The Control and Trust of CRISPR / Cas9 Genome Altering for Clinical Application with Gene Treatment and Treatment of Hereditary Diseases

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Abstract

Clustered Frequently Interspaced Brief Palindromic Rehashes (CRISPR) is determined from the bacterial natural safe framework and designed as a strong gene-editing apparatus. Due to the higher specificity and proficiency of CRISPR/Cas9, it has been broadly connected to numerous hereditary and non-genetic malady, counting cancers, hereditary hemolytic illnesses, obtained immunodeficiency disorder, cardiovascular illnesses, visual maladies, and neurodegenerative infections, and a few X-linked maladies. Besides, in terms of the restorative technique of cancers, numerous analysts have utilized the CRISPR/Cas9 procedure to remedy or lighten cancers through diverse approaches, such as quality treatment and resistant treatment. Here, we conclude the later application and clinical trials of CRISPR/Cas9 in non-cancerous illnesses and point out a few of the issues to be illuminated. Focus on the toughest barrier to potential in vivo use of CRISPR / Cas9 is then delivered. Shipping & Conveyance Vehicles Detailed to CRISPR / Cas9 Depict viral conveyance strategies (such as adenovirus-associated infection (AAV), full-size, non-viral adenovirus, and lentivirus. Gold), and we talk about their comparative focal points, which appear promising in this respect.CRISPR/Cas9, determined from the microbial natural safe framework, is created as a strong gene-editing device and has been connected broadly. Due to its tall exactness and proficiency, CRISPR/Cas9 strategies may give an awesome chance to treat a few gene-related maladies by disturbing, embeddings, rectifying, supplanting, or blocking qualities for clinical application with quality therapy.

Review

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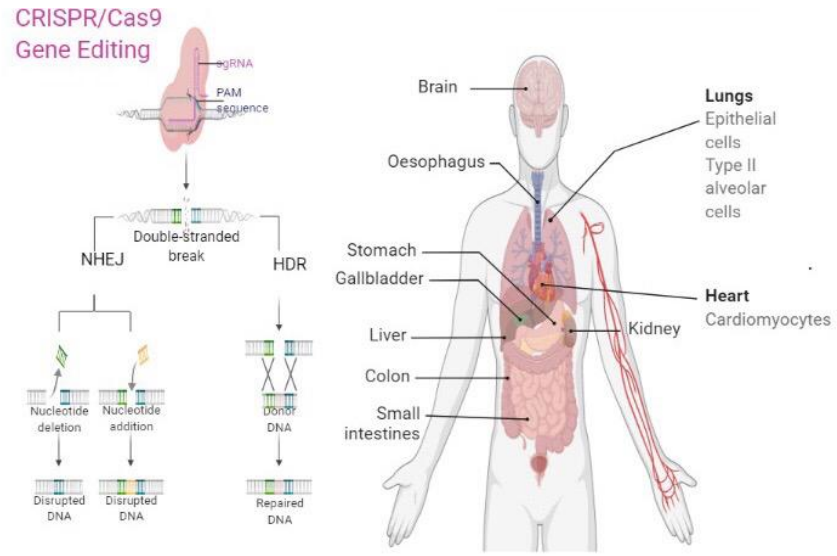
Graphical abstract



Abstract

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Keywords: Gene therapy, Clinical application, resistant therapy, CRISPR/Cas9

The advance improvement of CRISPR/Cas9 innovation

Taking after the fast advancement and the impressive victory of the CRISPR/Cas9 procedure,

CRISPR/Cas9 is re-engineered to apply in other viewpoints. For illustration, utilizing the focusing on capacity of CRISPR/Cas9, the CRISPR interference (CRISPRi) and catalytically dead Cas9 protein (dCas9)were outlined by evacuating nuclease spaces to diminish or stifle quality expression [1] whereas Cas9 nickase (nCas9) that may make a crevice in single DNA arrangement as it were misfortune the fractional capacities of nuclease movement [2]. One year afterward after creating dCas9, the same lab intertwined translation

variables with dCas9 to increase quality expression, specifically CRISPR enactment (CRISPRa) [3]. In expansion,more precise and more secure CRISPR/Cas9-based gene-editing strategies without presenting DSBs, like base editing[4,5] and prime editing[6],were too created. The base altering devices, counting cytidine base editor (CBE) and adenine base editor (ABE), ordinarily comprise of cytidine deaminase/evolved adenine deaminase-fused nCas9/dCas9. The CBE realizes the transformation of CG to TA whereas the ABE empowers the change of AT to GC, and the current created nNme2-CBE framework appears higher altering proficiency and flexibility [7]. In any case, the applications of base altering instruments are still restricted since they can as it were apply particular base conversion. Therefore, prime altering instruments are created by means of melding built switch transcriptase to dCas9 and including preliminary arrangements to gRNA (pegRNA) to realize the precise quality altering with lower off-target effect [6]. Both base altering and groundwork altering instruments are more secure than the conventional CRISPR/Cas9 so they are potential to be connected in clinical quality treatment. Interests, Zhang F and his colleagues created CRISPR-associated transposase in 2019, which too accomplishes quality addition without DNA cleavage [8]. In terms of quality treatment in vivo, various Inducible CRISPR frameworks were examined, such as photoactivated and chemically actuated Cas9 [9,8], and smaller-size CRISPR altering instruments were moreover found recently[10].

Ex vivo genome editing

Ex vivo genome altering may be a helpful approach in which the genome of particular cells are altered in vitro, and after that those adjusted cells are transplanted back into the patient to exert a restorative impact (particularly in which the helpful impact may be a result of the genome altering). This approach is in coordinate differentiate to in vivo genome altering approaches, in which the CRISPR/Cas9 or other genome altering components are specifically presented into the quiet through nearby or systemic conveyance and apply their helpful impact on-site [11,12]. Compared with the in vivo technique, the ex vivo altering procedure requires more steps (e.g. cell collection, confinement, extension, altering, determination, and transplantation) and may be way better suited for focusing on a particular organ instead of the complete living being [13]. Be that as it may, it to a great extent dodges the colossal in vivo conveyance challenges which have been depicted broadly in other survey papers [14,15]. Moreover, the ex vivo approach may have specific security benefits, particularly with respect to off target quality altering. In vivo approaches must stress almost unintended off-target altering occasions, either within the frame of unintended conveyance to an off-target cell sort, or within the shape of unintended altering of an off-target locus within the genome. Ex vivo approach dodges this issue by as it were altering precisely the aiming cell sort, and permitting an opportunity to screen for effective altering. In this area, we highlight the ex vivo applications of CRISPR/Cas9 for helpful genome altering. The focused on conditions, genome altering methodologies, and related references have been summarized in Table 1.

Clinical Trials Involving Ex Vivo CRISPR-Based Genome Editing

The primary clinical trial on CRISPR-based ex vivo genome altering endeavored to treat human immunodeficiency infection sort 1 (HIV-1) infection [16]. Disturbance of the CCR5 quality, which encodes a vital co-receptor for viral section, was initiated by nucleofection of ribonucleoprotein complexes focusing on CCR5 into patient-derived hematopoietic stem and begetter cells (HSPCs), which were hence exchanged back to the understanding. A 27-year-old male with HIV-1 disease and intense lymphoblastic leukemia gotten the treatment and appeared effective transplantation and long-term engraftment of CRISPR-edited HSPCs. CCR5 disturbance efficiencies extended from 5.2% to 8.3% in bone marrow cells over 19 months, which was not satisfactory to realize the corrective target (ClinicalTrials.gov, NCT03164135). Recently, a clinical trial endeavoring to treat serious monogenetic maladies with CRISPR-based genome altering detailed promising results [17]. Sickle cell malady and beta-thalassemia speak to unmistakable bunches of acquired hemoglobinopathies caused by changes within the hemoglobin beta-subunit (HBB) quality, which lead to mutant, decreased, or truant beta-globin proteins. In this think about, instead of the pathogenic HBB quality itself, an enhancer of the BCL11A quality, which could be a translation figure curbing gamma-globin amalgamation, was focused on to reestablish the generation of fetal hemoglobin and compensate for the changed HBB quality. Patient-

derived HSPCs were altered with CRISPR-Cas9 with a sgRNA focusing on the BCL11A enhancer to create gene-edited HSPCs called CTX001. One understanding with transfusion-dependent beta-thalassemia and one persistent with sickle cell malady were imbued with single measurements of CTX001 after myeloablation. Tall altered allele frequencies and levels of fetal hemoglobin were maintained, and both patients maintained a strategic distance from disease-related transfusion occasions over 21.5 and 16.6 months.In spite of the fact that some genuine unfavorable occasions were display in both patients (neutropenic pneumonia and veno-occlusive liver infection with sinusoidal hindrance disorder in Understanding 1; sepsis with neutropenia, cholelithiasis, and stomach torment in Quiet 2), they settled with suitable treatment. These trials are continuous; with additional preparatory comes about broadly steady with the first findings (ClinicalTrials.gov, NCT03655678, NCT03745287) .CRISPR-based ex vivo genome altering has moreover been connected to the treatment of headstrong cancers. [18-19-20].

The fundamental concept here is to upgrade the common anti-tumor reactions of cytotoxic T cells by the evacuation of resistant checkpoint modulator qualities through CRISPR-Cas9. Two clinical trials utilizing this technique were recently published, with blended victory. Within the to begin with report, analysts endeavored to treat different progressed, hard-headed cancers, counting different myeloma and liposarcoma, through CRISPR-Cas9 genome editing [21].

For this reason, T cells were disconnected from cancer patients and built with CRISPR-Cas9 to expel the endogenous T cell receptor (TCR) and safe checkpoint atom modified cell passing protein 1 (PD-1). Particularly, erasure of the TCR α chain (TRAC) quality, TCR β chain (TRBC) quality, and PDCD1 quality was initiated by electroporation of ribonucleoprotein complexes into patient-derived T cells. The altered T cells, named "NYCE" (NY-ESO-1–transduced CRISPR 3X altered cells), were in this way infused into the patients intravenously. A add up to of 3 patients gotten treatment and appeared steady engraftment of built T cells, in spite of the fact that altering frequencies of the target qualities in fringe blood mononuclear cells were moderately moo at 5% to 10%. No noteworthy off-target altering or genuine unfavorable occasions were famous. Clinically, as it were one understanding appeared tumor relapse constrained to early stages of treatment, and all tumors inevitably advanced, coming about in end of the trial (ClinicalTrials.gov, NCT03399448). Another comparative trial, detailed without further ado from that point, endeavored to treat hard-headed non-small cell lung cancer with CRISPR-engineered patient-derived T cells by focusing on the PD-1 gene [22].

