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CRISPR/Cas-9 and its therapeutic applications in Onco-viral diseases: A promising tool in cancer management

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Abstract---The CRISPR/Cas9 technique is one of the gene-editing tools. The CRISPR/Cas9 method is favoured by many due to its high degree of adaptability and precision when cutting and pasting DNA. It makes it possible to carry out genetic engineering on an

unprecedented scale at a very low cost, which is one of the reasons why it is so popular. It differs from previous methods of genetic engineering in that it permits the addition or deletion of multiple genes simultaneously. Additionally, it is unique in that it is not species-specific, allowing it to be applied to organisms that were previously resistant to genetic engineering. CRISPR/Cas9 has emerged as a potent strategy for altering the genome, and it has been extensively utilized in a variety of cell lines. CRISPR/Cas9 creation of cell and animal models laid the groundwork for the clinical trials that might have treated the tumor. The technology of genome editing through CRISPR-Cas9 holds great promise for preventing tumor migration, invasion, and even treatment. However, its clinical application is constrained by the possibility of an off-target effect, necessitating an effective ethical review. The research and limitations of cancer clinical trials are discussed, as are the molecular mechanisms of CRISPR/Cas9.

***Keywords*---**CRISPR/Cas9, cancer therapy, cancer immunotherapy, cancer-associated viruses, gene therapy-approved products.

Background

CRISPR (Cluster regularly interspaced short palindromic repeats) also called genome editing technology or system. CRISPR is associated with protein-9 recognized as (Cas-9) has created massive anticipation in numerous fields such as biotech, academia, and industries. The CRISPR Cas-9 system is developed based on prokaryotic, endogenous immune system response to a non-self-particles nucleic acid of such plasmids and genome of viruses (1). Preliminary a series of bioinformatics observations were identified that Cluster regularly interspaced short palindromic repeats (CRISPRs) were part of the defense system while the same sequences originate in phage and plasmids of organisms in later. Observation directed the hypothesis that adaptive immune systems contain central major constituents are called CRISPRs. The initial demo was conducted on adaptive immunity in bacterial culture contains Streptococcus thermophiles via observing the CRISPR loci in phage(2). CRISPR Cas-9 is Native gene-editing technology based on two potential constituents Cas-9 (CRISPR related protein-9) and sgRNA (a single guided-RNA). sgRNA recognized gene of interest while Cas-9 are endonucleases that cause a break in double standard DNA and create modification and alteration of the genome (3).

Concept of CRISPR Cas-9 system

Since the 1990s CRISPR system had been studied in prokaryotic organisms. In 2012 a key publication introduce a CRISPR system to suitable bacterial specie called Streptococcus pyogenes that could be highly efficient for DNA editing. Which lead or launched a new era of technology in the field of genetic engineering. (4) Bacteria and archaea encompass the CRISPR system that presenting the immune system and can recognize damage DNA by invading viruses and plasmids. Nuclease Cas-9 in Streptococcus pyogenes is responsible for destroying

or cleavage of foreign nucleic-acids, Cas-9 has directed two small short RNA complex molecules such as CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA) that target the DNA sequence *(fig.1).* These RNA molecules are complementary for each plasmid and viral DNA that create an account of 20 base pairs approximately. (5). The combination of (tracrRNA) and (crRNA) in chimeric Single-guide RNA (sgRNA) is prepared for genome engineering that is easy to design and synthesized as compare to (tracrRNA) and (crRNA) complex. Cas-9 can be recognized by a specific DNA sequence and these DNA sequences must lie near to upstream of the protospacer-adjacent-motif (PAM), PAM-adjacent site is found within the genome that create double-strand break (2).

