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Article

Utilizing CRISPR Technology to Develop Vaccinations and Treatments Against Viruses

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Abstract: CRISPR/Cas systems have attracted considerable attention because of their ability to detect and eliminate foreign nucleic acid, DNA and Genes; most amazingly, this type of process is also present within human cells here on Earth. It is like a system that has obtained immunity, such as this development over time. The recent discovery that CRISPR/Cas 9, 12 and 13 systems can be artificially altered leads to a new potential. The target of these altered systems is human diseases: DNA and RNA viruses. Current methods of prevention and treatment cannot deal with the RNA virus that caused the 2019 coronavirus pandemic, a serious public health concern. The CRISPR /Cas system offers a useful approach to gene editing in comparison with traditional techniques for managing viral infections. This comprehensive review summarizes the latest strategies for treating and preventing human viral infection via CRISPR/Cas technology. We also identify significant challenges and look to a hopeful future for this cutting-edge gene editing method.

Keywords: CRISPR-Cas Gene editing, Infection, Treatment Virus

1. Introduction

Diseases caused by viruses are a major cause of illness and death worldwide. If these illnesses were to increase and lead to widespread outbreaks, they would severely impact society and the economy [1,2]. The ongoing appearance of new harmful viruses and the development of drug-resistant versions have created a continuous need for new antiviral treatments [3,4,5]. However, creating these types of drugs is challenging. This is due to the fact that viruses often take over the machinery of the host cells to reproduce and spread. Therefore, any medication aimed at preventing the virus from replicating may interfere with the normal biological functions of the host [6].

Additionally, finding antiviral drugs that work broadly has been hindered by the highly diverse molecular characteristics of viruses [7.8]. Given these challenges, RNA interference has become a popular strategy for developing antiviral treatments [9.10]. Nevertheless, this method has limitations that make it ineffective for certain tasks, such as removing the hepatitis B virus cccDNA.

As a result, discontinuing RNAi treatment can result in the reactivation of the'' HBV'' [11,12]. When it comes to the construction of an infectious virion, the presence of nucleic acid is essential, in addition to the presence of structural proteins. Therefore, the successful replication of the viral genome is essential to the completion of the life cycle of the virus within the host cell. As a result, one may claim that the elimination of viral genetic material is one of the most effective antiviral approaches.

On the other hand, it is possible that his antiviral strategy, which aims to target the viral genome, could be susceptible to a severe impediment, which is the absence of a virus-specific gene degradation method. Those endonucleases that are unique to a sequence,

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such as clustered Zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENS), and regulatory interspaced short palindromic repeat (CRISPR)-associated nucleases (Cas) have been the primary subjects of study that have been conducted in recent preclinical studies [13,14]. Within the scope of these investigations into the therapeutic potential of endonucleases, the primary focus has been on the utiliszation of these enzymes to modify the viral genome directlydirectly modify the viral genome. The CRISPR/Cas system has garnered a significant amount of interest [15,16,17,18] attributable to the fact that it is straightforward, specific, and extremely versatile. The CRISPR/Cas system is known to exist in six different variations [19], with type II CRISPR/Cas9, which is associated with Streptococcus pyogenes (Sp), being the most significant of these variants. In several number of animal models, including nonhuman primates, mice, Drosophila, and zebrafish, the CRISPR/Cas9 system has demonstrated that it has the potential to insert genetic material at particular sites and knock out target genes [20,21]. It has been argued by a few individuals that this technique could be an effective method. It is possible that in vivo treatments, which are made available by this approach, could be appropriate for the treatment of some human diseases and genetic abnormalities. Over the course of the last twenty years, the CRISPR/Cas9 rewiring techniques have possessed a wealth of promising uses, including epigenetic regulation, gene activation, target gene inhibition, RNA monitoring, and the study of human disorders [22]. They have thus attracted considerable interest. A new era in the treatment of viral infections has begun with the discovery of the gene editing tool CRISPR-Cas. Enzymes that digest DNA were

once assumed to be a means by which bacteria and archaea could defend themselves against potential threats. As a result of this, nucleases have been utilised in the process of genome editing, which enables precise alteration of nucleic acid sequences in a wide variety of species [23]. Both the genomes of the virus and the genomes of the host are susceptible to editing by the nucleases [24]. One of the reasons for this is that they have the ability to change the sequences of DNA and RNA. Additionally, the nucleases have the ability to effectively modify a large number of genes all at once [25]. In contrast to other sequence-specific endonucleases like TALENs and zinc-finger nucleases (ZFNs), the CRISPR-Cas system does not require the creation, selection, and verification of target site-specific protein pairs. This is one of the disadvantages of the technology [26]. Short RNAs, which are simpler to design and create at a lower cost, are utilised instead in order to accomplish the sequence-specific cleavage action that they are capable of possessing. In addition to these characteristics, the CRISPR-Cas system possesses a high degree of precision and adaptability, which makes it a potentially useful therapeutic alternative for viral illnesses treatment. A summary of the most recent studies on CRISPR-Cas therapeutics for viral infections is presented in this article. Particular attention is paid to HIV, HBV, herpesviruses, HPV, and SARS-CoV-2 for consideration. The possibility of vector systems to improve the delivery of CRISPR-Cas was another topic that we discussed on this occasion. In conclusion, a number of potential future research areas were suggested, and one of the limitations of employing CRISPR-Cas for the treatment of viral infections was brought to light.