Particularly, disturbance of the moment exon of the PD-1 quality was actuated by electroporation of Cas9- and sgRNA-encoding plasmids into patient-derived T cells. A add up to of 12 patients were treated; they appeared steady reasonability and development of altered T cells, in spite of the fact that the middle quality altering proficiency was very moo at 5.81%. No critical off-target altering or genuine antagonistic occasions were display. Clinically, as it were 2 patients appeared steady illness at 8 weeks, all patients inevitably had infection movement, and 11 patients passed on of infection movement (ClinicalTrials.gov, NCT02793856).

In vivo genome editing

Within the to begin with report of CRISPR-based in vivo genome altering, this innovation was connected to transthyretin amyloidosis, or ATTR amyloidosis, which comes about from the collection of misfolded transthyretin (TTR) protein in tissues[23].

ATTR amyloidosis could be a monogenic infection, and almost all TTR proteins are created within the liver, which makes the condition an amazing target for CRISPR-based in vivo genome altering. NTLA-2001, a liver-trophic lipid nanoparticle framework containing Cas9 mRNA and sgRNA focusing on the human TTR quality, was outlined to diminish circulating TTR protein levels in people. Six patients with TTR changes and tactile polyneuropathy were treated with a single injection of NTLA-2001, which diminished serum TTR levels by 52% within the low-dose bunch and 87% within the high-dose bunch after 4 weeks, with negligible side impacts. The ponder is as of now continuous; serial estimations will proceed to affirm the long-term toughness and security of the treatment (ClinicalTrials.gov, NCT04601051). In spite of the fact that the comes about have not been detailed however, numerous bunches are endeavoring to utilize CRISPR-based in

vivo genome altering to treat monogenic infections, counting Leber innate amaurosis 10 (ClinicalTrials.gov, NCT03872479). Table 1 list continuous clinical trials utilizing ex vivo and in vivo CRISPR-based genome altering. Moreover, broad investigate on creature models proceeds to supply promising targets for advance applications, which can be examined within the following section.

Table 1. Clinical Trials Involving Ex Vivo/ In vivo CRISPR-Based Genome Editing

status	NCT Num	diseases
Completed	NCT04191148	Urinary Tract Infections, UTI
Unknown	NCT03728322	Thalassemia Genetic Diseases
Active, not recruiting	NCT03655678	Thalassemia Genetic Diseases
Active not recruiting	NCT04205435	Beta-Thalassemia
Enrolling by invitation	NCT05143307	HIV
Concerned	2018-001320-19	Sickle Cell Disease, Haematological Diseases, Haemoglobinopathies
Active	NCT03745287	Sickle Cell Disease, Haematological Diseases, Haemoglobinopathies
Active	ChiCTR2100052858	Transfusion Dependent Beta-Thalassaemia, TDT
Active	NCT04037566	Leukemia Lymphocytic Acute in Relapse, Leukemia Lymphocytic Acute (A
Active	NCT04560790	Herpes Simplex Virus Refractory Keratitis
Active	NCT03872479	Blindness, Leber Congenital Amaurosis
Active	NCT05210530	Type 1 Diabetes, T1D
Active	NCT05120830	Hereditary Angioedema, HAE
Active	NCT04601051	Hereditary Transthyretin Amyloidosis, ATTR

Animal studies in the laboratory

Leber inherent amaurosis (LCA, OMIM #204000) comprises a bunch of early-onset childhood retinal dystrophies, with each subtype caused by changes completely different qualities. LCA sort 10 (LCA10, OMIM #611755) is caused by transformations within the CEP290 quality. CRISPR-based genome altering has been connected to humanized LCA10 mouse models; wild-type CEP290 expression was successfully reestablished by subretinal infusion of a single AAV encoding both SaCas9 and sgRNA [24].

LCA sort 2 (LCA2, OMIM #204100) is caused by transformations within the RPE65 quality. The rd12 mouse show of LCA2 was subjected to subretinal infusion of two AAVs encoding 1) SpCas9 and 2) sgRNA and donor DNA, coming about within the recuperation of retinal function.38 As of late, the same mouse show was effectively treated with subretinal infusion of adenine base editors utilizing RNPs [25] or intein-mediated part AAV vectors, [26] and prime editors utilizing trans-splicing part AAV vectors, [27]

Appearing guarantee in helpful genome altering with unused genome editors. Retinitis pigmentosa (OMIM #268000), which alludes to a heterogeneous gather of acquired visual infections that result in dynamic retinal degeneration, comprises of 92 diverse phenotypes and is caused by changes in over 200 qualities. AAV-mediated quality exchange to treat retinitis pigmentosa has as of now been affirmed as the primary AAV quality treatment in history [28].

but fundamental ponders on CRISPR-based genome altering for this reason as it were begun in 2016. Since at that point, numerous CRISPR-based approaches, each focusing on a diverse quality (*NrlMertk* , *Pde6b* , *Rho* , and *RPGR*) [29-30-31-32-33-34-35], have accomplished victory in creature models. Particularly, the Nrl quality was exhausted through NHEJ or curbed by means of an approach called CRISPR obstructions, both affects by AAV vector conveyance of CRISPR components, and the Mertk quality was rectified with a novel strategy called homology-independent focused on integration. The Pde6b quality was redressed through homology-directed repair, and the Rho quality was exhausted through NHEJ, both actuated by in vivo electroporation of Cas9-encoding plasmids. Hereditary tyrosinemia sort 1 (HT1, OMIM #276700), a deadly hereditary clutter caused by transformations within the fumarylacetoacetate hydrolase quality, comes about

within the aggregation of harmful metabolites that lead to extreme liver harm. CRISPR-based genome altering was to begin with utilized in humanized mouse models of HT1 in 2014, and brought about in adjustment of the pathogenic transformations and protect of the deadly phenotype[36].Mutation-corrected hepatocytes, which show a development advantage over changed hepatocytes, can repopulate the liver indeed at a really moo altering recurrence. Taking after this starting work, Cas9 variations (NmeCas9, [37] St1Cas9 [38].), base editors [39-40] and prime editors [41].have effectively protected the deadly HT1 phenotype in grown-up mouse models. Phenylketonuria (PKU, OMIM #261600) is an autosomal latent liver illness caused by transformations within the phenylalanine hydroxylase quality, which may cause mental impediment due to the neurotoxicity of metabolites. In grown-up mouse models, intravenous infusion of AAVs encoding an intein-split cytosine base editor effectively reestablished blood phenylalanine levels and switched the PKU-associated hide color [42].Afterward, the ordinary homology-directed repair approach too effectively improved side effects with the assistance of chemical modifiers [43-44].Ornithine transcarbamylase (OTC) lack (OMIM #311250), an X-linked metabolic clutter characterized by hyperammonemia, is caused by transformations within the OTC gene (OMIM *300461). Employing a double AAV framework containing 1) SaCas9-encoding groupings and 2) sgRNA-encoding groupings and giver DNA, OTC changes were rectified by homology-directed repair, coming about in expanded survival in mouse models [45].Duchenne muscular dystrophy (DMD, OMIM #310200) is an acquired X-linked infection caused by changes within the dystrophin quality. CRISPR-based genome altering was to begin with utilized to adjust transformations and reestablish expression of dystrophin in mouse zygotes in 2014 [46].After this beginning work, numerous inquire about bunches detailed fruitful CRISPR-Cas9-mediated reclamation of dystrophin expression, in grown-up mouse [47-48-49] ,dog [50] ,and pig[51] models of DMD. Adenine base altering moreover successfully turned around DMD pathology in mouse embryos and grown-up mouse models [52].Amyotrophic sidelong sclerosis (ALS) could be a neurodegenerative clutter in which the dynamic passing of engine neurons comes about in loss of motion. A few causative qualities have been distinguished as basic innate ALS, and transformations in SOD1 (OMIM *147450) are dependable for most cases of ALS sort 1 (ALS1, OMIM #105400). As of late, intravenous infusion of AAV encoding SaCas9 and SOD1-targeting sgRNA was appeared to delay illness onset and make strides engine capacities in ALS mouse models [53].Glycogen capacity illness Ia (GSD1A, OMIM #232200), too known as von Gierke illness, is caused by pathogenic transformations within the glucose-6-phosphatase alpha subunit (G6PC) gene that result within the amassing of glycogen all through the body. As of late, the profoundly predominant G6PC p.R83C variation was subjected to in vivo CRISPR-based genome altering in mouse models utilizing two AAVs, one encoding SaCas9 and the other encoding sgRNA, [54] coming about in normalization of G6Pase movement, diminishments in serum affront levels, and long-term survival. Hutchinson-Gilford progeria disorder (HGPS, OMIM #176670) is caused by changes within the lamin A (LMNA) quality. As of late, the LMNA c.1824 C>T transformation, which is found in over 90% of patients with HGPS, was redressed in transgenic mouse models utilizing AAVs encoding part adaptations of the adenine base editor, coming about in change of vascular pathology and expansion of life span [55] This report illustrated the potential of modern genome editors for specifically rectifying point changes to treat hereditary disorders.

CRISPR/Cas9 delivery platforms

To completely abuse the quality altering potential of CRISPR/Cas9, they must be proficiently presented into target cells or tissues utilizing suitable vectors [56].This area will survey the merits and absconds of each conveyance method.

Viral vectors

Recombinant viral vectors have been created utilizing capacity of infections to exchange outside hereditary fabric into cells to convey helpful qualities to infected tissues (Table 2) [57]. Among numerous viral vectors, adeno-associated infection (AAV), lentivirus, and adenovirus play a pivotal part in genome altering treatment and have been broadly utilized in preclinical models and clinical trials. In spite of the fact that adjusted viral vectors don't cause extreme human malady, they can actuate safe system-mediated clearance, which may decrease conveyance proficiency [58]. Another include of viral vectors is the capacity to coordinated

DNA into the host genome to attain steady quality expression, which may lead to off-target impacts and embed transformation [59]. Subsequently, the application of infection conveyance strategies is advanced.

