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"Precision Revolution: Exploring Viral And Non-Viral Vectors For Enhanced Gene Editing In Clinical Trials And Mapping CRISPR/Cas9 Delivery Technologies".

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Abstract

The CRISPR/ Cas9 technology which can correct genes, control transcription, model diseases, and visualize nucleic acids. Nevertheless, this is not an easy task because it has a low transfection efficiency rate as well as the off target risk associated with it. The extensive report on the CRISPR/CaSN system is delivered outlining viral as well as non-viral vehicles. The technological landscape of applied delivery technologies in CRISPR/Cas9-inspired clinical trials is clearly presented via a thorough examination. The spotlight is directed towards rational designs for non-viral vectors tasked with delivering the three pivotal forms of the CRISPR/Cas9 system: These include plasmid DNA, mRNA, and RNP, Cas9 protein complexed with guide RNA. The complexities associated with vector-derived methods of alleviating the off target worries are comprehensively scrutinized. Surprisingly, it focuses on the underlying mechanisms, uses, and obstacles concerning using CRISPR/Cas9 system for genome editing. Specific inherent in the system-guided Cas9 for cutting, resulting in insertions, deletions or substitution are emphasised. Besides, CRISPR/Cas9 clinical therapeutic potential is considered in relation to recent findings utilizing the genetic tools of bacterial adaptive immunity for site-specific cleavage and mutagenesis. Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), and Cas proteins have changed genetics studies worldwide, transforming gene therapy landscapes. The conventional gene transfer with viral vector-mediated delivery is problematic due to the risk of insertional oncogenesis and immune-mediated toxicity whereas CRISPR makes it highly attractive substitution. Finally, recent gene therapy trials are considered and the ways through which one is able to navigate the intricacies of this revolutionary, yet nascent practice are contemplated. Addressing the limits of CRISPR/Cas9, as illustrated by its impact on moving gene therapy paradigms forward should point towards safe and efficient clinical translation.