2. The Revolution by CRISPR/Cas Techniques

The CRISPR/Cas9 system has the potential to change virtually any region of the genome. This is accomplished by means of the Cas9 protein and a brief gRNA. In comparison to other, less effective methods of gene editing, the CRISPR/Cas9 system is light- years ahead of the curve. Nearly simultaneously with the discovery of the gene for E. coli alkaline phosphatase isozyme conversion in 1987, five identical copies of a 24-nucleotide repetitive sequence were found. From 1993 to 2005, this study received a lot of attention, and it was initially said to have been the inspiration for CRISPR [27]. The functions of CRISPR were revealed in 2007, following the identification of the CRISPR locus in 2002 and the finding of genes around non-native viral DNA sequences in CRISPR spacers in 2005 and 2007, respectively. There were two of these discoveries in 2005. The most successful gene-editing technology known today, CRISPR, was developed in 2013 by two distinct groups. In 2016, CRISPR/Cas9 was first used in a clinical setting to treat

lung cancer in China. Over the past three years, numerous therapeutic medications with their origins in CRISPR/Cas9 technology have been made public. The CRISPR/Cas9 technology was responsible for J. Doudna and E. Charpentier's 2020 Nobel Prize in Chemistry [28].

3. CRISPR/ Cas9 Gene-Editing Technology

One of the newest advancements in genome engineering is CRISPR/Cas9 [29], which was introduced in 2014. In terms of helping researchers familiarise themselves with an organism's DNA sequence, this approach is among the fastest. The CRISPR gene family, which is present in bacteria, provides the inspiration for this method of gene editing. Bacteria derive their ability to detect viruses and mount defences against them from these genes [30]. When studying the DNA of' the foreign creature's performance within the cells, however, this procedure is highly suggested. The technique of detecting the virus can be done in vitro in the laboratory, and microbes that have this genome can identify the virus. The organism employs the nuclease Cas9 to induce a site-focused double-fibre DNA break by means of a tracer RNA molecule that acts as a pilot molecule. The Cas9 nuclease is able to damage double-stranded DNA by functioning as "a DNA strand breaker" [31].

4. Structure of CRISPR

CRISPR/Cas9 provides us with precision data. And easy to make. The relative importance of the nucleotide marks and a sequence known as "protospacer adjacent motif (PAM)" decide its specificity[32]. Twenty bases each CRISPR locus in the crRNA array contains a target sequence[33]. There is usually a broad set of target sequences to be found among crRNA arrays. By correlating the gene organization and host DNA sequence layout, the Cas9 proteins is able to find the right position on the host genome. Because it is not dependent on the Cas9 protein, the sequence is flexible and self-generating. [34] We see the PAM pattern in the coding region that Cas9 is attempting to target. It is by no means an easy job to redraft Cas9 so that it can recognise brand new PAM sequences. [32] For example, since it appears on average every 8 to 12 base pairs in the human genome, the 5-NGG-3 PAM region for SpCas9 is not particularly binding. [32] There are many places in the genome where it occurs, and the pattern is both brief and generic. After turning the plasmid into a cell, Cas9 uses the crRNA to find the correct sequence within the host DNA area.

5. CRISPR-Cas Systems

Inactivating foreign genomic elements is one function of CRISPR-Cas; the prokaryotice immune system is adaptable. Different effector proteins are used by the two primary types of CRISPR-Cas systems. Class 2 systems are different from Class 1 systems as they use only a single, multi-domain Cas effector--Cas9, Cas12 or Cas13--to conduct nucleic acid cleavage activity (36). The type of Cas protein employed determines many characteristics of the CRISPR-Cas system, including the target molecule itself and some trans-cleavage abilities, as well as the recognition site's sequence, etc. In particular, Cas9-cutting targets double- stranded DNA molecules while Cas13 performs cleavages on RNA molecules that have a single strand. Comparatively speaking, the majority of research and attention has been paid to CRISPR-Cas9. The CRISPR/Cas9 system includes essential components such as the endonuclease Cas9 and guide RNA (gRNA), which directs it to its specific target position. The Cas9 enzyme has two nuclease domains: the HNH domain as well as a similar RuvC-like domain. The job of each domain is to cut one strand of DNA. A truncated form of CRISPR RNA (crRNA), which also includes the gRNA-believes an essential role in determining target specificity for CRISPR-Cas9.

The crRNA is contained within a longer trans-activating crRNA (tracrRNA). Genome editing tools utilising CRISPR-Cas9 leverage the targeting specificity of crRNA to direct Cas9 for the cleavage of specific sequences, achieved through synthetic modifications of the crRNA sequence. The CRISPR-Cas9 system can effectively cleave any double-stranded DNA sequence within the genome, as long as it is situated close to a protospacer adjacent motif (PAM), which is essential for Cas recognition [37].

6. The Use Of CRISPR-Cas Technology To Combat Viral Diseases

The surface receptors of prokaryotic or eukaryotic cells allow viruses, which are obligatory parasites, to infect and invade these cells [38,39]. They have a genome that is

made up of either DNA or ribonucleic acid. A virion is a fully functional unit of a virus that contains all of the virus's components and is able to infect host cells and transmit the viral DNA and RNA. They are located inside cells and require specific proteins for replication and survival, making them potential targets for CRISPR-based therapies [40,41]. There are three main types of viral infections: lytic, latent, and chronic. The virus is able to actively replicate and generate new virions during lytic infection. The viral genomes of subsequent generations are freed from their capsids, which are protein coverings, after cell lysis, which results in the death of the host cell.