Table 2.Virus vectors for CRISPR / Cas9 delivery system

delivery	Packaging capacity	benefits	Flaws
Lentivirus	Approximately 10 kb	High transduction efficiency Large cargo size Low immunogenicity Can transduce dividing and non-dividing cells in different tissues	Non-specific DNA integration causing cancer risk Complex packaging structure
Adenovirus	Approximately 8-10 kb	Efficient delivery Large cargo size	Inflammatory response
Adeno-associated virus (AAV)	Approximately 4.7 kb	Multiple serotypes Low immunogenicity Can transduce dividing and non-dividing cells in different tissues	Pre-existing neutralizing antibodies Long-term expression of Cas9 causing off-target effects

Adenovirus

Adenovirus may be a double-stranded DNA infection with a distance across of 80–100 nm. Its genome is ˜ 34–43 kb in length and can bundle ˜ 8 kb of exogenous DNA [60]. Due to its fabulous capacity to carry huge hereditary cargo, conveyance proficiency of the adenovirus vector-mediated CRISPR/Cas9 module can be progressed by conferring extra atomic localization signals [61]. Nonstop progression of innovation has created adenoviral vectors missing the viral genome, permitting stacking of target DNA up to 37 kb [62]. Adenovirus can contaminate isolating and non-dividing cells, but one of its significant preferences is that its genome isn't coordinates into the have cell, decreasing off-target impacts and inclusion transformations [63]. By the by, due to its pathogenicity, presentation of adenovirus vectors can trigger the body's safe reaction [64]. In spite of the fact that this reaction may upgrade the murdering impact on tumor cells, the neutralizing counter acting agent reaction caused by enactment of B cells isn't conducive to consequent vector conveyance [65]. Hence, diminishing the have safe reaction to the adenoviral vector will significantly make strides security and conveyance effectiveness of this vector. Utilizing poly (lactic/glycolic corrosive) copolymer to typify recombinant adenovirus vectors diminishes the immunogenicity of adenoviruses and empowers in vitro disease within the nearness of neutralizing antibodies, giving unused bits of knowledge for improvement of progressed viral vectors [66].

GEMMs of human cancer are imperative instruments to analyze the atomic components of tumorigenesis [67]. Presenting CRISPR/Cas9 into physical cells of grown-up creatures utilizing adenovirus vectors actuates particular chromosomal improvements to produce a mouse demonstrate of Eml4-Alk-driven lung cancer [68]. This methodology extends how researchers recreate human cancer in demonstrate living beings by rearranging complex and time-consuming hereditary controls. So also, adenoviral vectors have been utilized to intercede quality altering focusing on Pten in a mouse show of nonalcoholic steatohepatitis (NASH), in which mice infused with adenoviral vector appear signs of hepatomegaly and NASH after 4 months. Indeed within the nearness of typical adenoviral vector-related immunotoxicity within the liver, adenoviral vectors can still intervene effective Pten quality altering, giving a novel strategy to imitate human liver malady in mice [69].GEMMs produced by site-specific recombinase innovation are exorbitant and time-consuming, but adenoviral vector-mediated CRISPR/Cas9 quality altering can successfully deliver numerous subtypes of delicate tissue sarcoma in wild-type mice and GEMMs. Whole-exome sequencing appears that sarcomas produced utilizing CRISPR/Cas9 are comparative to those produced utilizing conventional recombinase

innovation, demonstrating the system's potential to quickly create cancers with comparative genotypes and phenotypes as conventional innovations [70].

Adeno-associated virus (AAVs)

AAVs comprise of an icosahedral protein capsid with a breadth of ˜ 26 nm and ssDNA genome of ˜ 4.7 kb [71]. AAV vectors have various focuses of intrigued, such as require of pathogenicity, long-term quality expression, and the capacity to sully isolating and non-dividing cells, so they are utilized broadly for in vivo transport systems [71, 72].In expansion, AAV family is characterized by wealthy serotype differences and has variable tropism, particularly focusing on distinctive organs [73]. In spite of the fact that AAVs are fabulous quality treatment conveyance vehicles, they still have shortcomings when utilized to convey CRISPR/Cas9 in vivo. The ideal AAV vector estimate is 4.1–4.9 kb. In spite of the fact that AAV can bundle vectors bigger than its genome measure, bundling proficiency drops strongly [74]. For case, the estimate of the SpCas9 protein is ˜ 4.2 kb, and recombinant AAV must too contain administrative components vital for quality expression, so AAVs cannot be utilized to provide numerous expansive quality arrangements [71]. When utilizing AAVs for transfection, SpCas9 and sgRNA must be encoded on diverse vectors [75, 76].Another major issue of AAV is pre-existing neutralizing antibodies against AAV in patients with past AAV contamination, which significantly decreases helpful adequacy [77]. In any case, combining capsid adjustment and genome adjustment to deliver an optimized AAV serotype vector can decrease liking with neutralizing antibodies, subsequently lessening have resistant reaction and making strides conveyance proficiency [78]. In expansion, long-term transgene expression of AAV too may be a chance, since ceaseless expression of Cas9 nuclease may cause critical off-target impacts [79]. Hence, there stay troubles in mass generation and application of AAVs.In spite of the fact that there are still numerous challenges to overcome, individuals have started to investigate AAV-mediated CRISPR conveyance. The AAV dual-vector framework effectively targets a single quality or different qualities within the mouse brain and characterizes the impacts of genome adjustment on neurons [80], proposing that that AAV-mediated genome altering can be connected to consider brain quality work. Since distinctive AAV serotypes have wide tissue tropism, AAV vector-mediated genome altering can too be utilized to produce creature models of cancer [81]. Platt et al. conveyed a single AAV vector to the lungs of Cas9 knock-in mice to intervene p53, Lkb1, and KrasG12D changes, driving to adenocarcinoma. In extension, application of AAV to supply sgRNA to Cas9 knock-in mice can be utilized for high-throughput mutagenesis in vivo to create autochthonous mouse models of cancer [82].

Lentivirus

Lentivirus may be a subcategory of the retrovirus family, and the lentivirus genome contains a single-stranded RNA of 7–12 kb [83]. Lentiviral vectors give successful cell transduction in different cell sorts (counting separating and non-dividing cells) and abbreviate the culture time required for cell transfection. Compared with adenovirus or AAV vectors, lentivirus appears moo cytotoxicity and immunogenicity and has negligible effect on transduced cells [84]. Since of their relative ease of utilize, lentiviruses are promising as in vivo conveyance frameworks. Regularly, lentivirus coordinating its genome into the have genome, which can essentially amplify the time for transgene expression. In any case, nonstop expression of Cas9 may increment the chance of off-target impacts and prevent application in high-precision genome altering [85]. As an elective, integration-deficient lentiviral vectors produced by integrase change can significantly diminish the hazard of inclusion transformations [86]. Preclinical ponders appear that lentiviral conveyance Cas9 and direct RNA focusing on changed KRAS essentially restrains multiplication of cancer cells [87]. Assist, lentiviral conveyance of CRISPR/Cas9 focusing on BCR-ABL essentially hinders myelogenous leukemia cell development and tumorigenesis, so treatments based on ABL quality altering may give a potential technique for imatinib-resistant inveterate myeloid leukemia patients [88]. So distant, lentiviruses have been affirmed for utilize by the U.S. Nourishment and Sedate Organization (FDA) and the European Solutions Organization [89].

Non-viral vectors

Security issues stay a primary bottleneck to wide clinical application of viral quality conveyance, with inade-

quacies counting insertional mutagenesis [90], safe reaction [58], and wide tropism [91]. As elective, non-viral vectors have been investigated for cancer treatment due to their moo immunogenicity, tall biocompatibility, amazing deliverability, and moo taken a toll for large-scale generation [92, 93]. Nanotechnology-based medicate conveyance frameworks will advance broaden applications of CRISPR/Cas9 treatment and move forward security, giving a practical approach to overcome the challenges confronted by viral vectors (Table 3).

Table 3. Nanotechnology-based delivery system for CRISPR / Cas9

Delivery system	Cargo options	benefits	Flaws
Polymer nanoparticles	RNP plasmid DNA RNP complex Cas9 mRNA sgRNA Donor DNA	High biocompatibility Low immunogenicity Reduce off-target effects Can be mass produced Low cost	Toxicity Limited delivery efficiency
Golden nanoparticles	RNP plasmid DNA RNP complex Cas9 mRNA sgRNA Donor DNA	High biocompatibility Low immunogenicity Reduce off-target effects Can be mass produced Low cost	Limited delivery efficiency
Lipid nanoparticles	RNP plasmid DNA RNP complex Cas9 mRNA sgRNA Donor DNA	High biocompatibility Low immunogenicity Reduce off-target effects Can be mass produced Low cost	Degradation in vivo

Lipid nanoparticles (LNPs)

LNPs are amphiphilic systems composed of diverse hydrophobic and hydrophilic components, such as cationic or ionized lipids, fair-minded lipids such as phospholipids or cholesterol, and polyethylene glycol–lipids.LNPs are fundamentally diverse from liposomes since LNPs have no ceaseless lipid bilayer or huge inner pool [94]. LNPs were created as carriers to convey an assortment of particles to cells, particularly with one of a kind points of interest in nucleic corrosive conveyance. Since nucleic acids are amazingly unsteady exterior the cell and carry a expansive sum of anions, they cannot effortlessly pass through the cell film. Be that as it may, epitome in cationic liposomes permits simple conveyance of nucleic acids into cells. Compared with conventional medicate treatment, LNPs have interesting points of interest counting anticipating sedate debasement, empowering focused on sedate conveyance, and diminishing sedate poisonous quality, which has created tall intrigued in LNPs for conveyance of anti-cancer drugs [95]. Pre-clinical trials appear that LNPs can effectively convey siRNA or mRNA [96, 97], so LNPs appear to be a secure and compelling conveyance device.