Key Words: CRISPR/Cas9, Non-viral vehicles, Delivery technology, Genome

1. INTRODUCTION:

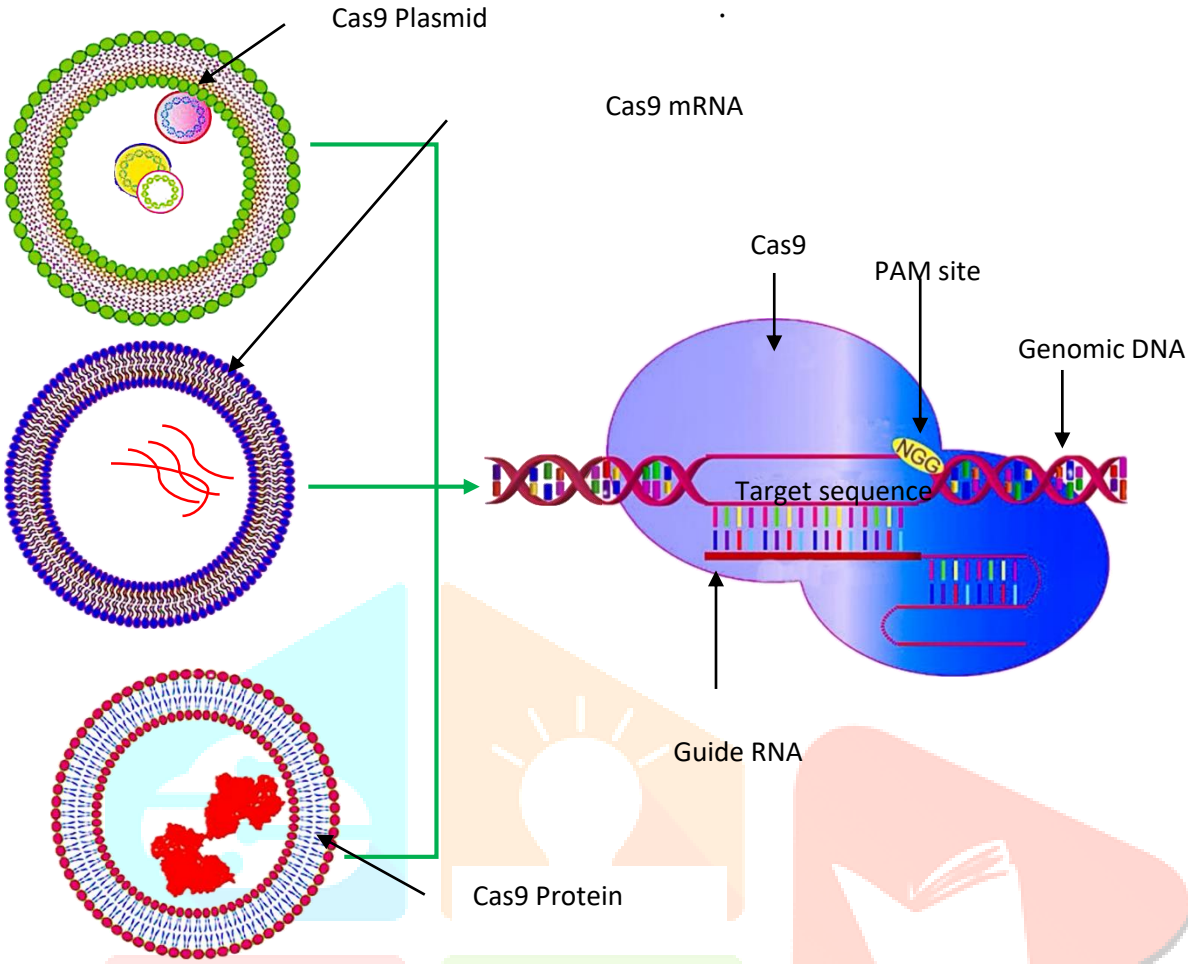
CRISPRs which comprise of the clustered regularly interspaced short palindromic repeats. DNA fragments which were first identified in prokaryotic organisms. The adaptive immune system in archaea and bacteria include the CRISPR, Cas. Plasmids and phages are harmful nucleic acids that can take over a given cell; thus, they need to be defended against. The CRISPR structure was Ishino reported it for the first time in 1987 [1]. It was not till 2002 that Janus suggested the acronym CRISPR. finally, several similar structures were found in different bacteria and archaea [2]. the milestone occurred in 2005, when hyper-variable spacers having homology for sequence was demonstrated. plasmids and viruses of different foreign origin to this day, the classification of CRISPR/Cas systems has been primarily determined by the composition and arrangement of their core elements, resulting in the identification of three primary types [3-5]. In Type I CRISPR/Cas systems, target loci are recognised and disrupted by CASCADE (CRISPR-associated complex for antiviral defence) complexes, which are composed of many Cas protein subunits and form complexes with crRNA. CrRNAs are integrated into a multi-subunit interference complex in type III systems. Cmr or Csm to identify and break down invading RNA. Quite the contrary, only theCas9 protein is necessary in type II systems for DNA interference [6–9]. Most remarkably, plasmids encoding the Cas9 Protein and its gRNA may be integrated into viral vectors—species that are naturally adapted to transport genetic material for cellular expression. Delivery formats for the CRISPR/Cas9 system can be classified as "viral" or "non-viral" due to the fact that a large percentage of delivery strategies have included viral techniques. Applications ranging from ex vivo transfection to animal model creation have successfully employed viral methods to deliver gene editing machinery [10-12].