Some viruses can induce a latent infection, entering a dormant state that may remain inactive for years [42,43]. A diverse range of human viruses is capable of inducing latent infections, such as SSV type 1, Epstein-Barr virus, CMV, HBV, and human herpesvirus 6. Finally, chronic infection is marked by persistent low-level viral replication that extends over time, resulting in organ damage. Numerous prominent human viruses, including HBV and HIV-1, demonstrate characteristics of persistent infection [44,45,46]. Eliminating the virus in latent or chronic infections presents significant challenges due to the viral genome's integration into the host genome or its existence within the host cell as free-floating viral mini-Mini chromosomes. Fortunately, viruses are unable to replicate or transcribe due to CRISPR-based technologies that enable precise targeting of the viral genome within the host cell. The CRISPR-Cas9 system has gained significant traction for its ability to target various human DNA viruses, such as HSV-1, EBV, CMV, HPV, and JC polyomavirus [47,48,49,50].

In order to effectively stop the reproduction of HSV-1 and HCMV, their research indicated that gRNAs should be directed against important viral genes, such as EBNA 1 and BARTs. By targeting HSV-1 simultaneously with multiple gRNAs, the formation of infectious viral particles was once again entirely eliminated [43]. Additionally, EBV was entirely eradicated from human tumour cells that had been latently infected. As a result of long-term replication and inefficient HCMV targeting with single gRNAs, viral escape mutants were selected [51]. Therefore, it is crucial to co-target many areas of the viral genome in order to stop viruses that are resistant to CRISPR/Cas9 digestion from escaping. The majority of Cs9-induced double-strand breaks are repaired by the non-homologous ''end joining (NHEJ) pathway''. This process turns off and disables the virus by randomly replacing, deleting, and inserting nucleotides, which disrupt essential regions of the viral genome that code for proteins and/or have cis-regulatory elements [52]. Beyond that, RNA viruses can be targeted using CRISPR-Cas complexes. In order to combat viral infections, scientists have created at least two CRISPR-Cas systems that target RNA. One such method relies on the Cas9 protein, which, as previously stated, does not typically target RNA [53]. The system can create the double-stranded target necessary for Cas9-mediated cleavage when it is supplied with a separate DNA oligomer bearing the PAM sequence [53]. The second CRISPR-Cas system makes use of the RNA-affinity Cas13 endonuclease [54,55]. One possible approach to treating RNA viruses is the CRISPR-Cas13 system [56].

Human Immunodeficiency Virus (HIV) 7.

The RNA virus that causes human immunodeficiency virus (HIV) originated from viruses that infected chimpanzees [57]. This retrovirus is a member of the Lentivirus family. The HIV genome consists of a pair of RNA molecules with a single strand. Proteins required for the virus to finish its life cycle are coded for by these components. Infected individuals are more likely to acquire cancer and other potentially deadly illnesses. Because the virus explicitly infects CD4+ T-cells, which is what leads to acquired immune deficiency syndrome (AIDS). One of the great obstacles facing public health officials is the growing global epidemic of HIV infection. The two major types of the human immunodeficiency virus (HIV) are HIV-1 and HIV-2. The majority of the world's AIDS patients have had this strain. More than 40 million people worldwide live with chronic HIV-1 status as of 2006, according to estimates [58].

After Highly active anti-retroviral treatment (HAART), cell-mediated HIV-1 replication is very low and patients are almost as good as uninfected. Resistance to antiretroviral drugs arises when HIV-1 achieves certain improvements in its

characteristics that provide it with an advantage, making treating HIV-1 infections effectively all the more difficult [59, 60]. The emergence of technologies such as ZFNs and TALENs and CRISPR-Cas systems that are good at editing insect genomes has given humans and hope for the eventual eradication of HIV. Through laboratory experiments, it has been found that when a CRISPR-Cas9 system with the help of RNA can cause indel mutations in specific genes, it is possible to lessen the state of active HIV infection [61]. Important co-receptors for HIV-1 entry into CD4+ T cells, the C-C chemokine receptor type 5 (CCR5) and the C-X-C chemokine receptor type 4 (CXCR4) are prime candidates for genome editing technology [62, 63].

Loss of function for these co-receptors increases the risk that immune cells may develop HIV resistance [64,65]. Modifying the genes that encode these co-receptors may have a negative impact on cell survival, there is limited evidence to support this claim [65,66]. Preliminary data from multiple clinical trials show that altering these genes in human patients is a safe and effective treatment option [67].

HTLV-1 8.

Disorders, these are caused by HTLV-1, a human retrovirus that is also an oncogenic. As a result, it generally results from CD4 + T-cell transformation and the pathogenesis example is adult T-cell leukaemia/lymphoma (ATL). This virus also causes HAM/TSP (HTLV-1-associated myelopathy/tropical spastic paraparesis) [67]. Some questions remain regarding HTLV-1-related disorders, sickness progression, and effective treatments, despite scientific advances. Hbz and Tax are two significant viral proteins involved in the pathophysiology of HAM/TSP and ATL, even though HTLV-1 encodes certain helper genes pertaining to the viral life cycle. Tax controls cellular immortalisation and new infection [68], whereas Hbz ensures the survival and proliferation of infected cells [69]. Several numbers of in-depth analyses have shown that these genes are targets for HTLV-1 anti-survival and anti-growth therapies. The viral long terminal repeat (LTR) sections serve as promoters for the expression of all viral genes and as sites for the integration of host chromatin with the viral genome. Patients with HAM/TSP and ATL, as well as fresh HTLV-1 infections, asymptomatic viral carriers, and LTR, Tax, and Hbz are the target regions that show the most promise for treatment. Using the 3'LTR and Tax and 3'LTR and Hbz overlapping reading frames to create gRNAs can simultaneously inhibit two viral components [70]. At this time, among retroviral proviruses, only HIV-1 is available for CRISPR editing [71]. On the flip side, HTLV-1 exhibits more concentrated gRNA targeting than other HTLVs since its genome is quite conserved and there is a great deal of sequence homogeneity among different HTLV isolates and within the same host.

