animals. To address the aforementioned issues, CRISPR-Cas9, a revolutionary genome editing tool, can help us develop better therapeutics, diagnostics, and vaccines in a short time. In addition, it can be used to achieve food security by improving productivity, adaptability, and resilience traits in plants and animals. In nature, most bacteria and archaea have CRISPR as a part of their adaptive immune system that helps bacteria defend themselves from phages, viruses, and other foreign genetic elements. CRISPR-Cas9 is a highly effective, simple, and accurate genome editing tool compared to previous genome editing tools such as meganucleases, Zinc finger nucleases (ZFNs), and transcription activators like effector nucleases (TALENs). This review article discusses the different components and working of the CRISPR-Cas9 system, and how CRISPR-Cas9 plays a significant role in the development of next-generation therapeutics, diagnostics, vaccine platforms, and improved crops and livestock species for food security.decrease medical as well as financial burden, hence improving the management of cirrhotic patients. These predictors, however, need further work to validate reliability.

Keywords: CRISPR-Cas9; diagnostics; food security; genome-editing; therapeutics; vaccines.

1. INTRODUCTION

The term “Genetic Scissors” is used for CRISPR-Cas9 system. On October 7, 2020, Emmanuelle Charpentier, a microbiologist and director of the Berlin-based Max Planck Unit for the Science of Pathogens, and Jennifer A. Doudna, a biochemist at the University of California, Berkeley, were awarded with Noble Prize in the field of chemistry for the discovery of CRISPR-Cas9. CRISPR stands for “Clustered Regularly Interspaced Short Palindromic Repeats”. In nature, most bacteria and archaea have CRISPR as a part of their adaptive immune system that helps bacteria defend themselves from phages, viruses, and other foreign genetic elements[1]. It was the year 1987, when a Japanese scientist, Yoshizumi Ishino, and his coworkers found the CRISPRs in the genome of *Escherichia coli* for the first time while they were looking for the gene responsible for the conversion of alkaline phosphatase. Initially, it was thought that these help in DNA repair mechanisms in thermophilic archaea and bacteria due lack of sufficient information related the bacterial genetics. Over the period, many such repeated sequences were identified in several other bacteria and archaea. Later on, Mojica and coworkers in early 2000 reported that spacer sequences in CRISPR-array were homologous to sequences in bacteriophages, viruses,

and plasmids [2]. Francisco Mojica and Ruud Jansen were the first to refer to them as CRISPRs [3].

CRISPR is the short identical DNA repeats present in the CRISPR loci of bacteria and archaeal genomes. CRISPR loci on the bacterial genome consist of a CRISPR array consisting of hundreds of palindromic repeats (35-45 bases) interspaced by unique spacers (30-40 bases) acquired from bacteriophages or foreign plasmids [4]. CRISPR system involves RNA-guided site-specific binding of Cas (CRISPR-associated) proteins to cleave DNA or RNA targets. CRISPR-Cas9 system can target and cleave double-stranded DNA [5]. CRISPR is highly specific, flexible, and simple, if compared with initial gene-editing tools such as meganucleases, Zinc finger nucleases (ZFNs), and transcription activators like effector nucleases (TALENs) [6]. The technique of editing genes using CRISPR-Cas9 gained prominence in the year 2012 when George Church, Jennifer Doudna, Emmanuelle Charpentier, and Feng Zhang used it to alter targeted regions in the genome. CRISPR-Cas9 genome editing technology has revolutionized genome editing with wide applications such treatment of genetic disorders and infectious diseases, the development of next-generation diagnostics, and the development of efficient vaccine platforms.
In addition to that, CRISPR-Cas9 genome editing technology can help to achieve sustainable development goals by resolving the glaring issues of human and animal health, environmental damage, and social inequality [7]. By harnessing the capabilities of CRISPR, we can achieve food security by enhancing crop yields, developing disease-resistant and climate-resilient varieties of crops, and developing highly productive animal and poultry breeds [8]. Additionally, CRISPR technology can contribute to environmental conservation by mitigating climate change impacts and protecting biodiversity [9]. This review summarizes the working principle of the CRISPR-Cas9 system and its applications in different fields of science including animal science.

2. CRISPR-CAS9 SYSTEM AND ITS WORKING

Based on the structure and function of Cas-proteins, CRISPR/Cas system can be classified into two classes: Class I and Class II. Further, the class I CRISPR/Cas system is divided into type I, III, and IV, whereas the class II CRISPR/Cas system is divided into type II, V, and VI [10]. Class I systems use multiple Cas-protein effectors, whereas class II systems use a single Cas-protein effector [11]. Because of the structural and functional simplicity of the type II CRISPR/Cas-9 system, it has been extensively investigated and employed in genetic engineering [10]. To differentiate between the self and non-self genetic material different CRISPR/Cas systems use different strategies. Type I, II, and V CRISPR/Cas systems recognize protospacer adjacent motif (PAM) on the target DNA to differentiate between the self and non-self, whereas type III CRISPR/Cas system uses CRISPR repeats to differentiate between the self and non-self target [12].

CRISPR is a part of a prokaryote's adaptive immune system which helps the prokaryote protect itself from phages, viruses, and other foreign genetic elements. Briefly, the CRISPR locus is comprised of a CRISPR array that contains short 30–40 base pairs direct repeats interspaced by short variable DNA sequences (spacers) acquired from previous virus infection (Figure 1). And, CRISPR array is preceded by an AT-rich sequence, called the leader sequence. The leader sequence guides the correct incorporation of the DNA sequence (protospacer) of the invading phage to the CRISPR array with the help of the Cas-1 and Cas-2 complex [13]. The acquisition of a spacer sequence requires recognition of a three-nucleotide protospacer adjacent motif in the virus DNA which is followed by excision and insertion of protospacer in the CRISPR-Cas system by Cas-1 and Cas-2 complex [13]. The DNA of recent phage infection is always incorporated near the leader sequence. Therefore, the relative distance of a spacer sequence from the leader sequence tells the

history of infection [14]. The spacer sequences encode for CRISPR RNAs (crRNAs) which helps in the recognition of the target DNA sequence. The leader sequence is usually flanked by a set of CRISPR-associated genes that encode for Cas proteins. The CRISPR-associated genes are preceded by genes that encode for trans-activating CRISPR RNA (tracrRNA) which serves as a binding scaffold for the Cas proteins [15]. CRISPR RNA and trans-activating CRISPR RNA (duplex) together act as single guide RNA. The bacterial CRISPR-Cas9 defense system works in three stages: (i) adaptation or acquisition of spacer from invading phages/ foreign genetic materials, (ii) biogenesis of guide RNA (CRISPR RNAs and trans-activating CRISPR RNAs) and Cas-9 proteins, and (iii) interference in which gRNA:Cas-9 complex look for the presence of foreign genetic material inside the bacterial cell and cut the invading virus DNA or any other foreign DNA at three nucleotides upstream to the PAM in the target DNA [1].