These artificial "carrier vehicles" are designed to contain and transport the CRISPR/Cas9 machinery in non-viral forms. The CRISPR/Cas9 machinery is available in non-viral forms like as mRNA-based, plasmid-based, and complexed Cas9-RNP [13-15]. Molecular biology and genetic engineering both rely heavily on plasmid DNA vectors, which are essential for many uses, such as the ground-breaking CRISPR-Cas9 gene editing technique. A variety of species' genomes may be precisely and specifically edited by using these vectors that have been altered to carry CRISPR components like the Cas9 nuclease and guide RNA. The ability to modify certain genes to investigate their activities or repair genetic mutations linked to illnesses has greatly increased genetic research and therapeutic advancements thanks to plasmid-based CRISPR technologies. Basically, plasmid DNA vectors are essential instruments that support the adaptability and efficiency of CRISPR technology in contemporary genetic engineering [16-20]. Ribonucleoprotein, or RNP, is an essential part of the CRISPR-Cas9 gene-editing machinery. In CRISPR, the Cas9 protein and guide RNA (gRNA) combine to create an RNP complex. This RNP complex is remarkably precise in targeting and editing certain genes. The Cas9 protein is guided by the gRNA to the targeted gene site, where it makes insertions, deletions, or alterations to the DNA sequence. This capacity to precisely alter genes has transformed genetic research and is a promising avenue for the development of novel treatments for the treatment of hereditary ailments. In this case, RNP vectors stand in for the directed pathway that the Cas9 protein takes to reach its target gene, highlighting the significance of precise genome navigation for targeted genetic alterations [21-25].

2. Improving the Accuracy of Genome Editing with Nanocarrier Approaches for CRISPR/Cas9 Delivery System

Two essential elements of the CRISPR/Cas system—gRNA and Cas protein—are required for efficient genome editing in eukaryotic cells. As of right now, Cas9 originating from Streptococcus pyogenes Because of its simplicity and great target specificity, Cas9 (SpCas9) is the endonuclease variation that causes the most concern [26]. While the RuvC domain cleaves the opposing DNA strand, the HNH nuclease site cuts the complementary strand to crRNA. The 5'-NGG base sequence-containing protospacer-adjacent motif (PAM), which is upstream of the Cas9 binding site, is situated. Additionally, the distinct tracrRNA and crRNA might be mixed to create a straightforward single-guide RNA (sgRNA) [27]. Cas9 protein can precisely target any genomic location via base pairing and cause a DSB under the supervision of sgRNA. The broken genome is then repaired by cells using either the homology-directed repair (HDR) or non-homologous end-joining (NHEJ) mechanism [28]. In general, the NHEJ mechanism aids in insertions or deletions (indels) at the cleavage site, whereas HDR may work with a DNA donor to cause specific changes. The CRISPR/Cas9 gene-editing mechanism is unique. entails delivering two entities—like Cas9 and another—at the same time. only one guide RNA (s). There are three different kinds

of cargoes available right now. provided (Figure 1). One of the delivered payloads is a protein. Specifically, the sgRNA was loaded by the Cas9 endonuclease and joined to produce a ribonucleoprotein RNP [29].



The simplest tactic is to deliver an RNP; no translation or transcription procedure is required. As soon as it enters the cell, it begins altering the genome, greatly lowering immune responses and off-target consequences. Nevertheless, this method necessitates the separation of extremely potent Cas9 protein. In addition, the enormous molecular weight of the Cas9 protein makes it difficult for it to enter cells. It is crucial to create nanodrug delivery systems that are capable of transporting RNP payloads. Currently, the only embryonic microinjection approach that is prioritised is protein delivery [30].

Plasmids encoding CRISPR/Cas9 or sgRNA can be transfected more easily by removing the plasmid and then inserting it into the nanocarrier than by using protein delivery techniques. The process of integrating the appropriate plasmids into the same delivery plasmid allows for knocking-in or gene modification. Low transfection effectiveness results from the gene fragment size encoding the CRISPR/Cas9 system being frequently too big. mRNA is another possible cargo that can be delivered. To carry out their genome editing activity, Cas9 mRNA and sgRNA can be synthesised in vitro, inserted into cells, and translated straight into protein in the cytoplasm. The primary barriers to mRNA application are its relatively poor stability and the resulting short expression time. Delivering mRNA in vivo to cure disease is difficult. This technique has only been applied thus far to modify the genomes of zygotes, embryos, and cultured cells [31].