9. Epstein-Barr virus (EBV)

Numerous viral diseases. Numerous viral diseases. Numerous viral diseases. There are numerous viral diseases that can affect humans, but none are more dangerous than the Epstein-Barr virus (EBV). Despite the fact that most people with EBV infection dont experience any symptoms, there are rare cases when it can cause significant illnesses such as B-cell lymphomas, T (NK)-cell lymphomas, as well as EBV-related lymphoproliferative diseases of the code[72] Epstein-Barr (EBV) infections and various types of epithelial cell carcinomas, such as nasopharyngeal, gastric, and breast cancer, also have close relations. [73] Because EBV is a latent herpesvirus, it has been one of many targeted ref targets using CRISPR/Cas9 technology. To generate a variety of editing targets, Wang et al. loaded seven guide RNAs that targeted six different regions within the EBV genome onto modified S. aureus Cas9 cleavage complexes.

Patients with Burkitt's lymphoma provided the B cell line that was transfected with these short guide RNAs. Aless episomal EBV genome was observed in infected cells using CRISPR-SpCas9/sgRNAs compared to uninfected ones. This intervention effectively prevented cell proliferation and lowered the virus burden [74]. Yuan and colleagues created two sgRNAs to specifically target a 558 bp location within the BART promoter region (BamHI A rightward transcript). It was a lengthy procedure. Cells harbouring a latent infection produce this area, which is a key viral transcript encoding the viral microRNA. The alteration of microRNA genes was observed in nasopharyngeal cells that

exhibited symptoms of latent EBV infection. This finding opened the door to a potential new way of treating EBV infection.

Furthermore, evidence suggests that a substantial portion of the Epstein-Barr virus (EBV) genome—roughly 40 to 60%—was disrupted when a small guide RNA (sgRNA) targeting the Epstein-Barr virus nuclear antigen 1 (EBNA1) and the origin of replication region was used. This was noticed in the Akata-Bx1 cells of Burkitt's lymphoma. By directing two distinct short guide RNAs towards EBNA1 at the same time, we were able to disrupt more than 90% of the gene. Here it is [75].

10. Human papillomavirus (HPV)

Not only does the human papillomavirus (HPV) account for the vast majority of cervical carcinoma cases, but it also plays a major role in the increasingly widespread anal and head and neck malignancies. [76] A large percentage of cervical cancer cases are thought to be caused by high-risk human papillomaviruses, or HR-HPVs. Herpes simplex virus types 16 and 18 are HR-HPVs. In the early stages of an HPV infection, the oncogenes E6 and E7 are produced. It is the job of these oncogenes to keep oncogenesis going. In order to split the HPV genome, the Cas9 nuclease has been effectively utilised in cancer cell lines that have been generated by HPV [77]. This triggers an upregulation of p53 or pRb, which, after targeting the E6 or E7 oncogenes, kills cancer cells. [78] in Results from treating cervical cancer with a combination of Cas9 and the anti-cancer drug cisplatin were encouraging, according to research by Zhen et al. Whether in a controlled environment or in a real-life thing, the goal was to attack E6 and E7. According to Yoshiba et al. [79], CRISPR/Cas9 could be an alternate, tailored treatment for cervical cancer. In animal models of high-risk HPV-positive cervical cancer, the transduction of adeno-associated viruses (AAVs) with Cas9/sgRNA targeting E6 reduced tumor cell proliferation. This was achieved by targeting the E6 gene with sgRNAs during intertumoral therapy in a mouse model.

11. Hepatitis C virus (HCV)

Liver cancer, cirrhosis, and fibrosis are mainly caused by persistent infections with the hepatitis C virus (HCV) RNA virus. [80] It has been discovered that eukaryotic cells can be protected from HCV infection using CRISPR-FnCas9. [81] For the purpose of determining whether or not Cas13a has an inhibitory effect on the replication of HCV RNA, Saeed et al. developed a sub- genomic viral replicon known as JFH1/SG-Feo. This replicon contains five HCV nonstructural (NS) proteins in addition to the firefly luciferase protein. Using neomycin phosphotransferase II (nptII), the replicon was fused in frame with the appropriate enzyme. HCV IRES was responsible for directing the expression of f firefly luciferase, whereas encephalomyocarditis virus (EMCV) IRES was responsible for controlling NS3-NS5B proteins [82]. After transfecting Huh 7.5 cells with in vitro produced JFH1/SG-Feo RNA for a brief period of time and then introducing the pCas13a-gRNA plasmid, the levels of viral RNA in all IRES-targeting crRNAs were significantly reduced. This was the result of the combination of these two processes. "Leptotrichia shahii (Lsh)" was tested for its knockdown efficacy 48 hours after the transfection. The levels of HCV RNA in the cells were reduced by a factor of 3.5 to 8 as a consequence of the Cas13a treatment. In light of these findings, it appears that Cas13a, which targets the HCV IRES, is extremely successful. Furthermore, the levels of HCV RNA translation were considerably lowered by plasmids that targeted the HCV internal regulatory element (Cas13a-crRNA) [83].