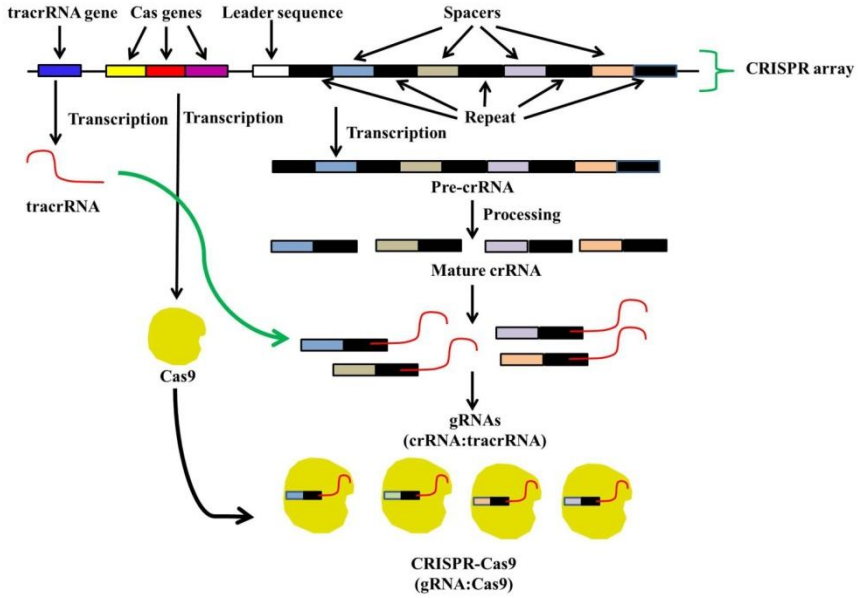

Fig. 1. CRISPR gene locus and formation of CRISPR-Cas9 complex

Since the discovery of the CRISPR-Cas9 system and its mechanism of action as a part of the bacterial adaptive immune system, it has been extensively investigated for genome editing and various other purposes. As aforementioned, the guide RNA (gRNA) and Cas-9 are two essential components of the CRISPR-Cas9 system for its working. The guide RNA (gRNA) and Cas-9 can be synthesized under laboratory conditions and can be introduced directly into the host cells using the appropriate delivery system to perform genome editing. Alternatively, the nucleotide sequences encoding gRNA and Cas-9 protein can be transfected into the host cells by using a plasmid vector. The plasmid vector, then, synthesizes functional gRNA and Cas-9 protein gRNA inside the host cell. Recognition of a specific DNA sequence in the gene of interest is very critical for the accuracy of the CRISPR-Cas9 system. And, this crucial step is performed by gRNA. The gene-specific gRNA can be designed and synthesized to recognize the desired DNA sequence in a gene. The structure of gRNA has two parts; CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) (Figure 2. A). The crRNA part of gRNA is 18–20 nucleotides long and helps in the recognition of the target DNA sequence. Whereas, the tracrRNA part of gRNA consists of a long stretch of loops that serve as a binding scaffold for the Cas9 protein [5].

Cas-9 protein consists of two recognition domains (REC I and II), an arginine-rich bridge helix, two endonuclease domains (HNH and RuvC), and a PAM Interacting domain (Figure 2. B) [16]. The REC domains are responsible for the binding of Cas-9 protein with gRNA (Figure 2. C), whereas the endonuclease domains of Cas-9 protein are responsible for cutting each strand of double-stranded DNA at three nucleotides upstream of the PAM sequence. The PAM interacting domain of Cas-9 protein interacts with the PAM sequence located 2-6 nucleotides downstream of the target DNA sequence. The PAM sequence is required for the binding of Cas-9 protein ontothe complementary strand of target DNA and subsequently creates the double-strand break (DSB) by endonuclease domains of Cas-9 at three nucleotides upstream of the PAM (Figure 3).

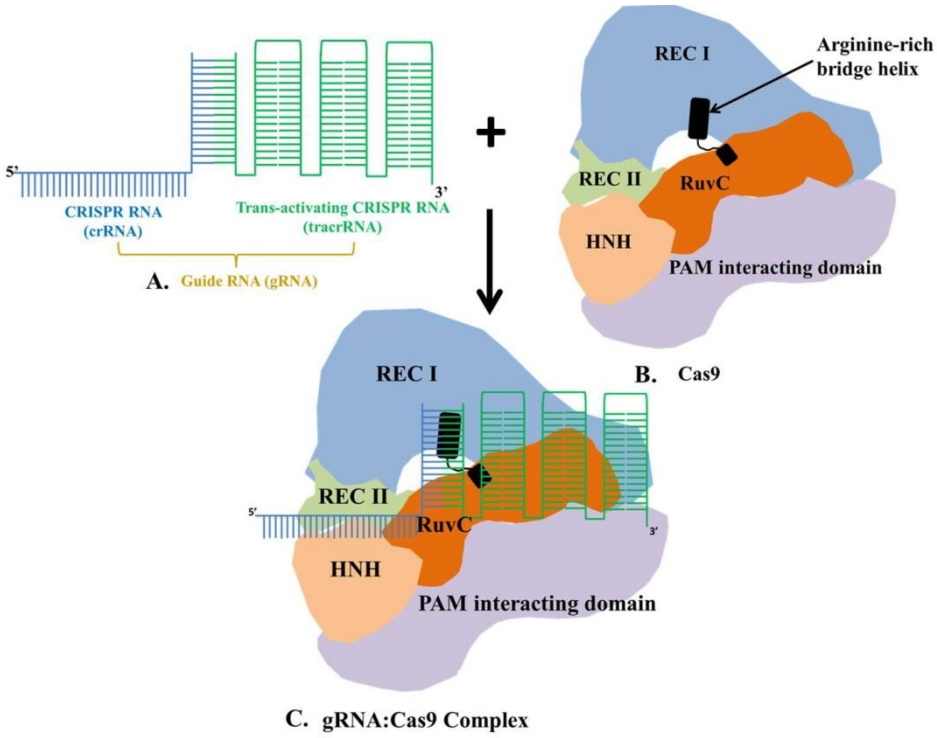

Fig. 2. Structure of CRISPR-Cas9.
A) gRNA consists of crRNA and tracrRNA, B) Cas9 consists of five domains; two recognition domains (REC I and REC II), an arginine-rich bridge helix, two endonuclease domains (HNH and RuvC), and PAM interacting domain, and C) Cas9 protein bind with tracrRNA scaffold to form gRNA:Cas9 complex.