Essentials of CRISPR Cas-9 in Genetic engineering

Ultimately CRISPR/Cas-9 is suitable, highly specific, and easy to design. The excited duration for genetic engineering technology that has simultaneously capacity to edit multiple genes. Technology holding numerous prospective for the various application that comprise function and systemic interrogation of genetic elements. Basically, to genera the gain of function or loss of function in oncogenes, a mutation in tumor suppressive cells can be hitched by the CRISPR system. CRISPR system (type-2) encoded for Cas-9 protein which can induce DSBs (double-stranded breaks) that target specific sites of the DNA. Twin RNAs or such guided RNA involves in Cas-9 which encodes for DNA cleavage at a specific site. These gRNAs are bind with Watson-crick base pairing and it is an important recognition site for Cas-9 protein. (6).

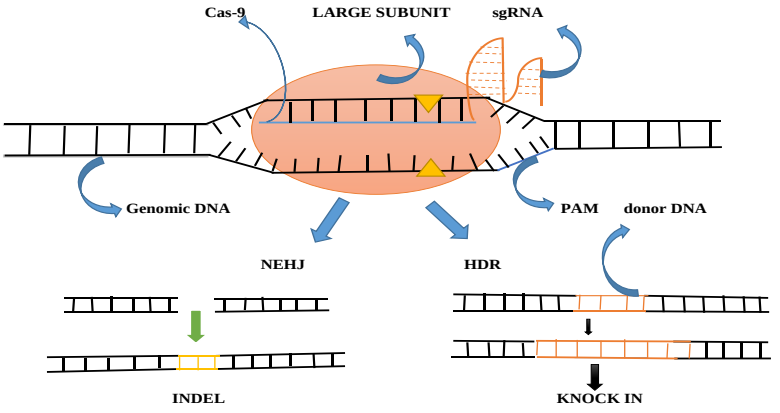

Figure 1. CRISPR Cas-9 understanding of machinery and constituents. Streptococcus pyogenes has been adopted such an efficient gene editing era called CRISPR editing technology. The cas-9 endonuclease is directed toward the recognition site into genomic DNA via single guide RNA (as shown in figure orange color representing the target site of sgRNA).

Operational mechanism and construction of CRISPR Cas-9 system

The achievement of CRISPR Cas-9 due to sequence specificity by a special structure and configuration of the Cas-9 protein. *(See in figure.2-B),* the structure of Cas-9 comprises conserved core, bi-lobed architect containing two nucleic-acid

binding grooves and adjacent active sites. (7).REC is a large recognition lobe while NUC is a little small nuclease lobe that is associated with a helix bridge. These REC and NUC performing distinguish functions include; NUC integrates both nuclease domains such as HNH, RvuC, and PAM-IP (protospacer-adjacent- motif-interacting domain). Normally Cas-9 system is inactive under the natural condition but can be activated when sgRNA is attributing at the REC lobe The complex of Cas-9-sgRNA examines a double standard DNA molecule for laborious PAMs while NGG is trinucleotide consuming Watson-crick pairing among sgRNA and DNA of interest, so at hand, HNH nuclease cleaves RNA-DNA hybrid while RvuC breaks the other strand and formed double-strand break also known as (DBS) *(Figure 2-C).* Homology-directed repair (HDR) and non-homologous end joining (NHEJ) mechanism can be repaired double-strand breaks (DSBs) that are endogenous in both eukaryotes and prokaryotes. NHEJ bearing activity DNA ligase-IV to repair the broken stand ends that can operate or introduce to mutation for insertion and deletion (indels), whereas homologous complementary templet of the DSBs repairs by HDR which results in a perfect repair. NHEJ has advantages only for knock-out the gene while for the replacement of gene and knock-in the gene in plants, HDR is used(8).