12. Influenza Virus

Over the course of each year, seasonal influenza virus epidemics claim the lives of between 290,000 and 650,000 individuals. On the other hand, significant pandemics occurred in 1918, 1957, 1968, and 2009, which caused enormous agony and death. [84 ,85]. The influenza virus. Both mammalian and avian species are susceptible to the catastrophic consequences of the influenza A virus (IAV), which is a virus with a negative-sense RNA transmission method. The Orthomyxoviridae family is the one to which this virus belongs to. A minimum of eleven proteins are encoded by each of the eight viral RNA segments that make up the genome of an infectious A virus (IAV). As a result of its propensity for mutation, IAV is constantly producing new strains, which brings about the need for the

creation of innovative vaccines and antiviral medicines. In addition, wild birds and ducks are a consistent source of extremely hazardous avian influenza viruses such as H5 and H7, which have the potential to occasionally cross species boundaries and put people in danger [86]. [88,87] More than sixty countries on three continents were affected by the H5N1 virus in 2003, which caused attacks on domestic and wild birds, respectively [89]. Positive outcomes for infection management were achieved by the utilisation of a DF1 cell line that can express Cas13a, crRNAs, and sequence-specific RNA targeting in a stable manner on a consistent basis. 64 Cas13a can therefore be exploited to down-regulate endogenous mRNA for the purpose of functional research, as opposed to the siRNA knockdown procedures that are more commonly utilised nowadays. There are a few clear benefits that come with using Cas13a rather than siRNAs. One of these benefits is the high level of accuracy that it provides while still allowing for robust mRNA breakdown. [91] [90] The information that was gathered lays the groundwork for additional research into the antiviral action of Cas13a against avian influenza viruses, such as the highly pathogenic H5N1 strain, as well as other viruses that are able to infect chickens. Following the effective incorporation of anti-viral CRISPR/Cas13a transgenes into the chicken genome through the utilisation of techniques such as the Tol2 transposon, in vivo challenge tests may demonstrate an antiviral impact. The result of this would be the production of transgenic hens that have germlines that express Cas13a and crRNAs that target H5N1. [90,91]

13. CRISPR/Cas System in Virology Research

There has been a remarkable increase in the number of publications describing research in the biological sciences and virology that is relevant to CRISPR and gene editing within the past decade. Not only does the CRISPR/Cas9 system have extensive experience editing genes in cellular species, but it has also been employed on certain non-cellular entities, like DNA viruses and RNA viral fragments that have been integrated into host genomes. A number of viral species have had their genes edited using the CRISPR/Cas9 system

These applications have centred on antiviral treatment, functional studies of viral virulence factors, and the restoration of viral vectors used for genetically engineered vaccine development. Further research into virus-host interactions has made use of the CRISPR/Cas9 system, which has allowed for whole-genome screening and the identification of host components critical for viral replication.

2. Materials and Methods

The Limitations and Practical Challenges

CRISPR technology has revolutionized geneediting , but it has several limitations and challenges. Efficient delivery of CRISPR components into target cells is therefore one of the primary challenges. Nevertheless, the application of CRISPR technologies as therapies confronts some significant challenges. These include how to deliver CRISPR effectively into target cells and whether or not the patient's immune system will react against it.

1-Delivery Methods: A major determinant of CRISPR technology’s efficiency is how well its components are delivered. Viral vectors, However, these vectors also have their problems. For example, the restricted packaging potential and possible immunogenicity[92]. Although delivery of viral vectors is naturally more efficient than their non-viral alternatives, safety concerns–including the risk of themselves being an immunogen as well as carrying insertional mutagenes–still loomed large. It was only after new, mature techniques had been worked out to use viral vectors that CRISPR might be attempted in humans [93]. Since adeno-associated viruses (AAVs) have a relatively low level of immunogenicity and stable genomic integration compared to other viral vectors during prolongued periods, even years after its launch treatment using AAVs [94].

Nevertheless, several recent studies have reported that CRISPR employing AAVs [adeno-associated viruses] results in incremental risk of AAV integration for clinical use, including severe insertional mutagenesis and the development of cancers [95,96].

Take AAV, for example. A study using it to treat hemophilia in dogs has run for up to 10 years and shows that the vector can insert itself in many sites in the host's genome, occasionally into genes that modulate cell growth [97] However, current delivery methods based on virology and liposomes are still relatively inefficient; and the specificity they provide is only moderate at best. What s more, they often cause toxicity when used in large doses. All of this poses major problems for translating nanotechnology means into clinical practice. The merging of health care with nanotechnology lead to the birth of nanomedicine or nanotherapy as it is also known [98]. Nanotherapy provides filming at an advanced stage, quicker diagnosis, effective drug and gene delivery systems, tissue repair in situ and individualised drugs [99].

So, around the cell types where these drugs or genes/proteins need to arrive, nanoparticles (NPs) are used as nanomedicine. A lot of NPs have passed clinical trials and others are being tested. Smart NPs are those which at the same time can be triggered by some form of energy or signal to deliver cargo in vivo efficiently and exactly [94].