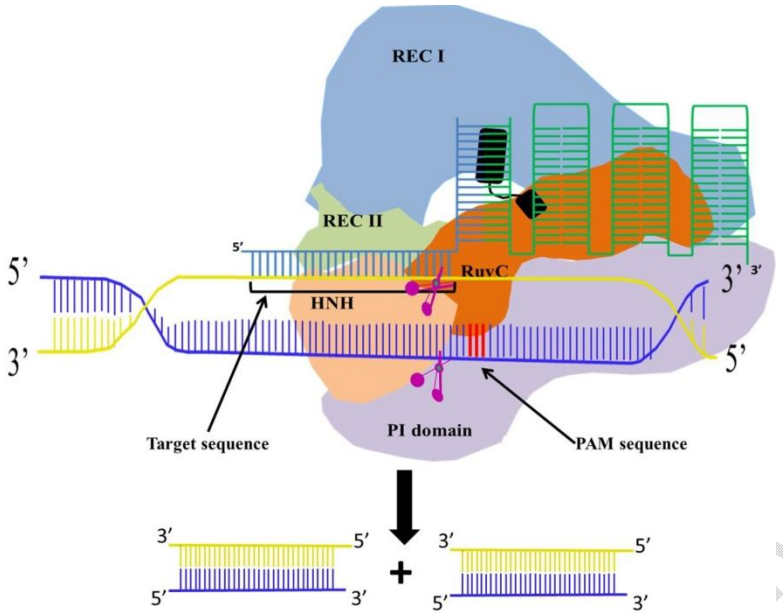

Fig. 3. Mechanism of genome editing using CRISPR-Cas9 technology

3. APPLICATIONS OF CRISPR-CAS9 SYSTEM

3.1 CRISPR-Cas9 based therapeutics

Besides tremendous advancements in the fields of molecular biology, biotechnology, and medicine, there are many diseases/conditions such as cancers, genetic disorders, diseases caused by viruses, etc. for which either we do not have any treatment or lack effective treatment options. For cancer treatment, surgery, chemotherapy, and radiation therapy are still considered gold-standard therapeutic measures. However, thesetherapeutic measures have serious side effects on patients [17]. Many researchers have applied several immunotherapies such as immune checkpoint inhibitors, CAR T-cells, monoclonal antibodies, and treatment vaccines for cancer treatment [18]. Even though several immunotherapeutics have been approved to treat many types of cancer, but are not widely used in cancer treatment as surgery, chemotherapy, and radiation therapy. Similar to surgery, chemotherapy, and radiation therapy, immunotherapeutics have side effects. Immunotherapeutic not only kills the cancerous cells but may also harm the normal healthy cells and tissues of the body (retrieved from the website: https://www.cancer.gov/about-cancer/treatment/types/immunotherapy on September 2024). At present, there is no treatment available to treat cancer without any side effects. The development of cancer is associated with changes in genes, particularly proto-oncogenes, tumor suppressor genes, and genes that regulate cell division [19]. Ever since the CRISPR/Cas9 gene editing technique was discovered, it has been evaluated to treat different types of cancerssuch as blood malignancies, brain cancer, renal cell carcinoma, colorectal cancer, hepatocellular carcinoma, urinary bladder cancer, and many more [20]. In a study conducted on mice, the CRISPR/Cas9 technique was used to knock out the Nf1, Pten, and Trp53 genes responsible for glioblastoma and the Ptch1 gene responsible for medulloblastomas in humans. CRISPR/Cas9 has been successfully used to knock out the lncRNA genes associated development of bladder cancers. The clinical trials of CRISPR-Cas9 mediated knockout of genes (*TRAC*, *TRBC*, and *PD-1*) that inhibit the T-cell to mount a strong immune response against cancer cells have shown promising results [21]. Approximately 20% of all human

cancer cases are caused by oncogenic viruses such as Epstein–Barr virus, human papillomavirus, hepatitis B virus, hepatitis C virus, etc. It has been reported that microRNAs from oncogenic viruses are involved in tumorigenesis by regulating the gene expression in host cells [22]. CRISPR-Cas9 has shown promising results for the treatment of cancers by knocking out the miRNA genes [23].

EDIT-101 is a CRISPR-Cas9 mediated gene-editing therapy undergoing clinical trials for the treatment of Leber congenital amaurosis 10 (LCA10, a *CEP290*-related retinal degenerative disorder)by repairing the IVS26 CEP290 mutant allele.EDIT-101 is given as a sub-retinal injection to reach and deliver gene editing machinery to photoreceptor cells. The results from undergoing clinical trials of EDIT-101 have shown optimistic results. CRISPR-Cas9 system has also been investigated in other retinal-related disorders such as age-related macular degeneration, Meesmann's epithelial corneal dystrophy, and Retinitis Pigmentosa [24]. The CRISPR-Cas9 system has been used to correct the mutation in the MYBPC3 gene in viable human embryos in the US. This mutation in the MYBPC3 gene is responsible for a condition, called hypertrophic cardiomyopathy (HCM), which can cause sudden cardiac death in people under the age of 30 years. Chang et al. [25] have recently rectified a point mutation in exon 14 of the JAK3 gene (cause of autosomal recessive form of T-B+ Severe combined immunodeficiency) in patient-derived induced pluripotent stem cells (iPSCs) using the CRISPR/Cas9-mediated HDR method. CRISPR-Cas9 has also been employed to treat inborn errors of metabolism (for example hereditary tyrosinemia type I, ornithine transcarbamylase, etc.), muscular dystrophy (Duchenne muscular dystrophy), neurological disorder (progressive neurodegenerative diseases), respiratory disorder (linked with mutation in CFTR), rheumatoid arthritis (RA), and skin disease (Dystrophic Epidermolysis bullosa) by correcting mutation in affected gene [26].

As we all know antimicrobial resistance poses a serious global threat to public health. According to a report, bacterial antimicrobial resistance was directly responsible for 1.27 million global deaths in 2019 and contributed to 4.95 million deaths [27]**.** Thus, it is a need of time to develop novel antimicrobials alternatives to conventional to tackle antimicrobial resistance. Among novel alternative antimicrobials, CRISPR-Cas has incredible antimicrobial potential. Antibiotics generally kill multiple bacteria without any discrimination. In contrast, CRISPR-Cas can be designed in such a way that only pathogenic bacteria will be killed without harming the surrounding bacterial population [28]. Among different types of CRISPR-Cas systems, Type II, type VI, and type I are most commonly explored to develop antimicrobials [29]. Over the past few years, researchers have been putting efforts into developing CRISPR-Cas9-based antimicrobials that can selectively inactivate the gene(s) involved in the development of antimicrobial resistance in bacteria. Kiga et al. [30]reported that they have developed a series of CRISPR-Cas13a-based antibacterial nucleocapsids [CapsidCas13a(s)] that are capable of sequence-specific killing of carbapenem-resistant *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* by recognizing corresponding antimicrobial resistance genes. Moreover, CapsidCas13a(s) can also be used to detect specific bacterial genes.