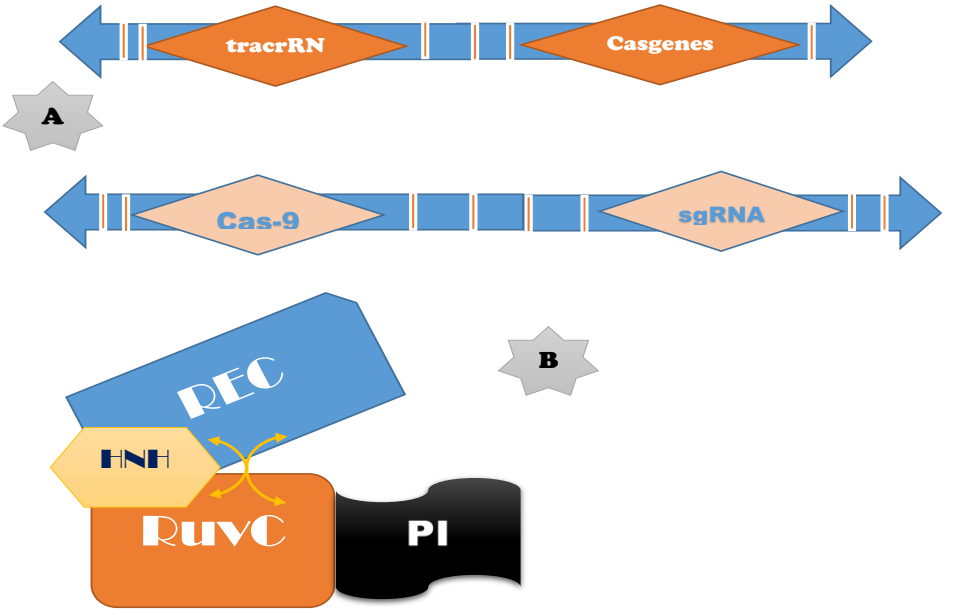

Figure 2. Architecture of the CRISPR Cas type-II system; (a) above structure is presenting the genome of native bacterial CRISPR/Cas system while the below structure is offering tracrRNA, trans-activator-RNA; sgRNA, single-guide-RNA; create engineered CRISPR Cas-9 system. (b) Diagrammatically performance of protein Cas-9 structure which comprises RvuC (nuclease domain) although REC (vast recognition lobe) that is connected with the arginine-rich region. HNH is another domain act as a nuclease. PI, PAM interacting domain

Configuration of Genetically engineered nucleases

In genetic engineering or gene therapy three most important and common nucleases are used such as TALENs, CRISPR Cas-9, and ZFNs. (a) TALENs; Transcription activator-like effector nucleases create huge capacity for genetic modification which increasing DNA double-strand-breaks (DSBs) by stimulating error-prone homology directed-repairs and non-homologous end-joining at the targeted location in the genome. ZFNs Target sequence are Repeat variable di-residues (RVDs) repeats, Protein-DNA interactions, even low-cytotoxicity, and nucleases compositions are 8–31 RVD repeats(9). (b) ZFNs; Zinc-figure-nucleases are prospective tools that redefining the limitation of ongoing research, also called chimeric nucleases based on the composition of programmable, High-Cytotoxicity. Target sequences are Zinc finger protein, protein-DNA interactions, and sequence-specific-DNA recognition components related to the non-specific-DNA cleavage domain. Targeting efficiency is Low. (c) CRISPR Cas-9; is directed two small short RNA complex molecules such as CRISPR RNA (crRNA) and trans-activating CRISPR RNA (tracrRNA), nucleases composition are sgRNA synthesis or cloning. Targeting efficacy is variable as compare to ZFNs and TELLANs (10).