However, it is difficult for CRISPR/Cas9 to enter cells, regardless of the delivery payload. Owing to its low stability and large molecular weight (Cas9's genetic size is around 4.5 kb), It is essential to create a better nano-delivery technique for the different Cas9 components. Maintaining the nuclease activity of Cas9 and shielding the RNP from proteases, nucleases, antibodies, and T cell identification in serum and bodily fluids are crucial considerations when planning and assembling a delivery system. The delivery mechanism should facilitate the release of the RNP from the endosome into the cytoplasm, so enabling its function, once it has entered the target cell. Currently, cationic LNPs, DNA nanoparticles, and lipid complexes are excellent nanocarriers for the CRISPR/Cas9 system., imidazole frameworks made of zeolite and gold-based nanoparticles. They have undergone substantial development and application efforts for the purpose of delivering RNP in vitro [32].

The Cas protein and gRNA, in particular the extensively utilised Cas9 from Streptococcus pyogenes, are necessary components of the CRISPR/Cas9 system (SpCas9). SpCas9 is a simple and highly selective target-directed RNA splicing enzyme that causes double-strand breaks (DSBs) in response to sgRNA. The results of genome editing are determined by repair processes like as homology-directed repair (HDR) and non-homologous end-joining (NHEJ). It is difficult to deliver Cas9 and sgRNA simultaneously, either as a ribonucleoprotein (RNP) or via plasmids/mRNA. To improve efficacy, nanodrug delivery techniques must be developed. A range of nanocarriers, including gold-based nanoparticles and cationic lipophilic nanoparticles, present viable ways to get over obstacles in the CRISPR/Cas9 delivery process, opening up new possibilities for sophisticated genome editing applications [33,34].

CRISPR/Cas9 Nano-Delivery Approaches areLipid-Supported Nanomedicines The traditional nucleic acid delivery method, lipid nanoparticles, has been thoroughly investigated. Through host-guest and electrostatic interactions, negatively charged nucleic acids and positively charged lipids form a complex that is then taken up by endocytosis. Plasmids or mRNAs can be delivered via nanoparticles, which can also shield them from nuclease breakdown. Small interfering RNA (siRNA) and mRNA may be transported by LNPs, a capability that has been well confirmed in preclinical and clinical research. However, due to their low delivery effectiveness to primary cells or in vivo animal trials, the editing efficiency of employing LNPs to package CRISPR/Cas9 plasmids has not satisfied the therapeutic criteria. The efficiency of the lipid nanodrug delivery system might be greatly increased by modification. Local administration of LNPs in vivo to the inner ear, muscle, brain, and malignancies is severely limited due to the challenges in controlling their size, homogeneity, and stability. It is currently difficult to create stable nanoparticles that would allow RNPs to be delivered systemically to the intended organs.

Researchers have created a universal engineering technique that involves incorporating permanent cationic additives, such DOTAP, into ionizable LNP formulations in order to preserve the integrity of RNP. Lipid components facilitate the encapsulation of C RNPs with intact activity and reroute DNA editing to the intended tissues. This allows low-dose intravenous injections to selectively target organs such as the brain, liver, lungs, and sphincter muscles [35]

Functionalized liposomes known as biodegradable lipid-like nanoparticles (LLNs) efficiently transport CRISPR/Cas9 payloads while lowering the biological toxicity of liposomes.[15(11)] created and produced a range of lipid-like, biodegradable substances with ester groups to transport Cas9 mRNA both in vivo and in vitro. Additionally, research has shown that the intracellular milieu including biodegradable lipids can enhance liposome escape and cargo release. Thus, adding reducible disulfide bonds to lipids' hydrophobic tails can increase the effectiveness of their gene delivery and encourage endosomal escape [36].