Besides non-infectious diseases, the potential of CRISPR-Cas9 has also been investigated to treat several infectious diseases such as human immunodeficiency virus, herpes simplex virus 1, Epstein-Barr virus, cytomegalovirus, human herpesvirus 6, hepatitis B virus, Zika virus, *Mycobacterium tuberculosis*, dengue virus, etc. [31]. As we all know Cas9 can target only DNA, however, CRIPSR-Cas9 can be programmed to target RNA viruses as well by the using DNA sequence incorporated with the PAM sequence that will hybridize with the target RNA sequence to form a double-stranded target required for Cas9-mediated cleavage [32]. In addition to CRIPSR-Cas9, CRISPR-Cas13 has also been explored to develop antiviral therapies [33]. PAC-MAN (Prophylactic Antiviral CRISPR in huMAN cells), CRISPR-

Cas13 mediated therapy, has been developed that can effectively degrade RNA from SARS-CoV-2 sequences and live influenza A virus (IAV) in human lung epithelial cells [34]. At present, we do not have effective antiviral therapy for AIDS despite continuous efforts over the past few decades. However, CRISPR-Cas9 has been successfully applied to block HIV entry into host cells by deleting chemokine co-receptor type-5 (*CCR5*) genes in the host cells [35]. In the year 2017, researchers from Temple University showed that the excision of the HIV-1 genome using CRISPR/Cas-9 can prevent HIV replication in animal models. In November 2018, He Jiankui, a Chinese biophysicist, announced that he had created the first human genetically edited babies, known by their pseudonyms “Lulu and Nana”. This news has stunned the science fraternity across the globe. He Jiankui was criticized for not considering the ethical issues and risks associated with this experimentation on babies, their parents, or humanity as a whole. Because He Jiankui had used CRISPR to modify the CCR5 gene in the embryo, the offspring of genetically edited babies might get the modified CCR5 gene and pass it down to their future generations. He and his two collaborators were found guilty of conducting illegal medical practices. He was sentenced to 3 years in jail and a penalty of 3 million Chinese yuanand the other two defendants in the same case were also sentenced. One was sentenced to imprisonment of 2 years with a fine of 1 million RMB Yuan, another was sentenced to imprisonment of 1 year and 6 months (with probation of 2 years) with a fine of 0.5 million RMB Yuan [36].He has used CRISPR/Cas9-mediated genome-editing technique on embryos created from serodiscordant couples (one partner is HIV-positive and another partner is HIV-negative) to edit the chemokine receptor (CCR5) to make HIV-resistant babies. One twin has modified the CCR5 gene on only one of two chromosomes, whereas the other twin has modified the CCR5 gene on both chromosomes. When the cells of both twins were examined, it was found that not all cells have modified CCR5 genes (genetic mosaicism) which raises a big question mark on He’s experimentation [37].

Although several experiments have documented the use of CRIPR-Cas9 gene-editing technology to treat many diseases, however, still there is a need to address real-life challenges such as a stable, efficient, and safe delivery system associated with CRIPR-Cas9 limiting its use in-vivo. Moreover, CRIPR-Cas9 gene editing is not stable in vivo, as seen in an experiment conducted byHe Jiankui on human embryos to modify the CCR5 gene. All the cells of babies borne from these embryos did not carry the modified CCR5 gene. Also, the off-site targeting by CRIPR-Cas9 is a major problem that needs to be addressed before its in-vivo use [38]. Many studies reported that off-site targeting by CRIPR-Cas9 can cause tumor development [38]. To date, Casgevy is the only CRISPR/Cas9 based therapy approved by the U.S. Food and Drug Administration for the treatment of sickle cell disease (SCD) in patients 12 years and older [39].

3.2CRISPR-Cas9 based diagnostics

From the recent outbreak of SARS-Cov2, we have learned the importance of diagnostic tools, particularly point-of-care diagnostics, for the diagnosis of infectious diseases which are capable of diagnosing the disease in a short period (60 minutes or less) and do not rely on sophisticated equipment. Timely and accurate diagnosis of any disease is very crucial to decide and adopt the best therapeutic and preventive measures for the treatment and control of that particular disease**.** At present, nucleic acid-based diagnostic tools are considered highly sensitive and specific for disease diagnosis.The polymerase chain reaction (PCR) is a gold-standard nucleic acid-based diagnostic tool with high sensitivity and specificity. To perform PCR, however, requires expensive equipment, chemical reagents, and skilled personnel. Thus, diagnosis based on PCR can only be done in specialized laboratories. However, the isothermal amplification of nucleic acid made it possible to amplify the nucleic acid in the laboratories with fewer resources or at the hospital level. The

isothermal amplification of nucleic acid does not rely on the availability of sophisticated equipment. However, the use of the isothermal amplification technique is limited because of its low specificity (false positive results) [40].

Since its discovery, the CRISPR-Cas system has been widely explored for its potential to treat infectious diseases, genetic disorders, and cancers. In addition to that, the potential of the CRISPR system as a diagnostic tool has also been explored to diagnose infectious diseases, cancers, and single nucleotide polymorphisms. CRISPR-Cas9, CRISPR-Cas12, and CRISPR-Cas13 are the most commonly explored as a diagnostic tool [41]. Because Cas12 and Cas13 have both cis-cleavage (cleavage of the target sequence) and trans-cleavage activities (collateral cleavage of sequences other than the target sequence), it is easier to develop diagnostics from Cas12 and Cas13 [42]. The diagnostics platform based on Cas12 and Cas13 consists of (1) a ribonucleoprotein (RNP) complex (crRNA and Cas protein) and (2) a nucleic acid signaling reporter [42]. Unlike Cas12 and Cas13, Cas9 has no trans-cleavage activity. However, recently a study by Chen et al.[43] has shown that Cas9 can also be programmed to perform trans-cleavage of ssDNA and ssRNA signaling reporter.

Moon et al.[44] were successfully able to detect SARS-CoV-2 and drug-resistant pH1N1 by directly detecting the RNA (without pre-amplification of target) of these viruses in virus RNA lysate using a colorimetric test based on CRISPR/dCas9. CRISPR-Cas9 has also been applied to differentiate the different strains of Zika virus in the plasma of a macaque. Broughton et al. [45] developed an assay, called DNA endonuclease-targeted CRISPR trans reporter (DETECTR), which is a CRISPR-Cas12a-based lateral flow assay for the detection of SARS-CoV-2 from respiratory swab RNA extracts. The DETECTR technique has also been used to detect the human papillomavirus (HPV). However, in this assay, pre-amplification of the target has to be performed using reverse transcription loop-mediated amplification (RT-LAMP). The LAMP-Cas12a-based method has been developed for accurate and rapid detection of the Hepatitis B virus (HBV) [46]. Unlike Cas9 and Cas12a, Cas13a cleaves single-stranded RNA. Thus, Cas13a is widely used for the detection of single-stranded viruses. The Cas13a-assisted virus expression and readout restriction system (CARVER) has been developed to diagnose a wide range of infectious diseases [47]. A single molecule of RNA or DNA can be detected using a Cas13a (for RNA) based assay, called specific highly sensitive enzyme report unlocking (SHERLOCK), in combination with pre-amplification of RNA through isothermal amplification technique via fluorescence and colorimetric readouts [48]. SHERLOCK is a highly sensitive, accurate, and rapid assay. This assay can be programmed to diagnose multiple diseases in a single assay [49].

CRISPR/Cas system can be utilized as a non-invasive technique for cancer diagnosis by detecting cancer biomarkers circulating in the blood and other body fluids [50]. The cancer biomarkers include tumor exosomes (small extracellular vesicles containing tumor-specific proteins, lipids, nucleic acids, mRNAs, microRNAs, and circular RNAs), circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), etc. [51]. Early diagnosis of cancers helps in the utilization and management of available resources in a more judicial way to treat the cancer patient. Several CRISPR/Cas13a system-based strategies have been developed with high sensitivity and specificity to diagnose cancer by detecting cancer-specific miRNA [52]. Zhao et al. [53] have utilized CRISPR/Cas13a to specifically recognize miRNA-17. A method based on CRISPR/Cas13a-triggered Cas12a was developed for miRNA-155 detection in cancer patients [54]. DETECTR and SHERLOCK are two commercially available CRISPR-Cas based diagnostic tools widely used techniques for the diagnosis of serval infectious diseases, genetic disorders, and cancers [55]. These diagnostic tools are highly sensitive, precise, and less time-consuming [55].