Contribution of CRISPR Cas-9 in cancer-biology and treatments

The Novel innovation of CRISPR Cas-9 technology in cancer biology thru the adjustment of genes in mice models, hydrodynamic injection is used for CRISPR plasmid DNA delivery to the liver for the expression of Cas-9 and sgRNA that directed the tumor suppressor genes P10 and p53, alone and combination by which results initiate the deletion of phenocopy of a gene by applying Cre-LoxP technology(11). A Research on adenocarcinoma of the lung in which particular, associated adenovirus was used as a model for dynamics of KRAS, LKB1 and p53 thus experiment generates a loss of function or mutation in Lkb1 and p53 very effectively, as well as responsible for adenocarcinoma tumor mutation formation(12). In vitro growth, SF3B1 mutant cells are not reliant on mutated alleles. The relationship between SF3B1 Splicing alteration and hot-spot mutation provided by SF3B1but depends upon the remaining wild-type alleles that are functional. The achievement enhances via CRISPR Cas-9 mediated-knock-in genes to the Degron-KL system which results in Degron-tags become reduce. CRISPR Cas-9 is the only system that proved the efficacy of chromosomal structural abnormalities and model them positively(13). CRISPR with twin sgRNAs successfully achieved the inversion of a directed fragment of DNA and replications of DNA fragment that are approximately 10 bp to 100 kb in human and mice genome, additionally deletions and replication of DNA fragments by CRISPR technology in-collaboration of trans-allelic recombination among the DSBs and Cas-9 in 2 homologous chromosomes and regulate the millions of DNA fragments as well as a huge amount of cluster of genes(14). Chromosomes rearrangement is helpful therapeutically by expressing gene fusion actions in the human pathogenesis of cancer. Genetic episodes have demonstrated been in a mouse that has proven stimulating and requires complex guidance of the germline. In vivo somatic cells of an adult, animals were used for Viral-mediated delivery due to CRISPR Cas-9 has sufficiently induced the specific rearrangement of chromosomes. Eml4-Alk gene cause Lung cancer, these Eml4-Alk genes was generated successfully in model mice that has the ability to a hidden tumor with

Eml4-Alk inversion and express the Eml4-Alk fusion gene which was showing molecular features of ALK(+) human and histopathology that responded to treatment with ALK inhibitors(15). Recent advances of CRISPR Cas-9 in synthetic biology made effective inhibition of miRNA. The expression of both cluster molecules such as monocistronic and polycistronic miRNA can be prevented by modifying the CRISPR interference system. The advantages of a Particular system have done numerous functions such as chemically modified oligonucleotides antisense complementary(16).

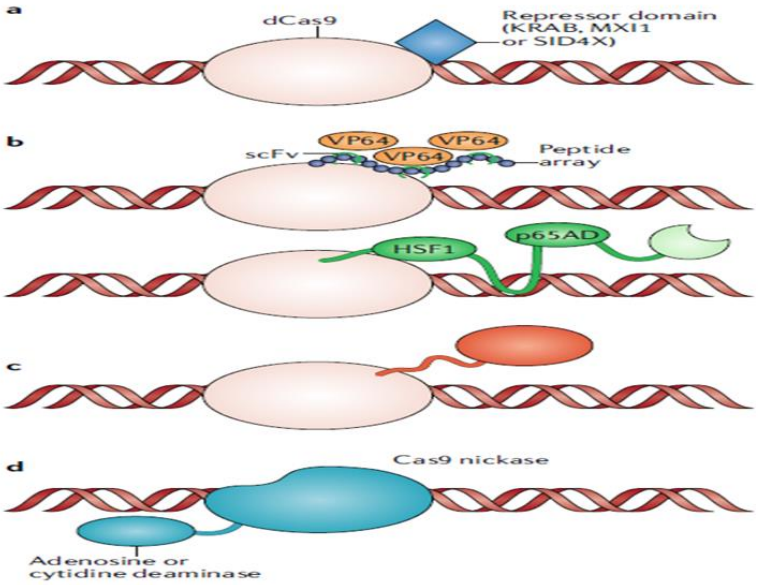

Figure 3. Application of CRISPR Cas-9 system in cancer therapy. (A) Deactivated

Cas-9 or (dCas-9) is fused with the domain of repressor that causes inhibition of the desired gene. (B) The activation site of the domain is fused with Cas-9 that induces expression of the desired gene, still the addition of transcriptional activators and binds to a sgRNA or dCas-9 to regulate the expression of particular exons. (C) Epigenetic regulators get a fusion of dCas-9 which leads to moreover activation and transcription. (D) dCas-9 get a combination with cytidine-deaminase and adenosine-deaminase which allow to absorbed point-mutation in the genome. (scFv, single-chain variable fragment), (KRAB, Kruppel- associated box)(17).