3. Non-Viral Vector Delivery Systems for CRISPR/Cas9: In Vitro Precision to In Vivo Therapeutic Potential.

The majority of research has depended on viral vectors, which are recognised to elicit immunogenic responses in the host and to carry the possibility of insertional mutagenesis [37,38]. Viral vectors can accomplish targeted delivery, however because of safety concerns, they might be better suited for application in cells for ex vivo therapy [39,40]. However, liposomes, polymers, cell-penetrating peptides (CPPs), and other nanoparticles are non-viral delivery vectors for CRISPR that are employed in research. For instance, it has been observed that lipofectamine and other lipid-based carriers can complex and transport CRISPR components [41]. Others have used DNA nanoclews to accomplish delivery [42], or gold nanoparticles [43]. In recent years, CRISPR/Cas9 systems have been widely employed to modify mouse zygotes and cultured cells both in vitro and ex vivo. Most of these delivery systems rely on physical techniques such as membrane deformation, electroporation, nucleofection, and microinjection, as well as viral vectors. Other non-viral vectors have therefore also been swiftly created in the interim. For the purpose of causing gene disruptions at the CCR5 locus in human cell lines, such as HEK293T, HeLa, NCCIT (a human pluripotent embryonal carcinoma cell line), human dermal fibroblasts, and human embryonic stem cells, Ramakrishna et al. developed CPP-conjugated recombinant Cas9 protein and CPP-complexed guide RNAs. This method effectively disrupted the CCR5 gene with few off-target alterations [44]. In the meanwhile, Mout and colleagues developed a nanoplatform based on gold nanoparticles modified with arginine for coasembly with designed Cas9 protein and sgRNA. Through membrane fusion, this nanoassembly can effectively infiltrate the targeted cells and allow the protein payload to be released directly into the cytoplasm,

avoiding endosomes. Using this method, they were able to effectively alter up to 30% of the AAVS1 and PTEN genes in the HeLa, HEK-293T, and Raw 264.7 cell lines [45]. To deliver a genome editing system based on CRISPR/Cas9, self-assembled DNA nanoclews were created. These nanoclews allowed for effective target disruption of the EGFP gene in vitro and in U2OS when they were loaded with CRISPR RNPs. After intratumoral injection, EGFP xenograft tumour models in vivo [46]. Lipid-like nanoparticles were created by Jiang et al. to specifically carry Cas9 mRNA and sgRNA to the liver. By using this method, the proprotein convertase subtilisin/kexin type 9 (pcsk9) gene and HBV DNA were disrupted in vivo [47]. Catalytic lipid nucleic acid transfection reagents were also used to deliver modified Cas9 protein topically for in vivo genome editing. Transgenic Atoh1-GFP animals had their cochleas injected with Cas9: sgRNA complexes targeting the GFP gene by Zuris et al. About 20% of hair cells underwent Cas9-mediated genomic alteration as a result of this method [48]. These are preliminary investigations into the delivery of CRISPR/Cas9 systems using non-viral vectors in vivo. These early research raises the prospect of using non-viral vectors to deliver CRISPR/Cas9 systems in vivo to cure genetic illnesses.