3.3 Vaccine development using CRISPR-Cas9

By looking at the current scenario, there is a dire need for a novel vaccine platform(s) that can be utilized to develop effective vaccines in a short period against a wide range of pathogens. New diseases are emerging at an unprecedented speed. Several factors are responsible for this situation. To date, vaccines, beyond doubt, are the only effective way to prevent and control the spread of diseases globally. If we look at history, vaccines were the key factor for successful eradication of smallpox (human disease) and rinderpest (animal). However, the development of vaccines is a very time-consuming, labor-intensive, and expensive affair. To bring a vaccine to market requires a minimum of 8-10 years and billions of dollars [56]. Even after that, the efficacy of that vaccine to prevent the susceptible population from all variants of that particular pathogen cannot be guaranteed.

Vaccines are generally classified into traditional vaccines and next-generation vaccines [57]. Traditional vaccines include live attenuated vaccines, killed vaccines, subunit vaccines, or toxoids. Whereas, next-generation vaccines include recombinant protein vaccines, recombinant vector vaccines, DNA vaccines, and RNA vaccines. Conventional methods for the production of both traditional vaccines and next-generation vaccines have many limitations such as complexity, time-consuming, laborious, less efficient, and costly [58]. However, CRISPR-Cas genome editing technology has the potential to conquer the limitations associated with conventional methods of vaccine production [58]. CRISPR/Cas9 is a highly precise, simple, and efficient genome editing technology compared to conventional recombination methods [58]. The vaccine development using CRISPR/Cas system utilizes different strategies such as gene knockout, gene knock-in, gene activation, gene deactivation, etc[31,59]. At present, a lot of research work is undergoing to harness the utility of the CRISPR-Cas system in vaccine development.

The live attenuated vaccines mount a great level of immune response and offer long-lasting immunity against the pathogen [60]. But manufacturing of these vaccines is a highly complex and costly process. However, CRISPR-Cas can be used efficiently to attenuate the pathogen by modification of target genes [58]. Recently, several live attenuated and recombinant viral vector vaccine candidates have been developed against some diseases. CRISPR/Cas9 system has been successfully used to create modifications in the genome of several vector viruses (such as the herpesvirus of turkey, infectious laryngotracheitis virus, and duck enteritis virus) to make them suitable to harbor gene encoding specific immunogenic antigen of one pathogen (in case of monovalent vaccines) or more pathogens (in case of multivalent vaccines) for the production of recombinant virus vectored vaccines [61]. The stability and expression of inserted genes in CRISPR-mediated recombinant virus vectors have been well-proven [11,5]. Zou et al. [62] have developed a trivalent recombinant duck enteritis virus (DEV) vector vaccine to provide protection against influenza virus (H5N1), duck enteritis virus (DEV), and duck Tembusu virus (DTMUV) infections. The recombinant duck enteritis virus (DEV) vector vaccine contains genes that encode for the hemagglutinin (HA) protein of the H5N1 virus, and pre-membrane proteins and glycoprotein of the duck Tembusu virus (DTMUV).Wang et al. [63] have developed a SARS-CoV-2 virus-like particles (VLPs) vaccine based on the Canarypox-virus vector (ALVAC-VLPs) using CRISPR/Cas9. Immunization of mice with ALVAC-VLPs showed the development of SARS-CoV-2 specific T and B cell responses to resist the lethal challenge of mouse adaptive strains.Zhang et al. [64] have developed a vaccine against an infectious bursal disease virus variant IBDV (G2d) by inserting the VP2 gene of IBDV (G2d) into recombinant turkey herpesvirus virus (HVT) using CRISPR/Cas9 gene-editing technology and claimed 100% protection against a challenge with the IBDV (G2d). Chang et al. [65] have investigated homology-directed repair (HDR)-dependent CRISPR/Cas9 as a tool for generating Turkey herpesvirus (HVT)-Avian influenza viruses (AIVs) bivalent vaccines. In this bivalent vaccine,

a region on the genome of turkey herpesvirus between UL45 and UL46 was modified to accept a gene encoding HA protein from the H7N9 virus. Zhu et al. [66] have developed a CRISPR modify the bacteriophage T4 which is suitable to harbor the immunogenic antigen-specific genes of pathogens, and expressed protein can be incorporated at appropriate locations such as spike epitope, envelope epitope, or nucleocapsid epitope of modify the bacteriophage T4 as per the need.

The animal and cell models play a critical role in studying the pathological changes caused by diseases at the molecular level, the effects of genetic alteration, the propagation of viruses, and the development of new therapeutics including vaccines. However, the creation of animal and cell models with a desired genetic modification using previous gene-editing tools (mega-nuclease, ZFNs, and TALENs) is a very challenging process. But CRISPR/Cas9, a versatile, efficient, and robust genome-editing tool, has made genome-editing very convenient to develop suitable animal and cell models in a short period. Niu et al. [67]created the first gene-modified cynomolgus monkey by applying CRISPR/Cas9 into a single-celled embryo; however, it was observed that the transgenic monkey was also carrying the wild allele of edited genes in some cells indicating genetic mosaicism. Komissarov et al. [68] used CRISPR-Cas9 to increase susceptibility of the HEK293FT cell line (isolated from the kidney of the human embryo) to influenza virus by CRISPR-Cas9 mediated knockout of AnxA6 gene in the HEK293FT cell line to enhance influenza A virus replication in low-permissive HEK293FT cell line, and also help in preservation of its glycosylation profile and antigenic properties.

4. ROLE OF CRISPR-CAS9 IN FOOD SECURITY

In simple words, the term “food security” means the availability of sufficient, safe, and nutritional food that must be accessible to all people at all times [69]. Despite great advancements in various fields of science, we are still unable to achieve food security. This is due to the accumulative effect of several factors such as the increasing human population, climate change, globalization, deforestation, shrinking of agricultural land due to urbanization, conflicts, the emergence of new diseases affecting agriculture as well as livestock, etc. To achieve food security, we have to fulfill the increasing demand for food by enhancing productivity. The best way to enhance productivity is by using high-yielding crops and livestock species. CRISPR-Cas9 can be utilized to improve productive traits of crops and livestock species. In addition to that, adaption and resilience traits can be enhanced by CRISPR-Cas9 to increase the adaptability to a particular environment and resistance to diseases and pests. CRISPR-Cas9 has been used for improvements of various crops and livestock species [70,71]. However, several hurdles need to be overcome before the commercial adoption of CRISPR technology to modify the genome of plants and animals.