Successful Clinical trials for gene-editing technology

Testation or trial of well-known gene-editing technology CRISPR Cas-9 in a patient with aggressive lung cancer. In trial defensive cells were removed from patient blood followed through editing ex vivo CRISPR Cas-9 thus deactivating PD-1 proteins. In vivo, ongoing gene-editing technology CRISPR Cas-9 shows promise but is yet to be confirmed in clinical trials(18). A new trial uses a combination of CRISPR Cas-9 and TALENs for the treatment of human papillomavirus-associated with cervical-neoplasm via directing DNA genes such-

as E6/E7, HPV-16, and HPV-18. The following approaches reduce the risk and effects(19).

Human Papilloma virus (HPV)

Viral oncogenes such as E6 and E7 are cause critical events and elaborate in pathogenesis, the progression of cervical cancer, and observed as perfect therapeutic objective sites in high-risk of human papilloma-virus individuals. Gene specific-therapy was developed for human papilloma virus related to cancer. Established the Promoter sites (E6/E7) of human papillomavirus (HPV-16) by CRISPR Cas-9 and also targeted E6 and E7 transcription which transduced the CRISPR Cas-9 into cervical (HPV-16) positive SiHa cell lines. The generated effect shows that CRISPR Cas-9 directing promoter sites and also targeting E6/E7 genes which result in p53 and p21 proteins are accumulated, and reduce the proliferation abilities of cervical cancer cells in-vitro. Subcutaneous cells were injected in mice for remarkable incubation thus establish the tumor transplanted animal model and found realistic inhibition of tumorigenesis and incubated the cells containing CRISPR Cas-9 directed promotors+E6/E7 transcript in the growth of mouse. These consequences providing therapeutic evidence for the implication of CRISPR Cas-9 target the HR-HPV which is key oncogenes, develop a native treatment strategy, in HPV-related cancer therapy(20).

Human immunodeficiency virus (HIV)

The first use of ex vivo in patients Transcription activator-like effector nucleases TALENs and Zinc-figure-nuclease (ZFN) are cutting enzymes, employed in gene-editing technology. ZFNs enzymes added to blood collected from the patient, these ZFNs censored-out those specific genes for a protein on T-cells focused by HIV, and while the researcher transfused these cells turn-back in the patient body and Positive results were expressed(21). When the researcher announced a positive result of gene-editing technology so after at that duration half of the population had been cleared to stop their antiretroviral therapy (22). 70 patients have now been treated with this therapy but few diseases it creates more-sense to edit the genome in vivo such as targeted cells are in tissue or organs of the body that are harder to remove than blood(23).

Hemophilia-B

In October a meeting of engineering and medicine in Washington DC and US national academies of sciences in which senior scientists of Sangano Urnov and Fyodor reported that their team had injected 16 monkeys with those viruses that carried gene coding for a ZFNs and normal version of IX-factors. Factor-IX normally produced in the liver serves as a blood-clotting protein that is become mutated in those peoples who suffering from hemophilia-B. These ZFNs are cut the genome at a location that encodes for a protein called albumin, albumin proteins are produced in the liver in massive amount and then introduce to healthy versions of the factor-IX gene. Monkeys continuously producing numerous factor-IX by an increased level of about 10%(23).

Table 1
Clinical testation for CRISPR Cas-9 in enormous cancer

Serial No.	Oncology	Recognition markers	Trial stages	Bibliography
i	Advanced stage (EBV) Epstein–Barr virus associated malignancies	PD-1 knockout EBV-CTLs	Phase II	(24)
ii	Mesothelin positive multiple solid tumors	PD-1 and TCR gene-knocked out mesothelin-directed CAR-T cells	Phase I	(25)
iii	Metastatic gastrointestinal epithelial cancer	CISH gene within tumor-infiltrating lymphocytes inactivated by CRISPR/Cas9	Phase II	(26)
iv	Multiple myeloma melanoma synovial sarcoma myxoid/ round cell liposarcoma	NY-ESO-1-redirected CRISPR (TCR-endo and PD1) edited T-cells (NYCE T-cells)	Phase I	(27)
v	Relapsed or refractory CD19+ leukemia and lymphoma	Gene-disrupted allogeneic CD19-directed BBζ CAR-T cells (UCART019	Phase II	(28)
vi	Metastatic non-small-cell lung cancer	PD-1 knockout engineered T Cells	Phase I	(29)
vii	Relapsed or refractory leukemia and lymphoma	Dual Specificity CD19 and CD20 or CD22 CAR-T Cell Immunotherapy	Phase II	(30)