3. Results and Discussion

Immune Responses:

There are some limitations and disadvantages associated with the use of viral vectors. For example, the first and perhaps foremost among them is that since viruses typically have a packaging limit of about 5 kilobases,[100,101,102]only sequences within that range will fit into one vector More recently, through using smaller Cas9 orthologs (e.g., StCas9, SaCas9, and cjCas9)9 or dual AAV vectors [103,104], some researchers have been able to bypass the packaging problem to some extent; however, getting exact genome editing via HDR under these conditions is still a major obstacle. The next problem is that viruses prompt potent negative immune responses which largely make it impossible to repeatedly dose cells with a new nucleotide load; this is vital in tissues with high turnover rates (which means most that are suitable for gene therapy).[101,102] Therefore, a single application of viral vectors must give rise to a number of permanently-mutated cells large enough for it to be therapeutically meaningful--which is hard to achieve without using more than one dose.

Strategies to mitigate immune responses are crucial. These include using non-viral delivery systems that inherently evoke lower immune responses and engineering CRISPR components to be less immunogenic.

The Applications of CRISPR Technology on Vaccine Development.

CRISPR technology has pioneered significant advancements in vaccine development across various applications. The CRISPR/Cas9-based gene-editing system offers high precision and efficiency, which is instrumental in modifying viral genomes to understand viral pathogenesis, ultimately contributing to vaccine research. For instance, it has been utilised in developing recombinant vaccines against large DNA viruses of humans and animals. The versatility of CRISPR/Cas9 allows it to bypass traditional vaccine development methods by directly engineering B-cells to produce specific antibodies against viral pathogens. This remarkable capacity of CRISPR technology has been pivotal in overcoming the limitations of conventional vaccine strategies [105.106]. Moreover, CRISPR technology's application extends beyond traditional vaccine development. It includes diverse roles such as in the translational research of human diseases, contributing to vaccine formulation by engineering genetic circuits and manipulating cellular events to strengthen immune responses. This adaptability makes CRISPR-Cas systems valuable in generating novel vaccine candidates and customising immune responses. Overall, the development of CRISPR/Cas9-edited vaccines represents a significant leap forward, offering precise genome editing capabilities that enhance vaccine efficacy and reduce development time. This innovation points to promising future prospects in battling viral infections by leveraging the inherent advantages of CRISPR technology [106].

Recent innovations such as the LNP or viral vector-based delivery methods, and more improved gene editing techniques, such as base editing and prime editing.

Recent advancements in lipid nanoparticles (LNPs) and viral vector-based delivery methods, as well as improved gene editing techniques like base editing and prime editing, have marked significant progress in the field of genetic and therapeutic research.

Lipid Nanoparticle (LNP) Delivery Systems: LNPs have rapidly emerged as a critical technology for delivering nucleic-acid-based therapies. This means that LNP provides a new weapon to develop therapies for genetic diseases. These also come quickly into view: its chemical properties are suitable for use in a number of ways. For it is no problem at all to produce milligrams of pure substance which can be lyophilized or reconstituted and then used. The Nucleic acid that can be encapsulated in LNP includes or is not limited to mRNA, microRNA(miRNA), siRNA, and single guide RNA (sgRNA). [107] LNP is composed of different lipids, so it is easier to quality control since lipids are easier to purify than other types of carriers (such as macromolecules or viruses)[108]. Furthermore, it is easy to develop new LNP by changing the lipid structures or compositions of fat, which adds the versatility the of LNP. [109] In general, LNP consisted of four types of lipids, including ionizable lipids, lipid helpmates, cholesterol, and lipids with PEG strands attached. The route of administration is vital to achieving delivery of mRNA loaded LNP. A variety of delivery routes have been used to deliver LNP where it is needed, such as oral or inhalation and local injection (intramuscular, intratumoral or intracerebral injection). Also, on the way there is research into the biomolecular corona of LNPs. This can have profoundserious influences on stability as well as targeting capabilities. [110].

Viral Vector-Based Delivery Methods

Another reason that viral vectors have pros and cons is that a virus can stably carry and transmit extracellular materials by traversing the cell wall [111]. AAV-mediated CRISPR/Cas delivery has low pathogenicity, long-term gene expression, and can be used in both clinical and vitro experiments [111, 112]. AAVs have icosahedral capsids, are 26 nm in diameter and have a single-stranded genome of about 4.7 kb [113]. AAVs' serotype diversity and tropisms , make for a wide range of therapeutic targets [114]. The AAV family, distinguished by serotype diversity but also characterized by its wide tropisms, makes it an attractive means for gene delivery [111]. There are more than 3000 gene therapy studies using AAV as a subject [115, 116]. For example, a number of drug therapies which are AAV vectors like Zolgensma, Luxturna and Glybera are AAV-based and have been approved for use in human beings [117]. AAV-mediated-CRISPR delivery is highly effective in treating both nervous gene therapy and Duchenne muscular dystrophy (DMD) in mice. In schemes where AAV-mediated delivery of Cas9 and sgRNA to mouse models was inadequate, By none sense point mutation in the dystrophin gene (exone 23) successfully deleted this exon and so reduced the DMD phenotype [118]. CRISPR-associated protein SpCas9 is used widely in gene editing.