4. Precision Genome Editing: The Advancements of CRISPR/Cas9.

In recent years, the groundbreaking genome editing technique known as clustered regularly interspaced short palindromic repeats (CRISPR)/associated protein 9 (CRISPR/Cas9) has garnered significant interest and quick development due to its extraordinary potential for treating genetic disorders. In order to defend themselves against invasion nucleic acids, bacteria and archaea used their natural CRISPR/Cas system as an adaptive immune system. In 1987, a series of repetitive short sequences in the genome of Escherichia coli (E. coli) were identified as the CRISPR [49]. Zhang et al. and Church et al. (2013) showed CRISPR/Cas9 gene editing in mammalian cells, providing a preprogrammed tool for genomic surgery [50]. Effective genome editing in eukaryotic cells requires the presence of two key components of the CRISPR/Cas system: gRNA and Cas protein. The Cas9 that comes from Streptococcus pyogenes can be found currently. Cas9 (SpCas9) is the most concerning endonuclease variant due of its ease of usage and high target selectivity [51]. The Cas9 shares two active sites termed HNH and RuvC, which could cleavage double-stranded DNA (dsDNA) and create site specific double-strand breaks (DSB) [52]. The HNH nuclease site cuts the complementary strand tocrRNA whereas the RuvC domain cleaves the opposite DNA strand. Located upstream of the Cas9 binding site is the protospacer-adjacent motif (PAM), which contains the 50-NGG base sequence. Furthermore, the different tracrRNA and crRNA might be combined to form a simple single-guide RNA (sgRNA) [53]. Under the guidance of sgRNA, the Cas9 protein can accurately target any genomic site via base pairing and create a DSB. Cells then use either non-homologous end-joining (NHEJ) or homology-directed repair (HDR) to repair the damaged DNA [54]. The CRISPR/Cas9 system is comprised of three fundamental elements: the Cas9 protein, CRISPR RNA (crRNA), and trans-activating CRISPR RNA (tracrRNA), which functions to enhance the repeat regions of crRNA [55]. The Cas9 protein attaches itself as a single-guide RNA to a mature dual RNA (tracrRNA: crRNA) in order to initiate DNA cleavage at the target (sgRNA). In order to pick the target DNA from the genome and limit the expression of target genes, Cas9 employs a short protospacer adjacent motif (PAM) identification to determine the viral DNA sequence [56]. Certain areas of the genome experience double-stranded DNA breaks (DSBs) as a result of the Cas9 protein binding to the target sequence [57]. Different DNA damage repair processes, namely homology-directed repair (HDR), classical non-homologous end joining (cNHEJ), and microhomology-mediated end joining, are employed by cells to repair double strand breaks (DSBs) (MMEJ) [58]. Asymmetric repair may be possible if different DNA repair pathways are employed to fix either end at a DSB [59]. These repair techniques are applied in therapeutic settings with the aim of treating or even curing illnesses. Because the repair mechanism is prone to mistake, DSBs are created and repaired at specified sites in CRISPR/Cas9-mediated targeted gene segments, resulting in targeted alterations. CRISPR/Cas9 gene editing has demonstrated great promise in oncology and has achieved a promising result by correcting the targeted mutation.

5. CRISPR/Cas9 Genome Editing for Therapeutic Intervention in Human Diseases

Screening, monitoring, and controlling genes is made possible by the strong and potent CRISPR/Cas system, which may also be used to modify a gene's expression and function. since 2012, when it was regarded as a technique for genome editing. [60,61]

The CRISPR/Cas system has been extensively employed in the study of human gene function and/or human-related features, with a focus on elements linked to human development and genetic diseases. Over the last eight years, research on CRISPR/Cas has demonstrated the enormous promise of this technology for gene therapy. CRISPR/Cas9 genome editing may be used to cure every human illness linked to genetic mutation or genetic element change. Here are just a few instances of how quickly CRISPR/Cas9 genome editing has developed and been used to fix genetic mutations linked to a variety of human illnesses. enormous promise for curing hereditary illnesses in humans with CRISPR/Cas genome editing. Nowadays, a wide range of human genetic illnesses, such as those pertaining to the liver, brain and neurogenetic disorders, heart disease, lung disease, and other organs, are treated with CRISPR/Cas9 genome editing technology [62]

CRISPR/Cas9 Genome Editing (Therapy) for Liver.

In individuals with loss-of-function FAH mutations who have hereditary tyrosinemia type I (HTI), harmful metabolites build up and damage the liver. Using hydrodynamic injection of plasmids expressing CRISPR/Cas components or a combination of AAV carrying sgRNA and HDR template and nanoparticles containing Cas9 mRNA, CRISPR/Casmediated HDR has been utilised to repair FAHmut/mut in the HTI mouse model [63, 64]. VanLith et al. treated HTI mice and FAH-knockout recipients by transplanting modified hepatocytes containing corrected FAH. [65]. Song et al. corrected an FAH point mutation in an adult mouse form of HTI with ABE [66]. To cure HTI metabolic illness and minimise toxic metabolite buildup, several groups have knocked down hydroxyphenylpyruvate dioxygenase (HPD), an upstream enzyme of FAH that works in the second phase of tyrosine catabolism, in addition to correcting FAH [67]. Due to a hazardous gain-of-function mutant allele, patients with alpha-1 antitrypsin deficiency (AATD) have progressive lung illness as well as liver disease from the loss of AAT antiprotease activity. To lessen the pathologic liver phenotype, mutant AAT has been disrupted using CRISPR/Cas-mediated NHEJ. [68], an AAT point mutation has been corrected using HDR, although [69].