Positive consequences of CRISPR Cas-9 in Onco-virology

Verity of viruses are contributing cancer carcinogenesis such as a hepatitis-b virus (HBV) and hepatitis-c virus (HCV) caused cancer in the human liver, Herpes simplex virus HSV is related with the human cervix , Human T-lymph tropic virus-1 (HTLV-1) associated with Adult T-cell leukemia. Human papillomavirus (HPV) causes cervical cancer (31). The process of oncogenic viruses can be destroyed or maybe reverse-back via clearance or inactivation employing a gene-editing system called CRISPR Cas-9. Which is derived from the native immune system of bacteria (as described in the introduction) and has a genetic key role in a defensive system against viral genome or clearance of viral associated infection such as; effect of CRISPR Cas-9 intervened anti-viral activity and anti-proliferation activity have already happened in EBV- positive Burkitt's lymphoma cell lines and HPV-positive cervical carcinoma cell line (for explanation see section 6.1). CRISPR is positive, potential technology in the treatment of diseases by reducing viral load and inhibit the proliferation of the cell, hence CRISPR Cas-9 is

cost-effective and providing an encouraging option for treatment and control or prevention in viral associated cancer(32).

Oncolytic viruses used in gene editing as vectors

Oncolytic viruses (OVs) are anti-tumor viruses that provoke the anti-tumor defensive system and also specifically target infectious cells and destroy them much safely even without disrupting the normal cells of the body. In both clinical trials and preclinical models, Oncolytic viruses are gigantic in caner-therapy such as the first success therapeutic episode of genetically-modified adenovirus H101 had directed been in 2005 which approved by the chinese FDA for neck and head cancer. OVs are genetically modified viruses that are selective for cancerous cells(33). A wide verity of oncolytic viruses have been explored as vehicle or vectors for gene therapy, thus various sorts of Oncolytic are being in the investigation for the treatment of cancer.

Recently, a therapeutic strategy of immunotherapy is utmost and hopeful especially in cancer, for instance, the Significance efficacy of t-cells has demonstrated in tumor immune-therapy to treat cancer. The progress involves several steps in which immune cells of the patient are extracted and modify them genetically in-vitro and transfused into patients to mediate specific recognition and destroy the cancerous cell(34). Adeno-associated viral vector (AAV), lentiviridae, adenoviridae, and retroviridae are potential viral vectors used in gene therapy, as compare to non-viral vectors. These viral vectors are highly effective due to the extensive amount of transgenic expression and also potential delivery efficacy, ex vivo Cas-9 deliver via lentivirus and adenovirus, and achieved such effectual gene disruption. However, frequently viruses are used in progression particularly adenovirus due to its immunogenicity that may obstruct their use infrequent treatment. Adeno-associated virus (AAV) is a comparative small that causes much mild defensive response, considering it a suitable delivery vector for gene therapy. AAV Cas-9 causes 35% disruption of gen in mice liver, based on that activity it is considered helpful in the treatment of malignancy tumors from various tissue(35).

The key character of CRISPR in Onco-immunotherapy

The CRISPR system is adopted to enhance the efficacy of immunotherapies by inducing their effectiveness, decreasing tread-cost, justifying toxicity, and develop new strategies for discoveries in immunotherapies. The adaptation of CRISPR Cas-9 in Onco-research to invent the novel targets, numerous genetic screening studies have performed been in-vitro and in-vivo by CRISPR Cas-9. CRISPR is inhibiting the tumor growth directly or/either indirectly(17). Currently, immunotherapy is leading heights by cumulative its trends in cancer treatment. In immunotherapy, Te employs as barrier inhibitors in the immune system that has been shown to inverse, unfunctional T-cells, and develop efficacy in hematological cancer and solid tumors. Immunotherapy contains a secondary line genetically engineer T-cells such as (CAR-T) chimeric antigen receptor(26).