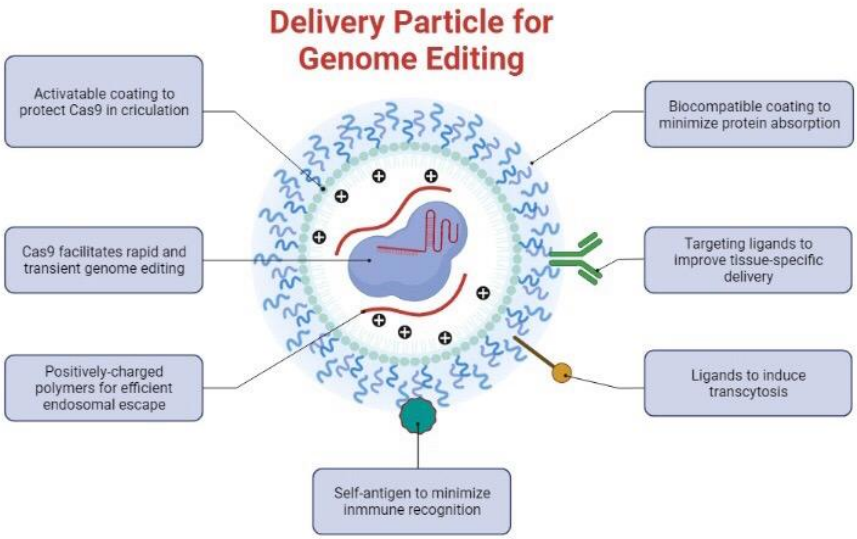
Within the past few a long time, numerous preclinical ponders of CRISPR/Cas9 conveyance utilized LNPs. Two fundamental strategies are utilized for LNP conveyance of CRISPR/Cas9 components: conveyance of Cas9 and sgRNA plasmid DNA or mRNA, or conveyance of Cas9: sgRNA RNP complex. Cas9 mRNA and sgRNA can be effectively stacked on LNPs and precisely transported to the liver of mice, successfully intervening mouse transthyretin (Ttr) quality altering [98]. In spite of a few advances, foreseeing and normally planning LNPs for conveyance to target tissues other than the liver for exact quality altering remains an issue. In 2020, Cheng et al. made a procedure called particular organ focusing on (SORT) by including supplementary components on the premise of conventional LNPs, absolutely changing the profile of RNA conveyance within the body and intervening tissue-specific quality altering [99]. SORT permits nanoparticles to provide quality altering frameworks to particular organs, which are anticipated to advance, encourage advancement of quality adjustment treatments.

Polymer nanoparticles

Polymer materials have long blood circulation; tall medicate bioavailability, amazing biocompatibility, and degradability, so they are considered a capable conveyance instrument [100]. Be that as it may, conventional strategies of conveying sgRNA: Cas9 RNPs are wasteful and have destitute soundness to proteases in cells. The protein center and lean penetrable polymeric shell shape a modern sort of nanocapsule, which can be falsely planned for corruption or steadiness at diverse pH values. Capsule corruption breaks down the external shell, permitting the center protein to enter the cell to perform natural capacities. This strategy can productively convey an assortment of proteins to cells conjointly has moo harmfulness, opening up a unused course for conveyance of sgRNA: Cas9 RNP and cancer treatment [101].Further, in 2019 Chen et al. synthesized a lean glutathione cleavable covalent cross-linked polymer coating around the Cas9 RNP complex to create a unused nanocapsule. This nanocapsule viably produces focused on quality altering in vitro without any self-evident cytotoxicity. Topical organization of the nanocapsules in mice produces effective quality altering capabilities [102]. In ensuing ponders, Cas9 RNP was effectively conveyed to 293 T cells and colorectal cancer cells and appeared tall genome altering movement. Critically, nanocomplex focusing on of transformed KRAS in cancer cells can successfully represses tumor development and metastasis in tumor-bearing mouse models [103] Guo et al. interceded compelling knockdown of known breast cancer oncogene lipocalin 2 (LCN2) in human TNBC cells through polymer nanoparticle movement of the CRISPR system. Misfortune of LCN2 altogether restrained the relocation and mesenchymal phenotype of human TNBC cells and debilitated their invasiveness [104]. In expansion, Zhang et al. combined nanotechnology and genome designing to disturb cyclin-dependent kinase 5 (Cdk5), coming about in extraordinarily diminished expression of PD-L1 on tumor cells, viably hindering development of mouse melanoma and TNBC lung metastasis [105]. A few considers appeared that this polymer nanoparticle has great prospects and wide potential in changing CRISPR genome altering into a modern sort of exactness pharmaceutical for cancer treatment.

Gold nanoparticles (GNPs)

GNPs are another alternative for conveying CRISPR/Cas9. GNPs can combine with distinctive components such as nucleic acids, lipids, or polymers; have relative biocompatibility; and can enter into various sorts of cells [106].Setting differentiated utilitarian components counting nucleic acids and glycoproteins on the molecule surface can effortlessly accomplish useful differences [107]. Assist, pharmacokinetics of GNPs can be controlled by altering their measure, shape, charge, and surface alteration [108–109]. GNPs prepared with designed Cas9 protein and sgRNA can accomplish ˜ 90% intracellular conveyance and ˜ 30% quality altering proficiency, giving a modern strategy for genomics inquire about [110]. Whereas HDR-based treatments likely remedy most hereditary infections, it has been challenging to create conveyance vehicles that can actuate HDR within the body. A conveyance vehicle composed of GNPs conjugated to DNA and complexed with cationic endosomal troublesome polymers can convey Cas9 RNP to essential cells and stem cells. This complex, called CRISPR-Gold can actuate HDR in mdx mouse essential myoblasts with negligible off-target impacts [111]. Since the safe framework is the primary obstruction for GNPs to enter the human body, it is significant to investigate this interaction. Take-up of GNPs by resistant framework cells actuates generation of pro-inflammatory cytokines, demonstrating that GNPs have an immunostimulatory impact [112]. Like most cells, interaction of GNPs with different receptors on the surface of safe cells and different sorts of endocytosis depend on surface adjustment of GNPs [113, 114]. In expansion, due to the special biophysical properties of metal particles, charge and electrostatic field on the particles' surface moreover essentially influence safe reactions. Assist inquire about is required to more totally characterize the components intervening the interaction of GNPs with the resistant framework.



Challenges and Future Bearings

Within the future, the application of base editors and prime editors in human trials will be exceptionally energizing to observe, since numerous hereditary disarranges are caused by point transformations, which can be rectified by modern sorts of genome editors with exceptionally moo levels of unintended genomic changes. Basic researchers are endeavoring to extend the altering proficiency of base editors and prime editors, and given that positive reports in creature ponders have already been accomplished, we anticipate a striking increment within the number of clinical trials including CRISPR-based genome altering with these unused instruments.

Problems of CRISPR/Cas9

Delivery challenge

In spite of the fact that CRISPR/Cas9 may be a develop quality altering innovation and has been utilized broadly, restorative CRISPR/cas9 keeps up numerous issues due to the off-target impact, proficiency, and bundling challenges. In terms of CRISPR-based quality treatment, the challenges of the conveyance framework in vivo are highlighted mainly [115]. A perfect conveyance strategy for restorative CRISPR/cas9 ought to have the highlights of tall conveyance effectiveness, extraordinary focusing on capacity, and ease of mass generation. Be that as it may, the current methodologies are still distant from coming to the perfect bay [45]. Physical procedures of CRISPR/Cas9 conveyance are as a rule connected in vitro or ex vivo but uncommon in vivo. But for conventional electroporation and microinjection, more proficient strategies, counting ultrasound-propelled nanomotors [116], microfluidic or nanofluidic approaches [117], and spear measure nanoinjection [118] are too utilized to provide CRISPR frameworks. But for the CRISPR/Cas9 intervened gene-editing in human essential safe cells, electroporation is still the primary choice in a few studies [119]. Interests, a few physical approaches are able to provide CRISPR/Cas9 in vivo. For case, hydrodynamic infusion (HDI) was detailed as a novel approach to CRISPR framework delivery [120], but its application is constrained to many organs, such as the liver, since the strategy may cause harm amid conveyance.

Separated from physical technique, analysts moreover provide CRISPR/Cas9 by means of diverse vectors. Concurring to the sorts of vectors, the strategy of CRISPR/Cas9 conveyance may well be isolated into two

sorts: viral methodologies and non-viral techniques. Considering the moreextraordinary conveyance and focusing on capacity, infections are as a rule utilized and built to convey CRISPR/Cas9. Adeno-associated infection (AAV) is the commonest vector for quality treatment in vivo and ex vivo due to its wide serotype, small immunogenicity, and toxicity [121], but the little payload (as it were 4.5–5 kb) limits its improvement. Compared with AAV, lentivirus (LV) and adenovirus (AdV) have distant better;a much better;a higher;a stronger;an improved">a much better capacity and permit conveyance of extra hereditary compounds, such as numerous promotors. Thereinto, one of the greatest focal points of AdV is its capacity to exchange a more extensive extends of cells than LV and AAV. In any case, the greater sizes of LV and AdV may trigger solid humoral and indeed cellular safe reactions, which propose productivity of conveyance and potential chance of inflammation [110]. Other viral vectors are too connected as of late and have their possess characteristics individually. For illustration, EBV vectors are able to specific exogenous qualities more stably [122], and Sendai viral vectors are competent of contaminating broader have types [123], whereas Baculovirus vectors have a greater payload [124]. Be that as it may, these vectors are as it were utilized ex vivo by presently, but they are still potential to be connected in vivo within the future after optimizing.