CRISPR/Cas9 Genome Therapy (Editing) on Cancer cell.

Tumor-specific immune checkpoint blocks (ICBs), adoptive cell transfer (ACT), and tumor-specific vaccinations are examples of actively or passively suppressing cancer through tumor-specific responses [70]. Although immunotherapy heralded a new era in the fight against cancer, it only proved effective against a small percentage of tumours and only certain cancer patients responded to it [71]. Conversely, the impact is less than anticipated due to the existence of immunological escape. Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and the CRISPR/Cas9 system are used in gene therapy to delete, insert, and modify the targeted gene in order to cure cancer [72]. Although they are difficult and time-consuming, ZFNs and TALENs have been used to modify specific genes in the body [73]. The CRISPR/Cas9 system is preferred for gene editing over ZFNs and TALENs because of its ease of use, practicality, and variety of applications. This is why the CRISPR/Cas system is so popular. CRISPR/Cas9 gene editing is anticipated to offer a novel approach to tumour treatment for tumour and immune cells.

Tumour cells employ CRISPR-Cas9 technologies to fight cancer. The primary techniques used in tumour cell editing are as follows:

- Because Cas9 can accurately and precisely cleave DNA locations in a planned way, gene knockout is a promising strategy for targeting pathogenic genes. By altering the CCR5 gene in CD4+ T cells, gene editing techniques can fight HIV infection; moreover, tampering with the BCL11A gene may be able to treat β-thalassemia and sickle-cell anaemia [74-79].
- Mutations are fixed by targeted DNA insertion, which returns the sequence to normal. In monogenic autoimmune disease cells, large DNA sequences (>1 kb) can rectify IL2RA mutation [80,81].
- Tumorigenic mechanism research has been made easier by modelling chromosomal translocations associated with cancer, such as the human alveolar rhabdomyosarcoma Pax3-Foxo1 chromosome translocation in mouse myoblast [82,83]
- To give the point mutation required for various gene editing procedures, including screening and monitoring, base editing [84]. The most prevalent kind of deleterious mutations in humans are point mutations, also referred to as single nucleotide polymorphisms, or SNPs. This meant that in order to accurately change a single base pair within a large genome, a new technique was required [85,86] David

R. Liu's research team pioneered the advancement of a CRISPR/Cas base editor (BE) technique, commonly referred to as cytosine base editors (CBEs), which facilitates the targeted substitution of particular bases. This innovative technology enables the conversion of cytidine to thymine or guanine to adenine. Subsequently, the development of adenine base editors (ABEs) expanded the repertoire of base pair modifications by allowing the conversion of A·T to G·C base pairs. Consequently, this breakthrough offers a comprehensive solution to rectify point mutations, presenting four potential alterations [87-89].

Conclusion:

In summary, the development of CRISPR/Cas9 technology has unquestionably transformed genetic research, providing previously unheard-of precision in gene editing and great potential for the treatment of inherited disorders. The plasmid-based genome editing method has emerged as a key tool for analysing gene functions and correcting genetic alterations linked to a range of diseases. It has explored the intricacies involved in selecting viral and non-viral vectors to improve gene editing during clinical trials.

The choice of suitable vectors is crucial in establishing the efficacy and security of gene therapy treatments. We have emphasized the continuous attempts to improve and optimize these techniques by examining the state-of-the-art ways that are now being used to augment gene editing in clinical contexts. Successful integration of this innovative, yet still-emerging approach into standard medical treatments requires navigating its complexities. It's critical to remain up to date on the most recent advancements and techniques as gene therapy progresses. To guarantee the appropriate and efficient use of CRISPR/Cas9 technology in clinical trials, researchers and practitioners must take safety, ethical, and regulatory considerations into account.

Additionally, the study clarified current discoveries that use the genetic resources of bacterial adaptive immunity for mutagenesis and site-specific cleavage. This demonstrates the inventive ways in which biological processes may be used for therapeutic ends, as well as the multidisciplinary character of genetic research.

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