Products approved for gene therapy

Recently, industry and companies spinout the level of attentiveness in gene-therapy is very precedential based on progress and frequent report of therapeutic efficacy(36). A huge amount of products are approved for gene therapy (*see in table.2*) from concern organization and research institution(37) such FDA (Food and Drug Administration), EMA (European Marketing Authorization), NIH (National Institute of Health) (38), OCTGT (Office of Cellular, Tissue, and Gene Therapies) (39) and CGT(Cancer Gene Therapy) (40).

Table 2
Approved brands for gene therapy

Brand-names	Manufacturer	Indications	Announcement date	Approving organization	References
Strimvelis	GlaxoSmithKline [Middlesex, United Kingdom]	Adenosine deaminase deficiency (ADA-SCID)	June 2016	European Marketing Authorization	41
Yescarta [axicabtagene Ciloleucel]	Kite Pharma, Incorporated [Santa Monica, California, USA]	B-cell lymphoma	October 2017	Food and Drug Administration	42
Luxturna [voretigene neparvovec-rzyl]	Spark Therapeutics, Inc. [Philadelphia, Pennsylvania, USA]	Retinal dystrophy [biallelic RPE65 mutation]	December 2017	Food and Drug Administration	43
Gendicine	Shenzhen SiBiono GeneTech (Shenzhen, China	Head and neck squamous cell carcinoma	October 2003	State Food and Drug Administration of China	44
Glybera [alipogene tiparvovec]	uniQure [Amsterdam, Netherlands]	Lipoprotein lipase deficiency	November 2012	European Marketing Authorization	45

Limitation of CRISPR Cas-9 systems

Off-targeted properties of the CRISPR Cas-9 system is a key limitation that may cause instability of genome, epigenetic modification, and disrupt the function of a gene. Hence, when CRISPR Cas-9 is used for therapeutic purposes then off-target effects of CRISPR Cas-9 must be reduced by the division of off-target effects into off-target cleavage and off-target binding. Cas-9 could bind with the Target sequence even without cleavage which is semi-complementary to sgRNA and cause the deactivation of the transcription of a specific gene(14).

Conclusion

It is concluded that the CRISPR Cas-9 only system which through genetic diseases specifically cancer-associated illness can be reduced by employing the CRISPR Cas-9 because it has no side effects and overexpression of genes. CRISPR Cas-9 providing accuracy over other chemotherapy or treatment, well success cases have been reported worldwide. CRISPR/Cas9 gene editing technology as a strategy to therapy disease successfully entered preclinical and clinical stages. With the continuous improvement of gene editing tools and the identification of new effective targets for diseases, the clinical translation and application research of gene editing technology has been expanded. Not only in insects and plants, but also in animals and even in humans, the CRISPR/Cas9 gene editing technology proves its powerful utility. Specific gene mutation improved tumor migration, invasion, and angiogenesis, which could be reversed by targeting editing genome. At present, the in vivo gene-editing based on the CRISPR/Cas system is currently being used for diseases, such as tumor and immune diseases. At present, the clinical programs are being carrying out to verify the effects of CRISPR/Cas9 and have made outstanding achievements.

Authors Contributions

All the authors contribute equally

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Informed Consent Statement

Not applicable

Data Availability Statement: We encourage all authors of articles published in Science Scholar journals to share their research data. In this section, please provide details regarding where data supporting reported results can be found, including links to publicly archived datasets analyzed or generated during the study. Where no new data were created, or where data is unavailable due to privacy or ethical restrictions, a statement is still required.

Conflicts of Interest

The authors declare no conflict of interest

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