-With respect to non-viral techniques, liposomes are utilized most as often as possible since they have been merchandised broadly, and a few analysts too attempt to utilize gold nanoparticles (AuNPs) as the vectors for CRISPR/Cas9 conveyance. Other non-viral conveyance vectors, counting Lipofectamine RNAiMAX [125], PolyJet In Vitro DNA Transfection Reagent[126], and X-tremeGENE HP DNA Transfection Reagent [126], are too commercial but are as it were appropriate for in vitro or ex vivo tests by presently. In terms of in vivo conveyance, thousands of thinks about center on finding and synthesizing high-efficient and low-cytotoxic non-viral vectors. Show techniques incorporate common nano-sized arrangements (e.g., self-assembled micelles [127] and polyethylene glycol phospholipid-modified cationic lipid nanoparticle[128] for CRISPR/Cas9 plasmid conveyance, and DNA nanoclews [129] and dark phosphorus nanosheets[130] for CRISPR/Cas9 complex conveyance), receptor-mediated conveyance procedures (e.g., folate receptor-targeted liposomes convey CRISPR/Cas9 plasmids[131]), cell-penetrating peptides (CPPs)-mediated conveyance methodologies (e.g., Kim combined Cas9 protein with a moo molecular-weight protamine and an atomic localization arrangement to convey CRISPR/Cas9 complex [132], whereas Wang built up adjusted cationic α-helical polypeptides based PEGylated nanoparticles to provide CRISPR/Cas9 plasmids and sgRNA[133]), and multi-model conveyance procedures (e.g., R8-dGR peptide altered cationic liposome for the conveyance of CRISPR/Cas9 and sgRNA plasmids [134], and near-infrared upconversion–activated framework for CRISPR/Cas9 complex delivery [135]). Still, a number of inconveniences within the field of non-viral procedures, like conveyance obstructions or endosome avoidance, stay uncertain, which stagnates the assist improvement of helpful CRISPR/cas9 [110].

In expansion to different conveyance vectors, the shapes of cargos moreover play an imperative part in CRISPR/Cas9 conveyance. Customarily, Cas9 is conveyed within the shape of DNA or mRNA with sgRNA and layout sequence together. In arrange to extend quality altering productivity, Yin and his colleagues conveyed Cas9 mRNA by lipid nanoparticles whereas conveying sgRNA and layout arrangement by AAV separately [136]. Additionally, Cas9 proteins can too be conveyed into cells straightforwardly by combination or enlistment strategies. It dodges the hazard of genome integration and diminishes the off-target impact due to the brief half-life of Cas9 protein, which is considered a safer approach for quality therapy [137]. In conclusion, there's an inverse issue in CRISPR conveyance challenges, counting the littler measure of the conveyance vectors to maintain a strategic distance from resistant reaction but the greater necessity of cargo loads to carry more CRISPR or expressive modules.

Off-target effect

The off-target impact is one of the CRISPR/Cas9 application confinements and is considered a critical hazard calculate amid quality treatment in vivo. In spite of the fact that a few computer programs have optimized the plan of sgRNA, its specificity cannot be guaranteed completely. Moreover, but for sgRNA plan; the length of the Cas9 chemical too plays a vital part. In this manner, expendable Cas9 plan, such as conveying protein specifically, might essentially diminish the off-target impact. In expansion, optimized Cas9 chemical

is additionally an approach to diminish the off-target impact, counting SpCas9-HF1 [138] and eSpCas9 [139]. Be that as it may, the off-target impact in vivo keeps unsolved, and it is additionally exceedingly related to the conveyance technique.

Pam limitation

As portrayed over, the Pam arrangement is fundamental for CRISPR/Cas9 focusing on, and as it were the DNA groupings that contain Pam can be focused on by the Cas9 chemical. Be that as it may, Pam limits the plan of sgRNA and diminishes the adaptability of CRISPR/Cas9 altogether. In spite of the fact that an expanding number of CRISPR sorts are found, causing more Pam parts is selectable at display. The obligatory Pam addition still influences the plan of sgRNA in a few circumstances. Subsequently, how to create a designable Pam is critical to broaden the application of CRISPR/Cas9.

Immune response

As a remote protein, Cas9 may actuate the safe reaction. In spite of the fact that there are not numerous reports approximately the extreme safe response caused by Cas9, the antibodies of Cas9 have been broadly recognized in human bodies [140], which recommends the potential hazard of aggravation amid CRISPR/Cas9-based quality treatment. At show, analysts pay more consideration to the immunogenicity caused by conveyance vectors, particularly the viral vectors, since the human body may have been tainted by these infections some time recently and contain the comparing antibodies as of now. Collectively, the safe response possibly actuated by CRISPR/Cas9 gene-editing framework is one of the major hazard components within the improvement of CRISPR-based quality treatment in vivo.

Multiple gene-editing

CRISPR/Cas9 is a proficient gene-editing device but as it were alters one quality with a sgRNA at the same time. Subsequently, numerous gene-editing utilizing CRISPR/Cas9 ought to depend on different sgRNAs, which diminishes the altering effectiveness whereas increments the conveyance trouble. Later CRISPR/Cas12a may overcome the challenge, but other ensuing issues are still unsolved, counting inactivation of cells and cell cycle capture after different gene-editing. Within the future, compelling different gene-editing techniques will significantly advance the quality treatment of polygenic maladies and cancers.

Outlook

CRISPR/Cas9, determined from the microbial intrinsic resistant framework, is created as a strong gene-editing apparatus and has been connected broadly. Due to its tall precision and proficiency, CRISPR/Cas9 strategies may give an incredible chance to treat a few gene-related infections by disturbing, embeddings, rectifying, supplanting, or blocking genes. Cas9-mediated quality altering has been utilized to treat different non-cancerous maladies. Monogenetic infections and X-linked illnesses caused by quality transformation are the foremost coordinate and clear sorts that CRISPR/Cas9 can be connected to. A number of ponders have demonstrated quality adjustment in monogenetic maladies and X-linked illnesses is an viable helpful procedure, and a few related clinical trials have been within the prepare as of late. So also, the chance of CVDs is diminished, and the indication of NDDs is diminished drastically after focusing on related qualities utilizing CRISPR/Cas9. At the same time, the treatment of visual illnesses by Cas9 has entered into clinical stages. Recognizably, Helps May gotten to be treatable through thumping out the viral qualities by Cas9, which benefits millions of patients within the world.

In terms of cancer treatment, CRISPR/Cas9 was at first connected in medicating targets screen, causing a fast revelation of parcels of novel sedate targets. Combined with computer and information methods, Cas9-based target screening gives a progressed approach to get it cancers superior. Disturbing oncogene or rectifying tumor silencer qualities alone or in combination are the major methodology to treat cancers whereas thumping out viral genomes like HPV diminishes the hazard of virus-induced tumors. Besides, a few investigates moreover illustrate a few controller qualities, epigenetic qualities, and microenvironmental qualities moreover play crucial parts in cancerization and are created as successful restorative targets. As of late, more analysts center on the resistant treatment of cancers. Particularly CAR-T treatment has been

connected in clinical treatment and accomplishes victory to a few degrees, and the restorative impact may be progressed by restraining a few related qualities by Cas9.

In fact, CRISPR/Cas9 may be a vigorous gene-editing apparatus. In any case, a few issues keep unsolved, counting off-target impact, conveyance challenges, PAM confinement, and immunogenicity, which pieces its application in clinical treatment. When creating quality treatment, the off-target effect and altering effectiveness are two of the foremost concerning issues because the off-target impact may cause unforeseen altering of ordinary qualities and after that lead to extreme illnesses or indeed passing, whereas editing efficiency specifically influences the restorative impact. To fathom these two issues, parts of ponders work totally different angles. Firstly, the conveyance framework of CRISPR/Cas9 is basic for CRISPR-based treatment. For case, the conveyance proficiency decides the proficiency of Cas9-mediated quality altering to a significant degree, and the targetability, soundness, and discharge time of conveyance vectors are exceedingly related to the off-target impact.

As said over, expendable plans (such as conveying Cas9 protein) and all-in-one plans (such as conveying Cas9 plasmids and sgRNA at the same time) are compelling ways to diminish the off-target impact. Furthermore, re-engineered or optimized Cas9 proteins diminish the off-target impact as well. Compared with conventional CRISPR/Cas9-based quality altering, base altering and preliminary altering instruments don't make DSBs when altering qualities, which drastically diminish the off-target impact. And their altering effectiveness is ceaselessly improved in later thinks about by means of optimizing chemicals or pegRNA, recommending the awesome potential for clinical application. At last, sgRNA plan is still fundamental since it plays a key part in quality focusing on. But for optimizing sgRNA plan rules and computer programs, the ponders to maintain a strategic distance from PAM confinement may move forward the detail and adaptability of sgRNA, which is advantageous to move forward altering productivity whereas diminishing the plausibility of off-target. At show, most clinical trials fair restrain in altering qualities in patient-derived cells ex vivo, and after that the cells are infused back into the patient's bodies, such as the treatment of SCDs and safe treatment. This strategy dodges the hazard of off-target effect and conveyance challenge but isn't reasonable for all illnesses. And later research prefers to disturb or thump out qualities instead of redressing since the extra DNA layouts increment the conveyance trouble. The clinical application of CRISPR/Cas9 is still at an early arrange, and the prioritized issues of clinical quality treatment by Cas9 in vivo are off-target impact and conveyance challenges.

Compared with monogenetic illnesses, Cas9-based quality treatment of cancers are more challenging due to numerous quality transformations. In spite of the fact that it is accessible to apply numerous quality altering by CRISPR/Cas9 after including the comparing sgRNAs. CRISPR/Cas9-mediated numerous quality altering isn't broadly connected in clinical treatment or indeed quality work thinks about since it seem lead to a few potential issues, such as extreme off-target impact and the erasure of enormous DNA fragments[141]. In this manner, novel approaches in multiple gene altering got to be created advance to overcome the display challenges. But for CRISPR/Cas9, other CRISPR frameworks, counting Cas12a, Cas3 (with Cascade), Cas13, dCas9, and nCas9, too contain colossal possibilities for quality therapy [142]. For case, comparable to Cas9, Cas12a moreover has a place to the course II CRISPR framework. But Cas12a creates a amazed cut instead of the level conclusion that Cas9 creates, which could be a incredible advantage when joining DNA groupings. Within the Cas3 framework, the Cascade complex ties and recognizes the target DNA arrangement at that point Cas3 proteins are selected to produce a single-strand scratch. Due to the wanton acknowledgment of PAM within the Cas3 framework, it is more adaptable to target particular DNA arrangements than Cas9. Diverse from Cas9, Cas12a, and Cas3 frameworks, Cas13 is an RNA-guided RNA focusing on framework. Cas13 seem alter single-strand RNA proficiently, whereas nuclease-inactive dCas13 is able to direct protein interpretation. Both dCas9 and nCas9 lose the nuclease action but keep up the capacity to target DNA groupings, so a part of re-engineered CRISPR/Cas9 devices, such as CRISPRi, CRIPARa, base altering device, and preliminary altering instruments, etc., are based on dCas9 or nCas9.In conclusion, CRISPR/Cas9 is an proficient gene-editing instrument but not a idealize treatment approach at display. Parts of issues got to be developed assist until its unwavering quality and security keep up a better level. Cell treatment by Cas9 appears to be more basic to design whereas dodging a few inconveniences

that in vivo quality treatment meets. However, only some of hundreds of maladies may be treated by cell treatment. To supply a broader helpful procedure for hereditary maladies, quality treatment by Cas9 is one of the major viewpoints to create within the future. Hence, how to effectively and securely alter qualities by CRISPR/Cas9 in vivo will be recorded at the beat within the following decade.