Recent advances have focused on overcoming limitations such as vector immunogenicity and off-target delivery by modifying the viral surface with nanomaterials. Nanomaterial-based vector systems are not immunogenic. They have characteristics that make structure and molecular weight easy to adjust. Moreover tendencies toward facile conjugation of different functional groups with the nanomaterial backbone have been demonstrated. Therefore, the process of nanomaterials generation is simple, and they can be preserved for long-term storage. Nevertheless, the transgene expression efficiency mediated by nanomaterial-based vectors is notably lower than that of viral-vectors, limiting their therapeutic efficacy. So to address the problems of systemic delivery by viral vectors and low therapeutic index for nanoparticles, a Viral Vector Surface with various liposomes (lipids), polymers and particles as hybrid car [119,120] were used. With hybrid vectors combining the strengths of both viral vectors and nanomaterial-based systems, it is possible to avoid shortcomings of each while combining

their advantages. A nanomaterial coat can act as a shield for viral vectors in vivo, so that they are invisible to host immune-surveillance systems or degraded enzymatically. In addition, nanomaterials may possess a targeting moiety that improves target-specific localization and accumulation of the viral vectors. After nanomaterial-mediated delivery and internalisation into target cells the viral component of this hybrid system can escape from endosomes (where it is usually trapped) and go straight to cell nucleus, so causing high expression levels of your transgene [121].

These modifications help improve therapeutic efficacy by enhancing vector accumulation at target sites and reducing immune responses.

Gene Editing Techniques - Base Editing and Prime Editing

Recent developments in gene editing offer highly precise tools like base editors and prime editors, which allow targeted repairs without needing to create double-strand DNA breaks. As prime editing can make insertions, deletions, and all sorts of substitutions, it has the potential to correct every single mutation that causes a hereditary disease. At the same time, prime editing can make cell lines or animal models for specific mutations--so research into a few genetic diseases underway.

This review gives a summary of what has been achieved in preclinical using prime editing for human gene therapy. Up to now, prime editing has made attempts at treating familial diseases of liver and eye, skin and muscle, neurodegenerative diseases--in addition to cystic fibrosis, beta-thalassemia, X-inked severe combined immunodeficiency and cancer.

Base editors, such as DNA base editors, composed of cytosine base editors (CBEs) (122) and subsequent adenine base editors (ABEs) (123), are unique for their aptitude for accurate point mutations, avoiding the need for donor DNA templates or the requirement for double-strand breaks. This technological advance enables to carry out pinpointed editing all the way down to the level of bases on the genomic level, thus preserving the surrounding genetic sequence intact. The Cytosine Base Editor (CBE), a derivative of the CRISPR/ Cas9 system known as the Base Editor (BE), consists of two essential components: a single-guide RNA (sgRNA) and a fusion protein comprising Cas9,

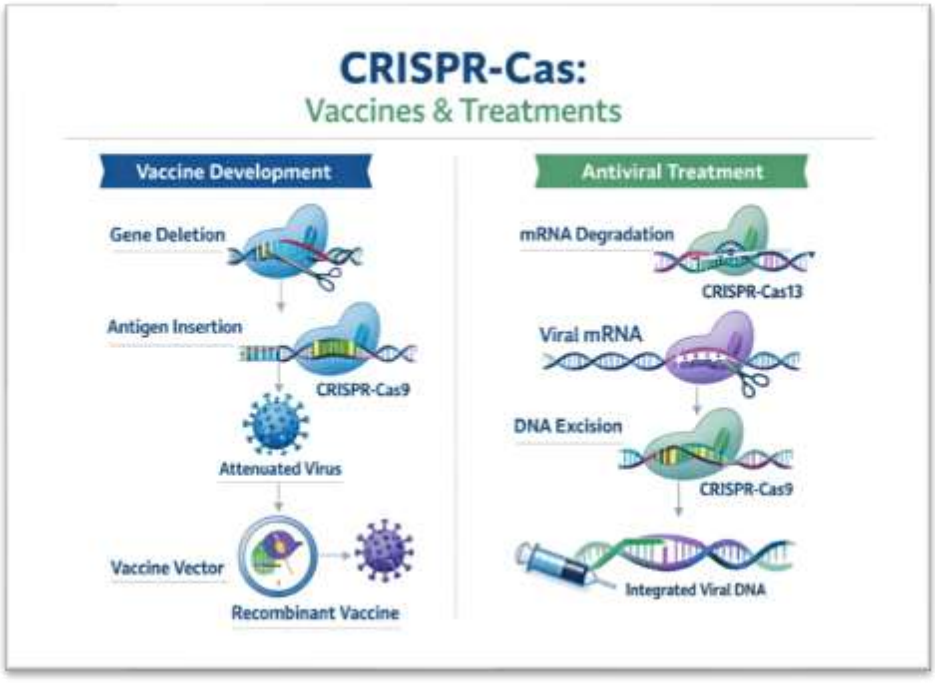
(dCas9 or nCas9) and an enzyme having the property of modification that targets cytosine " The dCas9 (dead Cas9) or nCas9 (nickase Cas9) mutants are activated by mutations in RuvC and HNH domains of the Cas9 nuclease. dCas9 holds onto its DNA-binding capability without clipping the backbone, while nCas9 exclusively cleaves one of the DNA strands, resulting in a single-strand break. Since researchers use Cas9 proteins that are defective for cutting or for which only one strand has been altered in this fashion they can achieve site-specific base changes at the target site without relying on the generation of double-strand breaks. The guided by sgRNA Cas9 protein fusion is directed to genomic DNA, turning cytosine （C） into uracil(U) and repairs or replicates DNA.U. During this process, the uracil is converted into thymine （T ）.One result of this mechanism is a change in the base from C—T (Komor et al., 2016). The natural enzyme produces a significant amount of base changes in cytidine to uridine and therefore can be a suitable DNA cytidine deaminase for base editing applications. So what researchers did was to evaluate the single-stranded DNA (ssDNA) dC to dU activities of several different natural cytidine deaminases (hAID, hAPOBEC3G, rAPOBEC1, and pmCDA1) in efforts to find such a suitable enzyme. In this group of proteins, rAPOBEC1 from rats appeared to have the highest activity on ssDNA. The original base editor BE1 (rAPOBEC1–XTEN–dCas9) pairs rAPOBEC1 with dCas9, using an XTEN linker to connect them. This is 16 amino acids long [122].