5. ETHICAL ISSUES ASSOCIATED WITH CRISPR

The CRISPR-Cas genome editing technology is highly simple, flexible, time-saving, and efficient. The characteristics of the CRISPR-Cas system are being utilized in different fields of science such as medicine, biotechnology, and vaccine development. But at the same time, there is a need to address many ethical issues associated with the use of CRISPR-Cas genome editing technology. CRISPR-Cas is such a powerful tool with the help of which the genome of any organism can be manipulated (by removing, adding, or altering a stretch of the DNA sequence) either in a good way (therapeutics, diagnostics, or vaccines) or bad way (development of bioweapon or enhancement purpose). The major ethical issues associated with CRISPR-Cas technology include safety, equal accessibility, risk of germline editing, non-therapeutics use, etc. [72,73,74]. The offsite targeting by CRISPR-cas9 poses a major safety issue [75]. The offsite targeting by CRISPR-cas9 has been reported to produce

cancerous changes in the subject during clinical trials [38]. Also, we have to make sure that CRISPR should be accessible to all without any type of discrimination. The scientific community across the world is highly concerned over the use of CRISPR-Cas technology in germline editing. The genetic changes made in the germ cells can be passed down to future generations. Therefore, the use of CRISPR-Cas technology in germline editing must be prohibited or if required in any extreme situations should be applied only after thoroughly analyzing the risks associated with its use on future generations. Genetic mosaicism has been reported by many scientists in individuals born from embryos that have been genetically modified by using CRISPR-Cas. It is very difficult to ignore the possibility of the use of CRISPR-Cas for non-therapeutic (enhancement) purposes such as the enhancement of human physical ability, features, intelligence, etc. Moreover, it may also be used to modify infectious agents to develop bioweapons. Many countries have rules and regulations to regulate the genome-editing. However, there is a need to frame unified international regulations and laws to regulate the use of CRISPR-Cas or any genome-editing tools across the world. The misuse of CRISPR-Cas should be criminalized to avoid its use for non-therapeutic purposes.

6. CONCLUSIONS

It is a well-established fact that the susceptibility of an individual to any disease depends on the genetic makeup of that individual. In other words, by making desirable changes in the DNA of a susceptible host, we can make that susceptible host resistant to a particular disease. For example, modification of the CCR5 gene (responsible for uptake of HIV) in host cells can make those host cells resistant to HIV infection as compared to host cells that do not have a modified CCR5 gene. Before the discovery of the CRISPR-Cas9 system, mega-nucleases, zinc finger nucleases (ZFNs), and transcription activator-like effector nucleases (TALENs) are being used for genome editing. However, mega-nucleases, ZFNs, and TALENs lack specificity (off-targets). On the other hand, the CRISPR-Cas9 system is more robust and efficient than other genome editing tools. CRISPR-Cas9 genome editing tool has the following advantages over other genome editing tools: (1) easy to use, (2) able to target specific DNA sequences of the desired gene, (3) able to target more than one gene at a time, (4) flexibility, (5) less time consuming, (6) less laborious, and (5) inexpensive. The CRISPR-Cas9 system has transformed genome editing with wide applications in the development of next-generation therapeutics, diagnostics, and vaccine platforms. Many therapeutics, diagnostics, and vaccines have already been developed using the CRISPR-Cas9 and many of them are under clinical trials system.Casgevy, a type of genome editing technology, is the first U.S. Food and Drug Administration-approved therapy utilizing CRISPR/Cas9 for the treatment of sickle cell disease in patients 12 years and older.Out of several commercially available CRISPR-Cas based diagnostic tools, DETECTR and SHERLOCK are two widely used techniques for the diagnosis of serval infectious diseases, genetic disorders, and cancers. Many candidate vaccines are undergoing clinical trials to get approval for their commercial use. Besides these applications CRISPR-Cas9 system, many ethical issues are associated with the use of CRISPR-Cas technology in genome editing that need to be addressed properly.There is a need to frame rules and regulations to prevent the misuse of CRISPR-Cas for enhancement purposes.

REFERENCES

1. Loureiro A, da Silva GJ. Crispr-cas: Converting a bacterial defence mechanism into a state-of-the-art genetic manipulation tool. Antibiotics. 2019;8(1):18.

2. Ishino Y, Krupovic M, Forterre P. History of CRISPR-Cas from encounter with a mysterious repeated sequence to genome editing technology. J Bacterial. 2018;200(7):10-1128.

3. Morange M. What history tells us XXXVII. CRISPR-Cas: The discovery of an immune system in prokaryotes. JBiosci. 2015;40(2):221-223.

4. Tao S, Zhou D, Chen H, Li N, Zheng L, Fang Y, Xu Y, Jiang Q, Liang W. Analysis of genetic structure and function of clustered regularly interspaced short palindromic repeats loci in 110 *Enterococcus* strains. FrontMicrobiol. 2023;14:1177841.

5. Asmamaw M, Zawdie B. Mechanism and applications of CRISPR/Cas-9-mediated genome editing. Biol.: Targets Ther.2021;21:353-361.

6. Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. Drug Deliv. 2018;25(1):1234-1257.

7. Woźniak-Gientka E, Tyczewska A. Genome editing in plants as a key technology in sustainable bioeconomy. EFB Bioeconomy Journal. 2023;3:100057.

8. Hussain B, Lucas SJ, Budak H. CRISPR/Cas9 in plants: at play in the genome and at work for crop improvement. Brief FunctGenom.2018;17(5):319-328.

9. Matinvafa MA, Makani S, Parsasharif N, Zahed MA, Movahed E, Ghiasvand S. CRISPR-Cas technology secures sustainability through its applications: a review in green biotechnology. 3Biotech. 2023;13(11):383.

10. Xu Y, Li Z. CRISPR-Cas systems: Overview, innovations and applications in human disease research and gene therapy. Comput Struct Biotechnol J. 2020;18:2401-2415.

11. Tang Y, Fu Y. Class 2 CRISPR/Cas: an expanding biotechnology toolbox for and beyond genome editing. Cell Biosci. 2018;8(1):59.

12. Gleditzsch D, Pausch P, Müller-Esparza H, Özcan A, Guo X, Bange G, Randau L. PAM identification by CRISPR-Cas effector complexes: diversified mechanisms and structures. RNA Boil. 2019;16(4):504-517.

13. Hille F, Charpentier E. CRISPR-Cas: biology, mechanisms and relevance. Philos Trans R Soc B: Biol Sci. 2016;371(1707):20150496.

14. Bhattacharya S, Satpati P. Insights into the mechanism of CRISPR/Cas9-based genome editing from molecular dynamics simulations. ACS Omega. 2022;8(2):1817-1837.

15. Liao C, Beisel CL. The tracrRNA in CRISPR biology and technologies. Annu Rev Genet. 2021;55(1):161-181.

16. Hillary VE, Ceasar SA. A review on the mechanism and applications of CRISPR/Cas9/Cas12/Cas13/Cas14 proteins utilized for genome engineering. Mol Biotechnol. 2023;65(3):311-325.

17. Debela DT, Muzazu SG, Heraro KD, Ndalama MT, Mesele BW, Haile DC, Kitui SK, Manyazewal, T. New approaches and procedures for cancer treatment: Current perspectives. SAGE Open Med. 2021;9:20503121211034366.