Data availability statement: Data openly available in a public repository that issues datasets with DOIs

References

1- Qi LS et al. Repurposing CRISPR as an RNA-cuided platform for sequencespecific control of gene expression. Cell 2013;152:1173–83.

2- Standage-Beier K, Zhang Q, Wang X. Targeted Large-Scale Deletion of Bacterial Genomes Using CRISPR-Nickases. ACS Synth. Biol. 2015;4:1217–25.

3- Tanenbaum ME, Gilbert LA, Qi LS, Weissman JS, Vale RD. A Protein-Tagging System for Signal Amplification in Gene Expression and Fluorescence Imaging. Cell 2014;159:635–46.

4- Gaudelli NM et al. Programmable base editing of A T to G C in genomic DNA without DNA cleavage. Nature 2017;551:464–71.

5- Komor AC, Kim YB, Packer MS, Zuris JA, Liu DR. Programmable editing of a target base in genomic DNA without double-stranded DNA cleavage. Nature 2016;533:420–4.

6- Anzalone AV et al. Search-and-replace genome editing without double-strand breaks or donor DNA. Nature 2019;576:149–57.

7- Liu Z et al. Efficient and high-fidelity base editor with expanded PAM compatibility for cytidine dinucleotide. Sci. China Life Sci. 2021; 64:1355–67.

8- Strecker J et al. RNA-guided DNA insertion with CRISPR-associated transposases. Science (80-.) 2019;365:48–53.

9- Liu Z et al. Efficient and high-fidelity base editor with expanded PAM compatibility for cytidine dinucleotide. Sci. China Life Sci. 2021;64:1355–67.

10- Burstein D et al. New CRISPR-Cas systems from uncultivated microbes.Nature 2017;542:237–41.

11- Mout R, Ray M, Lee YW, Scaletti F & Rotello VM In Vivo Delivery of CRISPR/Cas9 for Therapeutic Gene Editing: Progress and Challenges. Bioconjugate Chem 28, 880–884 (2017).

12- Ho BX, Loh SJH, Chan WK & Soh BS In Vivo Genome Editing as a Therapeutic Approach. Int J Mol Sci 19, 2721 (2018).

13- Naldini L Ex vivo gene transfer and correction for cell-based therapies. Nat Rev Genet 12, 301–315 (2011).

14- Wang HX et al. CRISPR/Cas9-Based Genome Editing for Disease Modeling and Therapy: Challenges and Opportunities for Nonviral Delivery. Chemical reviews 117, 9874–9906 (2017).

15- Glass Z, Lee M, Li YM & Xu QB Engineering the Delivery System for CRISPR-Based Genome Editing. Trends in biotechnology 36, 173–185 (2018).

16- Xu L, Wang J, Liu Y, Xie L, Su B, Mou D, et al. CRISPR-edited stem cells in a patient with HIV and acute lymphocytic leukemia. N Engl J Med 2019;381:1240–1247.

17- Frangoul H, Altshuler D, Cappellini MD, Chen YS, Domm J, Eustace BK, et al. CRISPR-Cas9 gene editing for sickle cell disease and β-thalassemia. N Engl J Med 2021;384:252–260.

18-Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, et al. CRISPR-engineered T cells in patients with refractory cancer. Science 2020;367:eaba7365

19-Lu Y, Xue J, Deng T, Zhou X, Yu K, Deng L, et al. Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. Nat Med 2020;26:732–740.

20-Jing Z, Zhang N, Ding L, Wang X, Hua Y, Jiang M, et al. Safety and activity of programmed cell death-1 gene knockout engineered t cells in patients with previously treated advanced esophageal squamous cell carcinoma: an open-label, single-arm phase I study; 2018 ASCO Annual Meeting I; American Society of Clinical Oncology; 2018. pp. 3054.

21- Stadtmauer EA, Fraietta JA, Davis MM, Cohen AD, Weber KL, Lancaster E, et al. CRISPR-engineered T cells in patients with refractory cancer. Science 2020;367:eaba7365

22- Lu Y, Xue J, Deng T, Zhou X, Yu K, Deng L, et al. Safety and feasibility of CRISPR-edited T cells in patients with refractory non-small-cell lung cancer. Nat Med 2020;26:732–740.

23- Gillmore JD, Gane E, Taubel J, Kao J, Fontana M, Maitland ML, et al. CRISPR-Cas9 in vivo gene editing for transthyretin amyloidosis. N Engl J Med 2021;385:493–502

24- Maeder ML, Stefanidakis M, Wilson CJ, Baral R, Barrera LA, Bounoutas GS, et al. Development of a gene-editing approach to restore vision loss in Leber congenital amaurosis type 10. Nat Med 2019;25:229–233.

25- Jo DH, Song DW, Cho CS, Kim UG, Lee KJ, Lee K, et al. CRISPR-Cas9-mediated therapeutic editing of Rpe65 ameliorates the disease phenotypes in a mouse model of Leber congenital amaurosis. Sci Adv 2019;5:eaax1210

26- Jang HK, Jo DH, Lee SN, Cho CS, Jeong YK, Jung Y, et al. High-purity production and precise editing of DNA base editing ribonucleoproteins. Sci Adv 2021;7:eabg2661

27- Jang H, Jo DH, Cho CS, Shin JH, Seo JH, Yu G, et al. Application of prime editing to the correction of mutations and phenotypes in adult mice with liver and eye diseases. Nat Biomed Eng. 2021 Aug 26;[Epub]. Available at: https://doi.org/10.1038/s41551-021-00788-9.

28- Smalley E. First AAV gene therapy poised for landmark approval. Nat Biotechnol 2017;35:998–999.

29- Yu W, Mookherjee S, Chaitankar V, Hiriyanna S, Kim JW, Brooks M, et al. Nrl knockdown by AAV-delivered CRISPR/Cas9 prevents retinal degeneration in mice. Nat Commun 2017;8:14716

30-Moreno AM, Fu X, Zhu J, Katrekar D, Shih YV, Marlett J, et al. In situ gene therapy via AAV-CRISPR-Cas9-mediated targeted gene regulation. Mol Ther 2018;26:1818–1827.

31-Goossens R, van den Boogaard ML, Lemmers RJLF, Balog J, van der Vliet PJ, Willemsen IM, et al. Intronic SMCHD1 variants in FSHD: testing the potential for CRISPR-Cas9 genome editing. J Med Genet 2019;56:828–837.

32-Vagni P, Perlini LE, Chenais NAL, Marchetti T, Parrini M, Contestabile A, et al. Gene editing preserves visual functions in a mouse model of retinal degeneration. Front Neurosci 2019;13:945

33-Latella MC, Di Salvo MT, Cocchiarella F, Benati D, Grisendi G, Comitato A, et al. In vivo editing of the human mutant rhodopsin gene by electroporation of plasmid-based CRISPR/Cas9 in the mouse retina. Mol Ther Nucleic Acids 2016;5:e389

34-Bakondi B, Lv W, Lu B, Jones MK, Tsai Y, Kim KJ, et al. In vivo CRISPR/Cas9 gene editing corrects retinal dystrophy in the S334ter-3 rat model of autosomal dominant retinitis pigmentosa. Mol Ther 2016;24:556–563.

35-Hu S, Du J, Chen N, Jia R, Zhang J, Liu X, et al. In vivo CRISPR/Cas9-mediated genome editing mitigates photoreceptor degeneration in a mouse model of X-linked retinitis pigmentosa. Invest Ophthalmol Vis Sci 2020;61:31

36- Yin H, Xue W, Chen S, Bogorad RL, Benedetti E, Grompe M, et al. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat Biotechnol 2014;32:551–553.

37- Ibraheim R, Song CQ, Mir A, Amrani N, Xue W, Sontheimer EJ. All-in-one adeno-associated virus delivery and genome editing by Neisseria meningitidis Cas9 in vivo. Genome Biol 2018;19:137

38-Agudelo D, Carter S, Velimirovic M, Duringer A, Rivest JF, Levesque S, et al. Versatile and robust genome editing with Streptococcus thermophilus CRISPR1-Cas9. Genome Res 2020;30:107–117.

39- Song CQ, Jiang T, Richter M, Rhym LH, Koblan LW, Zafra MP, et al. Adenine base editing in an adult mouse model of tyrosinaemia. Nat Biomed Eng 2020;4:125–130.

40-Yang L, Wang L, Huo Y, Chen X, Yin S, Hu Y, et al. Amelioration of an inherited metabolic liver disease through creation of a de novo start codon by cytidine base editing. Mol Ther 2020;28:1673–1683.

41- Jang H, Jo DH, Cho CS, Shin JH, Seo JH, Yu G, et al. Application of prime editing to the correction of mutations and phenotypes in adult mice with liver and eye diseases. Nat Biomed Eng. 2021 Aug 26;

https://doi.org/10.1038/s41551-021-00788-9.

42- Villiger L, Grisch-Chan HM, Lindsay H, Ringnalda F, Pogliano CB, Allegri G, et al. Treatment of a metabolic liver disease by in vivo genome base editing in adult mice. Nat Med 2018;24:1519–1525.

43- Richards DY, Winn SR, Dudley S, Nygaard S, Mighell TL, Grompe M, et al. AAV-mediated CRISPR/Cas9 gene editing in murine phenylketonuria. Mol Ther Methods Clin Dev 2020;17:234–245.

44-Yin S, Ma L, Shao T, Zhang M, Guan Y, Wang L, et al. Enhanced genome editing to ameliorate a genetic metabolic liver disease through co-delivery of adeno-associated virus receptor. Sci China Life Sci. 2020 Aug 17;

https://doi.org/10.1007/s11427-020-1744-6.