Overall, these innovations are paving the way for more effective and precise therapeutic strategies, highlighting the importance of continued research and development in these areas for future clinical applications.

Non-viral approaches, like nanoparticles, have emerged as promising alternatives. These systems, including lipid-based and polymer-based nanoparticles, offer advantages

such as improved safety and reduced immune responses. They allow for better control over size, surface characteristics, and payload release, essential for precision medicine applications., Nanoparticles enhance the therapeutic index of a wide variety of medications and can improve access to better health care through more effectual drug administration by patients. A lower risk of side effects helps clinics provide even more effective medical services as consumer satisfaction ratings soar. [124] Actually, nanocarriers potentially represent the future direction for this profession. They could also allow presently used medications to increase bioavailability while needing fewer doses and at less frequent intervals. [125] The development of nanocarriers offers numerous advantages: precise targeting of drugs to a certain location; at such places control over release, easy penetration of health protective barriers inside the body, combined treatment with multiple drugs for diseases, and increased stability and capacity to carry pharmaceuticals. [21] Liposome formulations have been used to deliver both types of therapeutics: small molecules and macromolecular. A liposomal delivery device is round and has a hydrophilic polar head group (positively charged) on the equator of its membrane bilayer. Water-soluble substances are well encapsulated in the hydrophilic core of liposomes, while water-insoluble entities can be similarly lodged in their lipid membrane. [22] The first cationic lipid used to make liposomes was N- [1-(2,3-dioleyloxy)propyl]-N ,N ,N-trimethylammonium chloride (DOTMA).

Compared to calcium phosphate and diethylaminoethyl-dextran for which plasmid DNA transfection rates were usually about 2 to 5 percent dripping out into the cell culture medium as enzymatically active product, the new type of complex showed slightly higher efficiency but equally low levels of free DNA inside the cells after transfection[23]. Lipoplexes are DOTMA-based liposomes that electrostatically adhere to nucleic acids, and with them form stable particles. In addition to an optimal lipid chemistry to create stable, particle-sized liposomes, various efforts have gone into determining the entrapment and release of nucleic acids from lipid bilayers.



This visual summary shows how CRISPR-Cas technology can be used in both immunology and virology, concentrating on creating vaccines precisely and targeting viruses for treatment .Vaccine Creation **(Prophylactic** (Use): The left side explains how CRISPR-Cas9 is used as a tool to make vaccines safer and more effective. Gene Removal & Antigen Addition: CRISPR-Cas9 performs exact "genetic surgery. " By removing harmful genes that make a virus dangerous, researchers can develop a weakened version

of the virus. At the same time, they can add specific antigens to boost the immune response. Vaccine Vector Creation: The altered genetic material is then put into a Vaccine Vector. This leads to a Recombinant Vaccine, which resembles a natural infection enough to prepare the immune system without leading to actual illness.

Antiviral Therapy (Treatment Use): The right side shows how different Cas enzymes can be designed to eliminate viral parts inside an already infected person. mRNA Destruction (CRISPR-Cas13) Method: Unlike the famous Cas9, which focuses on DNA, Cas13 is designed to find and cut RNA. Effect: By detecting and breaking apart viral mRNA, it stops the virus from converting its genetic information into proteins, effectively stopping the virus from multiplying at the stage of transcription. DNA Removal (CRISPR-Cas9) Method: This method targets viruses that embed their genetic material into the host's DNA (like HIV or some herpes viruses). Effect: CRISPR-Cas9 locates the Viral DNA that is integrated into the host's chromosomes and creates a double-strand break. This removal takes the viral material out of the host cell, providing a possible "functional cure" instead of merely suppressing the virus.

4. Conclusion

Challenges and potential solutions

Despite its many benefits, CRISPR/Cas technology is not without its drawbacks, which is the cherry on top. There is insufficient preclinical and clinical data to support the approach's suitability for application in humans using a delivery technique. Considering the size, charge, and composition of the CRISPR/Cas9 payload, problems are inevitable that the cargo is too heavy to move. The CRISPR/Cas9 gene has been effectively delivered into cells by various physical, viral, and non-viral techniques. Viral vectors for delivering CRISPR/Cas9 are employed in many different types of environments, both within and outside the laboratory. Size limits prevent them from carrying foreign genes and plasmids; the viral genome, once integrated into the host genome, can cause cancer; it is difficult to mass-produce them; robust immune responses are among the many challenges they face. Because they are able to do so, these obstacles are no longer an issue. "Considering this, a different way to distribute the virus could be to use non-viral vectors, like lipid or polymer nanocarriers."

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