18. Sharma A, Campbell M, Yee C, Goswami S, Sharma P. Immunotherapy of cancer. In *Clinical Immunology* (pp. 1033-1048). Elsevier; 2019.

19. Chial H. Proto-oncogenes to oncogenes to cancer. Nature Education. 2008;1(1):33

20. Rabaan AA, AlSaihati H, Bukhamsin R, Bakhrebah MA, Nassar MS, Alsaleh AA, Alhashem YN, Bukhamseen AY, Al-Ruhimy K, Alotaibi M, Alsubki RA. Application of

CRISPR/Cas9 technology in cancer treatment: a future direction. Curr Oncol. 2023;30(2):1954-1976.

21. Yamamoto H, Yamamura K, Nagasaki H, Suzuki T, Ninomiya F, Matsubara K, Harada N, Ohkubo, S. Genome editing of Nf1, Pten, and Trp53 in neonatal mice induces glioblastomas positive for oligodendrocyte lineage transcription factor 2. J ToxicolPathol. 2021;34(4):359-365.
22. Kandeel M. Oncogenic viruses-encoded microRNAs and their role in the progression of cancer: Emerging targets for antiviral and anticancer therapies. Pharm. 2023;16(4):485.
23. Hussen BM, Rasul MF, Abdullah SR, Hidayat HJ, Faraj GSH, Ali FA, Salihi A, Baniahmad A, Ghafouri-Fard S, Rahman M, Glassy MC. Targeting miRNA by CRISPR/Cas in cancer: advantages and challenges. Mil Med Res. 2023;10(1):32.
24. Peddle CF, MacLaren RE. Focus: genome editing: the application of CRISPR/Cas9 for the treatment of retinal diseases. Yale J Biol Med. 2017;90(4):533.
25. Chang CW, Lai YS, Westin E, Khodadadi-Jamayran A, Pawlik KM, Lamb LS Jr, Goldman FD, Townes TM. Modeling human severe combined immunodeficiency and correction by CRISPR/Cas9-enhanced gene targeting. Cell Rep. 2015;12:1668-1677
26. Schneller JL, Lee CM, Bao G, Venditti CP. Genome editing for inborn errors of metabolism: advancing towards the clinic. BMC Med. 2017;15:1-12.
27. Murray CJ, Ikuta KS, Sharara F, Swetschinski L, Aguilar GR, Gray A, Han C, Bisignano C, Rao P, Wool E, Johnson SC. Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. The lancet. 2022;399(10325):629-655.
28. Sen D, Mukhopadhyay P. Antimicrobial Resistance (AMR) management using CRISPR-Cas based genome editing. Gene and Genome Editing. 2024;7:100031.
29. Tao S, Chen H, Li N, Liang W. The application of the CRISPR-Cas system in antibiotic resistance. Infect Drug Resist.2022;4155-4168.
30. Kiga K, Tan XE, Ibarra-Chávez R, Watanabe S, Aiba Y, Sato'o Y, Li FY, Sasahara T, Cui B, Kawauchi M, Boonsiri T. Development of CRISPR-Cas13a-based antimicrobials capable of sequence-specific killing of target bacteria. Nature Commun. 2020;11(1):2934.
31. Najafi S, Tan SC, Aghamiri S, Raee P, Ebrahimi Z, Jahromi ZK, Rahmati Y, Nahand JS, Piroozmand A, Jajarmi V, Mirzaei H. Therapeutic potentials of CRISPR-Cas genome editing technology in human viral infections. BiomedPharmacother. 2022;148:112743.
32. Price AA, Sampson TR, Ratner HK, Grakoui A, Weiss DS. Cas9-mediated targeting of viral RNA in eukaryotic cells. Proc. Natl Acad. Sci. USA. 2015;112, 6164–6169.
33. Lotfi M, Rezaei N. CRISPR/Cas13: A potential therapeutic option of COVID-19. BiomedPharmacother. 2020;131:110738.
34. Abbott TR, Dhamdhere G, Liu Y, Lin X, Goudy L, Zeng L, Chemparathy A, Chmura S, Heaton NS, Debs R, Pande T. Development of CRISPR as an antiviral strategy to combat SARS-CoV-2 and influenza. Cell. 2020;181(4):865-876.
35. Xiao Q, Guo D, Chen S. Application of CRISPR/Cas9-based gene editing in HIV-1/AIDS therapy. Front Cell InfectMicrobiol. 2019;9:69.
36. Liu S. Legal reflections on the case of genome-edited babies. Global Health Research and Policy. 2020;5(1):24.

37. Raposo VL. The first Chinese edited babies: a leap of faith in science. JBRA AssistReprod. 2019;23(3):197.

38. Guo C, Ma X, Gao F, Guo Y. Off-target effects in CRISPR/Cas9 gene editing. Front BioengBiotechnol. 2023;11:1143157.

39. Parums DV. First regulatory approvals for CRISPR-Cas9 therapeutic gene editing for sickle cell disease and transfusion-dependent β-thalassemia. Medical Science Monitor: Int J Clin Exp Med. 2024;30:e944204-1.

40. [40] Kundu S, Varshney R, Sulabh S. Exploration of Isothermal Nucleic Acid Amplification Techniques in the Biomedical Field. Gene and Genome Editing.2024;7:100032.

41. Lou J, Wang B, Li J, Ni P, Jin Y, Chen S, Xi Y, Zhang R, Duan G. The CRISPR-Cas system as a tool for diagnosing and treating infectious diseases. Mol Biol Rep. 2022;49(12):11301-11311.

42. Feng W, Zhang H, Le XC. Signal amplification by the trans-cleavage activity of CRISPR-Cas systems: kinetics and performance. Anal Chem. 2023;95(1):206-217.

43. Chen J, Chen Y, Huang L, Lin X, Chen H, Xiang W, Liu L. Trans-nuclease activity of Cas9 activated by DNA or RNA target binding. Nat. Biotechnol.2024;1-11.

44. Moon J, Kwon HJ, Yong D, Lee IC, Kim H, Kang H, Lim EK, Lee KS, Jung J, Park HG, Kang, T. Colorimetric detection of SARS-CoV-2 and drug-resistant pH1N1 using CRISPR/dCas9. ACS Sens. 2020;5(12):4017-4026.

45. Broughton JP, Deng X, Yu G, Fasching CL, Servellita V, Singh J, Miao X, Streithorst JA, Granados A, Sotomayor-Gonzalez A, Zorn K. CRISPR–Cas12-based detection of SARS-CoV-2. NatBiotechnol. 2020;38(7):870-874.

46. Ding R, Long J, Yuan M, Zheng X, Shen Y, Jin Y, Yang H, Li H, Chen S, Duan G. CRISPR/Cas12-based ultra-sensitive and specific point-of-care detection of HBV. Int J Mol Sci. 2021;22(9):4842.

47. Huang Z, Fang J, Zhou M, Gong Z, Xiang T. CRISPR-Cas13: A new technology for the rapid detection of pathogenic microorganisms. FrontMicrobiol. 2022;13:1011399.

48. Kaminski MM, Abudayyeh OO, Gootenberg JS, Zhang F, Collins JJ. CRISPR-based diagnostics. Nat Biomed Engin. 2021;5(7):643-656.