45- Yang Y, Wang L, Bell P, McMenamin D, He Z, White J, et al. A dual AAV system enables the Cas9-mediated correction of a metabolic liver disease in newborn mice. Nat Biotechnol 2016;34:334–338.

46- Long C, McAnally JR, Shelton JM, Mireault AA, Bassel-Duby R, Olson EN. Prevention of muscular dystrophy in mice by CRISPR/Cas9-mediated editing of germline DNA. Science 2014;345:1184–1188.

47- Nelson CE, Hakim CH, Ousterout DG, Thakore PI, Moreb EA, Castellanos Rivera RM, et al. In vivo genome editing improves muscle function in a mouse model of Duchenne muscular dystrophy. Science 2016;351:403–407.

48-Tabebordbar M, Zhu K, Cheng JKW, Chew WL, Widrick JJ, Yan WX, et al. In vivo gene editing in dystrophic mouse muscle and muscle stem cells. Science 2016;351:407–411.

49-Bengtsson NE, Hall JK, Odom GL, Phelps MP, Andrus CR, Hawkins RD, et al. Muscle-specific CRISPR/Cas9 dystrophin gene editing ameliorates pathophysiology in a mouse model for Duchenne muscular dystrophy. Nat Commun 2017;8:14454

50- Amoasii L, Hildyard JCW, Li H, Sanchez-Ortiz E, Mireault A, Caballero D, et al. Gene editing restores dystrophin expression in a canine model of Duchenne muscular dystrophy. Science 2018;362:86–91.

51- Moretti A, Fonteyne L, Giesert F, Hoppmann P, Meier AB, Bozoglu T, et al. Somatic gene editing ameliorates skeletal and cardiac muscle failure in pig and human models of Duchenne muscular dystrophy. Nat Med 2020;26:207–214.

52- Ryu SM, Koo T, Kim K, Lim K, Baek G, Kim ST, et al. Adenine base editing in mouse embryos and an adult mouse model of Duchenne muscular dystrophy. Nat Biotechnol 2018;36:536–539.

53- Gaj T, Ojala DS, Ekman FK, Byrne LC, Limsirichai P, Schaffer DV. In vivo genome editing improves motor function and extends survival in a mouse model of ALS. Sci Adv 2017;3:eaar3952

54- Arnaoutova I, Zhang L, Chen HD, Mansfield BC, Chou JY. Correction of metabolic abnormalities in a mouse model of glycogen storage disease type Ia by CRISPR/Cas9-based gene editing. Mol Ther 2021;29:1602–1610.

55- Koblan LW, Erdos MR, Wilson C, Cabral WA, Levy JM, Xiong ZM, et al. In vivo base editing rescues Hutchinson-Gilford progeria syndrome in mice. Nature 2021;589:608–614.

56- Wilbie D, Walther J, Mastrobattista E. Delivery aspects of CRISPR/Cas for in vivo genome editing. Acc Chem Res. 2019;52:1555–64.

57-Kay MA, Glorioso JC, Naldini L. Viral vectors for gene therapy: the art of turning infectious agents into vehicles of therapeutics. Nat Med. 2001;7:33–40.

58-Bessis N, GarciaCozar FJ, Boissier MC. Immune responses to gene therapy vectors: influence on vector function and effector mechanisms. Gene Ther. 2004;11(Suppl 1):S10–7.

59-Samulski RJ, Zhu X, Xiao X, Brook JD, Housman DE, Epstein N, et al. Targeted integration of adeno-associated virus (AAV) into human chromosome 19. EMBO J. 1991;10:3941–50.

60-Yan J, Kang DD, Turnbull G, Dong Y. Delivery of CRISPR-Cas9 system for screening and editing RNA binding proteins in cancer. Adv Drug Deliv Rev. 2021;180:114042.

61-Maggio I, Zittersteijn HA, Wang Q, Liu J, Janssen JM, Ojeda IT, et al. Integrating gene delivery and gene-editing technologies by adenoviral vector transfer of optimized CRISPR-Cas9 components. Gene Ther. 2020;27:209–25.

62-Palmer D, Ng P. Improved system for helper-dependent adenoviral vector production. Mol Ther. 2003;8:846–52.

63-Jager L, Ehrhardt A. Persistence of high-capacity adenoviral vectors as replication-defective monomeric genomes in vitro and in murine liver. Hum Gene Ther. 2009;20:883–96.

64-Byrnes AP, Rusby JE, Wood MJ, Charlton HM. Adenovirus gene transfer causes inflammation in the brain. Neuroscience. 1995;66:1015–24.

65-Yang Y, Greenough K, Wilson JM. Transient immune blockade prevents formation of neutralizing antibody to recombinant adenovirus and allows repeated gene transfer to mouse liver. Gene Ther. 1996;3:412–20.

66-Beer SJ, Matthews CB, Stein CS, Ross BD, Hilfinger JM, Davidson BL. Poly (lactic-glycolic) acid copolymer encapsulation of recombinant adenovirus reduces immunogenicity in vivo. Gene Ther. 1998;5:740–6.

67-Tuveson DA, Jacks T. Technologically advanced cancer modeling in mice. Curr Opin Genet Dev. 2002;12:105–10.

68-Maddalo D, Manchado E, Concepcion CP, Bonetti C, Vidigal JA, Han YC, et al. In vivo engineering of oncogenic chromosomal rearrangements with the CRISPR/Cas9 system. Nature. 2014;516:423–7.

69-Wang D, Mou H, Li S, Li Y, Hough S, Tran K, et al. Adenovirus-mediated somatic genome editing of Pten by CRISPR/Cas9 in mouse liver in spite of Cas9-specific immune responses. Hum Gene Ther. 2015;26:432–42.

70-Huang J, Chen M, Whitley MJ, Kuo HC, Xu ES, Walens A, et al. Generation and comparison of CRISPR-Cas9 and Cre-mediated genetically engineered mouse models of sarcoma. Nat Commun. 2017;8:15999.

71-Wang D, Tai PWL, Gao G. Adeno-associated virus vector as a platform for gene therapy delivery. Nat Rev Drug Discov. 2019;18:358–78.

72-Luo J, Luo Y, Sun J, Zhou Y, Zhang Y, Yang X. Adeno-associated virus-mediated cancer gene therapy: current status. Cancer Lett. 2015;356:347–56.

73-Nelson CE, Gersbach CA. Engineering delivery vehicles for genome editing. Annu Rev Chem Biomol Eng. 2016;7:637–62.

74-Dong JY, Fan PD, Frizzell RA. Quantitative analysis of the packaging capacity of recombinant adeno-associated virus. Hum Gene Ther. 1996;7:2101–12.

75-Senís E, Fatouros C, Große S, Wiedtke E, Niopek D, Mueller AK, et al. CRISPR/Cas9-mediated genome engineering: an adeno-associated viral (AAV) vector toolbox. Biotechnol J. 2014;9:1402–12.

76-Yang Y, Wang L, Bell P, McMenamin D, He Z, White J, et al. A dual AAV system enables the Cas9-mediated correction of a metabolic liver disease in newborn mice. Nat Biotechnol. 2016;34:334–8.

77-Sun JY, Anand-Jawa V, Chatterjee S, Wong KK. Immune responses to adeno-associated virus and its recombinant vectors. Gene Ther. 2003;10:964–76.

78-Huttner NA, Girod A, Perabo L, Edbauer D, Kleinschmidt JA, Büning H, et al. Genetic modifications of the adeno-associated virus type 2 capsid reduce the affinity and the neutralizing effects of human serum antibodies. Gene Ther. 2003;10:2139–47.

79-Vakulskas CA, Behlke MA. Evaluation and reduction of CRISPR off-target cleavage events. Nucleic Acid Ther. 2019;29:167–74.

80-Swiech L, Heidenreich M, Banerjee A, Habib N, Li Y, Trombetta J, et al. In vivo interrogation of gene function in the mammalian brain using CRISPR-Cas9. Nat Biotechnol. 2015;33:102–6.

81-Winters IP, Chiou SH, Paulk NK, McFarland CD, Lalgudi PV, Ma RK, et al. Multiplexed in vivo homology-directed repair and tumor barcoding enables parallel quantification of Kras variant oncogenicity. Nat Commun. 2017;8:2053.

82-Chow RD, Guzman CD, Wang G, Schmidt F, Youngblood MW, Ye L, et al. AAV-mediated direct in vivo CRISPR screen identifies functional suppressors in glioblastoma. Nat Neurosci. 2017;20:1329–41.

83-Naldini L. Lentiviruses as gene transfer agents for delivery to non-dividing cells. Curr Opin Biotechnol. 1998;9:457–63.

84-Kantor B, Bailey RM, Wimberly K, Kalburgi SN, Gray SJ. Methods for gene transfer to the central nervous system. Adv Genet. 2014;87:125–97.

85-Ling S, Yang S, Hu X, Yin D, Dai Y, Qian X, et al. Lentiviral delivery of co-packaged Cas9 mRNA and a Vegfa-targeting guide RNA prevents wet age-related macular degeneration in mice. Nat Biomed Eng. 2021;5:144–56.

86-Wanisch K, Yáñez-Muñoz RJ. Integration-deficient lentiviral vectors: a slow coming of age. Mol Ther. 2009;17:1316–32.

87-Kim W, Lee S, Kim HS, Song M, Cha YH, Kim YH, et al. Targeting mutant KRAS with CRISPR-Cas9 controls tumor growth. Genome Res. 2018;28:374–82.

88-Chen SH, Hsieh YY, Tzeng HE, Lin CY, Hsu KW, Chiang YS, et al. ABL genomic editing sufficiently abolishes oncogenesis of human chronic myeloid leukemia cells in vitro and in vivo. Cancers (Basel). 2020;12(6):1399.

89-High KA. Turning genes into medicines-what have we learned from gene therapy drug development in the past decade? Nat Commun. 2020;11:5821.

90-Baum C, Kustikova O, Modlich U, Li Z, Fehse B. Mutagenesis and oncogenesis by chromosomal insertion of gene transfer vectors. Hum Gene Ther. 2006;17:253–63.

91-Waehler R, Russell SJ, Curiel DT. Engineering targeted viral vectors for gene therapy. Nat Rev Genet. 2007;8:573–87.

92-Pack DW, Hoffman AS, Pun S, Stayton PS. Design and development of polymers for gene delivery. Nat Rev Drug Discov. 2005;4:581–93.

93-Pahle J, Walther W. Vectors and strategies for nonviral cancer gene therapy. Expert Opin Biol Ther. 2016;16:443–61.

94-Witzigmann D, Kulkarni JA, Leung J, Chen S, Cullis PR, van der Meel R. Lipid nanoparticle technology for therapeutic gene regulation in the liver. Adv Drug Deliv Rev. 2020;159:344–63.

95-Sercombe L, Veerati T, Moheimani F, Wu SY, Sood AK, Hua S. Advances and challenges of liposome assisted drug delivery. Front Pharmacol. 2015;6:286.

96-Mehnert W, Mäder K. Solid lipid nanoparticles: production, characterization and applications. Adv Drug Deliv Rev. 2001;47:165–96.

97-Pardi N, Hogan MJ, Pelc RS, Muramatsu H, Andersen H, DeMaso CR, et al. Zika virus protection by a single low-dose nucleoside-modified mRNA vaccination. Nature. 2017;543:248–51.

98-Finn JD, Smith AR, Patel MC, Shaw L, Youniss MR, van Heteren J, et al. A single administration of CRISPR/Cas9 lipid nanoparticles achieves robust and persistent in vivo genome editing. Cell Rep. 2018;22:2227–35.

99-Cheng Q, Wei T, Farbiak L, Johnson LT, Dilliard SA, Siegwart DJ. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR-Cas gene editing. Nat Nanotechnol. 2020;15:313–20.

100-Song R, Murphy M, Li C, Ting K, Soo C, Zheng Z. Current development of biodegradable polymeric materials for biomedical applications. Drug Des Devel Ther. 2018;12:3117–45.

101-Yan M, Du J, Gu Z, Liang M, Hu Y, Zhang W, et al. A novel intracellular protein delivery platform based on single-protein nanocapsules. Nat Nanotechnol. 2010;5:48–53.

102-Chen G, Abdeen AA, Wang Y, Shahi PK, Robertson S, Xie R, et al. A biodegradable nanocapsule delivers a Cas9 ribonucleoprotein complex for in vivo genome editing. Nat Nanotechnol. 2019;14:974–80.

103-Wan T, Chen Y, Pan Q, Xu X, Kang Y, Gao X, et al. Genome editing of mutant KRAS through supramolecular polymer-mediated delivery of Cas9 ribonucleoprotein for colorectal cancer therapy. J Control Release. 2020;322:236–47.

104-Guo P, Yang J, Huang J, Auguste DT, Moses MA. Therapeutic genome editing of triple-negative breast tumors using a noncationic and deformable nanolipogel. Proc Natl Acad Sci U S A.

105-Deng H, Tan S, Gao X, Zou C, Xu C, Tu K, et al. Cdk5 knocking out mediated by CRISPR-Cas9 genome editing for PD-L1 attenuation and enhanced antitumor immunity. Acta Pharm Sin B. 2020;10:358–73.

106-Gindy ME, Prud'homme RK. Multifunctional nanoparticles for imaging, delivery and targeting in cancer therapy. Expert Opin Drug Deliv. 2009;6:865–78.

107-Bowman MC, Ballard TE, Ackerson CJ, Feldheim DL, Margolis DM, Melander C. Inhibition of HIV fusion with multivalent gold nanoparticles. J Am Chem Soc. 2008;130:6896–7.

108-Chompoosor A, Saha K, Ghosh PS, Macarthy DJ, Miranda OR, Zhu ZJ, et al. The role of surface functionality on acute cytotoxicity, ROS generation and DNA damage by cationic gold nanoparticles. Small. 2010;6:2246–9.

109-Ahmad S, Zamry AA, Tan HT, Wong KK, Lim J, Mohamud R. Targeting dendritic cells through gold nanoparticles: a review on the cellular uptake and subsequent immunological properties. Mol Immunol. 2017;91:123–33.

110-Lino CA, Harper JC, Carney JP, Timlin JA. Delivering CRISPR: a review of the challenges and approaches. Drug Deliv. 2018;25:1234–57.

111-Lee K, Conboy M, Park HM, Jiang F, Kim HJ, Dewitt MA, et al. Nanoparticle delivery of Cas9 ribonucleoprotein and donor DNA in vivo induces homology-directed DNA repair. Nat Biomed Eng. 2017;1:889–901.

112-Yen HJ, Hsu SH, Tsai CL. Cytotoxicity and immunological response of gold and silver nanoparticles of different sizes. Small. 2009;5:1553–61.

113-Bastús NG, Sánchez-Tilló E, Pujals S, Farrera C, López C, Giralt E, et al. Homogeneous conjugation of peptides onto gold nanoparticles enhances macrophage response. ACS Nano. 2009;3:1335–44.

114-Lee JY, Park W, Yi DK. Immunostimulatory effects of gold nanorod and silica-coated gold nanorod on RAW 264.7 mouse macrophages. Toxicol Lett. 2012;209:51–7.

115-Mout R, Ray M, Lee YW, Scaletti F, Rotello VM. In Vivo Delivery of CRISPR/ Cas9 for Therapeutic Gene Editing: Progress and Challenges. Bioconjug. Chem. 2017;28:880–4.

116- Hansen-Bruhn M et al. Active Intracellular Delivery of a Cas9/sgRNA Complex Using Ultrasound-Propelled Nanomotors. Angew. Chemie 2018;130:2687–91.

117- Hur J, Chung AJ. Microfluidic and Nanofluidic Intracellular Delivery. Adv. Sci. 2021;8.

118- Sessions JW et al. CRISPR-Cas9 directed knock-out of a constitutively expressed gene using lance array nanoinjection. Springerplus 2016;5:1521.

119– Song X et al. Delivery of CRISPR/Cas systems for cancer gene therapy and immunotherapy. Adv. Drug Deliv. Rev. 2021;168:158–80.

120-Yin H et al. Genome editing with Cas9 in adult mice corrects a disease mutation and phenotype. Nat. Biotechnol. 2014;32:551–3.

121- Samulski, R. J. & Muzyczka, N. AAV-Mediated Gene Therapy for Research and Therapeutic Purposes. doi:10.1146/annurev-virology-031413-085355.

122-Jiang, S. et al. CRISPR/Cas9-Mediated Genome Editing in Epstein-Barr Virus- Transformed Lymphoblastoid B-Cell Lines. Curr. Protoc. Mol. Biol. 121, 31.12.1-31.12.23 (2018).

123-Park A et al. Sendai virus, an RNA virus with no risk of genomic integration, delivers CRISPR/Cas9 for efficient gene editing. Mol. Ther. - Methods Clin. Dev. 2016;3:16057.

124- Mansouri M, Ehsaei Z, Taylor V, Berger P. Baculovirus-based genome editing in primary cells. Plasmid 2017;90:5–9

125 - Zuris JA et al. Cationic lipid-mediated delivery of proteins enables efficient protein-based genome editing in vitro and in vivo. Nat. Biotechnol. 2015;33:73–80.

126-Hu Z et al. Disruption of HPV16-E7 by CRISPR/Cas System Induces Apoptosis and Growth Inhibition in HPV16 Positive Human Cervical Cancer Cells. Biomed Res. Int. 2014;2014:1–9.

127-Lao Y-H et al. HPV Oncogene Manipulation Using Nonvirally Delivered CRISPR/Cas9 or Natronobacterium gregoryi Argonaute. Adv. Sci. 2018;5:1700540.

128-Zhang, L. et al. Lipid nanoparticle-mediated efficient delivery of CRISPR/Cas9 for tumor therapy. NPG Asia Mater. 9, e441–e441 (2017).

129-Sun W et al. Self-Assembled DNA Nanoclews for the Efficient Delivery of CRISPR-Cas9 for Genome Editing. Angew. Chemie Int. Ed. 2015;54:12029–33.

130-Zhou W, Cui H, Ying L, Yu X-F. Enhanced Cytosolic Delivery and Release of CRISPR/Cas9 by Black Phosphorus Nanosheets for Genome Editing. Angew. Chemie Int. Ed. 2018;57:10268–72.

131-He Z-Y et al. In Vivo Ovarian Cancer Gene Therapy Using CRISPR-Cas9. Hum. Gene Ther. 2018;29:223–33

132-Kim SM et al. Simple in Vivo Gene Editing via Direct Self-Assembly of Cas9 Ribonucleoprotein Complexes for Cancer Treatment. ACS Nano 2018;12:7750–60.

133-Wang H-X et al. Nonviral gene editing via CRISPR/Cas9 delivery by membrane-disruptive and endosomolytic helical polypeptide. Proc. Natl. Acad. Sci. 2018;115:4903–8.

134-Li M et al. Knockdown of hypoxia-inducible factor-1 alpha by tumor targeted delivery of CRISPR/Cas9 system suppressed the metastasis of pancreatic cancer. J. Control. Release 2019;304:204–15.

135-Pan Y et al. Near-infrared upconversion–activated CRISPR-Cas9 system: A remote-controlled gene editing platform. Sci. Adv. 2019;5.

136-Yin H et al. Therapeutic genome editing by combined viral and non-viral delivery of CRISPR system components in vivo. Nat. Biotechnol. 2016;34:328–33.

137-Lyu P, Wang L, Lu B. Virus-like particle mediated crispr/cas9 delivery for efficient and safe genome editing. Life 2020;10:1–16.

138-Kleinstiver BP et al. High-fidelity CRISPR–Cas9 nucleases with no detectable genome-wide off-target effects. Nature 2016;529:490–5.

139- Slaymaker IM et al. Rationally engineered Cas9 nucleases with improved specificity. Science 2016;351:84–8.

140- Charlesworth CT et al. Identification of preexisting adaptive immunity to Cas9 proteins in humans. Nat. Med. 2019;25:249–54.

141- Li C, Brant E, Budak H, Zhang B. CRISPR/Cas: a Nobel Prize award-winning precise genome editing technology for gene therapy and crop improvement. J. Zhejiang Univ. Sci. B 2021;22:253–84.

142- Pickar-Oliver A, Gersbach CA. The next generation of CRISPR–Cas technologies and applications. Nat. Rev. Mol. Cell Biol. 2019;20:490–507.

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