49. Zahra A, Shahid A, Shamim A, Khan SH, Arshad MI. The SHERLOCK platform: an insight into advances in viral disease diagnosis. MolBiotechnol. 2023;65(5):699-714.

50. Sornambikai S, Divya KP, Vasanth S, Viswanathan C, Ponpandian N. CRISPR based biosensing: An ultrasensitive theranostic tool for the detection of early Breast Cancer biomarkers–A mini review. Biosens. Bioelectron.: X. 2023;14:100367.

51. Lone SN, Nisar S, Masoodi T, Singh M, Rizwan A, Hashem S, El-Rifai W, Bedognetti D, Batra SK, Haris M, Bhat, AA. Liquid biopsy: a step closer to transform diagnosis, prognosis and future of cancer treatments. Mol Cancer. 2022;21(1):79.

52. Durán-Vinet B, Araya-Castro K, Calderón J, Vergara L, Weber H,Retamales J, Araya-Castro P, Leal-Rojas P. CRISPR/Cas13-based platforms for a potential next-generation diagnosis of colorectal cancer through exosomes micro-RNA detection: a review. Cancers. 2021;13(18):4640.

53. Zhao L, Qiu M, Li X, Yang J, Li J. CRISPR-Cas13a system: A novel tool for molecular diagnostics. FrontMicrobiol. 2022;13:1060947.

54. Zhao D, Tang J, Tan Q, Xie X, Zhao X, Xing D. CRISPR/Cas13a-triggered Cas12a biosensing method for ultrasensitive and specific miRNA detection. Talanta. 2023;260:124582.

55. Mustafa MI, Makhawi AM. SHERLOCK and DETECTR: CRISPR-Cas systems as potential rapid diagnostic tools for emerging infectious diseases. J Clin Microbiol. 2021;59(3):10-1128.

56. Gouglas D, Le TT, Henderson K, Kaloudis A, Danielsen T, Hammersland NC, Robinson JM, Heaton PM, Røttingen JA. Estimating the cost of vaccine development against epidemic infectious diseases: a cost minimisation study. Lancet Glob Health. 2018;6(12):e1386-e1396.

57. Pollard AJ, Bijker EM. A guide to vaccinology: from basic principles to new developments. Nat Rev Immunol. 2021;21(2):83-100.

58. Naeem M, Alkhodairy HF, Ashraf I, Khalil AB. CRISPR/Cas System Toward the Development of Next-Generation Recombinant Vaccines: Current Scenario and Future Prospects. Arab J Sci Eng. 2023;48(1):1-11.

59. Bhujbal S, Bhujbal R, Giram P. An overview: CRISPR/Cas-based gene editing for viral vaccine development. Expert Rev Vaccines. 2022;21(11);1581-1593.

60. Chen Z, Gao X, Yu D. Longevity of vaccine protection: Immunological mechanism, assessment methods, and improving strategy. View. 2022:3(1):20200103.

61. Vilela J, Rohaim MA, Munir M. Application of CRISPR/Cas9 in understanding avian viruses and developing poultry vaccines. Front Cell Infect Microbiol. 2020;10:581504.

62. Zou Z, Huang K, Wei Y, Chen H, Liu Z, Jin M. Construction of a highly efficient CRISPR/Cas9-mediated duck enteritis virus-based vaccine against H5N1 avian influenza virus and duck Tembusu virus infection. Sci Rep. 2017;7(1):1478.

63. Wang W, Wang S, Meng X, Zhao Y, Li N, Wang T, Feng N, Yan F, Xia X. A virus-like particle candidate vaccine based on CRISPR/Cas9 gene editing technology elicits broad-spectrum protection against SARS-CoV-2. Antivir Res. 2024;225:105854.

64. Zhang JF, Park JY, Kim SW, Choi YR, Cha SY, Jang HK, Wei B, Kang M. Development of a highly efficient CRISPR/Cas9-mediated herpesvirus of Turkey-based vaccine against novel variant infectious bursal disease virus. Vaccines. 2024;12(3):226.

65. Chang P, Ameen F, Sealy JE, Sadeyen JR, Bhat S, Li Y, Iqbal M. 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.

66. Zhu J, Ananthaswamy N, Jain S, Batra H, Tang WC, Lewry DA, Richards ML, David SA, Kilgore PB, Sha J, Drelich A. A universal bacteriophage T4 nanoparticle platform to design multiplex SARS-CoV-2 vaccine candidates by CRISPR engineering. Sci. Adv. 2021;7(37):eabh1547.

67. Niu Y, Shen B, Cui Y, Chen Y, Wang J, Wang L, Kang Y, Zhao X, Si W, Li W, Xiang AP. Generation of gene-modified cynomolgus monkey via Cas9/RNA-mediated gene targeting in one-cell embryos. Cell. 2014;156(4):836-843.

68. Komissarov A, Sergeeva M, Zhuravlev E, Medvedev S, Malakhova A, Andreeva E, Shurygina AP, Gorshkov A, Timofeeva M, Balakhonova E,Grudinin M. CRISPR-Cas9 mediated knockout of AnxA6 gene enhances influenza A virus replication in low-permissive HEK293FT cell line. Gene. 2022;809:146024.

69. Odey GO, Adegbite MA, Denkyira SA, Alhaj SM, Lucero-Prisno III DE. Women and food security in Africa: The double burden in addressing gender equality and environmental

sustainability. In: Cohen MJ, editor(s). Advances in Food Security and Sustainability (Volume 7, pp. 35-59). Elsevier; 2022.

70. Kafle S. CRISPR/CAS9: A new paradigm for crop improvement revolutionizing agriculture. J Agric Food Syst Community.2023.11:100484.
71. Lu, Z., Zhang, L., Mu, Q., Liu, J., Chen, Y., Wang, H., Zhang, Y., Su, R., Wang, R., Wang, Z. and Lv, Q., Progress in Research and Prospects for Application of Precision Gene-Editing Technology Based on CRISPR–Cas9 in the Genetic Improvement of Sheep and Goats. Agriculture. 2024.14(3):487.
72. Caplan AL, Parent B, Shen M, Plunkett C. No time to waste—the ethical challenges created by CRISPR: CRISPR/Cas, being an efficient, simple, and cheap technology to edit the genome of any organism, raises many ethical and regulatory issues beyond the use to manipulate human germ line cells. EMBO Rep. 2015.16(11):1421-1426.
73. Lorenzo D, Esquerda M, Palau F, Cambra FJ. Ethics and genomic editing using the crispr-cas9 technique: Challenges and conflicts. NanoEthics. 2022;16(3):313-321.
74. Ayanoğlu FB, Elçin AE, Elçin YM. Bioethical issues in genome editing by CRISPR-Cas9 technology. Turk J Biol. 2020;44(2):110-120.
75. Rasul MF, Hussen BM, Salihi A, Ismael BS, Jalal PJ, Zanichelli A, Jamali E, Baniahmad A, Ghafouri-Fard S, Basiri A, Taheri M. Strategies to overcome the main challenges of the use of CRISPR/Cas9 as a replacement for cancer therapy. Mol Cancer. 2022;21(1):1-30.