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Review

Precision Gene Editing Strategies with CRISPR-Cas9 for Advancing Cancer Immunotherapy and Alzheimer's Disease

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Abstract

Precision medicine approaches such as gene editing via clustered regularly interspaced short palindromic repeat (CRISPR) and their associated protein (Cas-9) are revolutionizing treatment strategies for complex diseases such as Alzheimer and cancer. In this review, we explore the application of CRISPR-Cas9 in advancing therapies for these conditions, as well as its potential in targeting senescent cells. Since the risk of Alzheimer's disease is strongly associated with genetic mutations and variations, the use of gene editing technologies to rectify these genetic errors by modifying disease-associated proteins becomes relevant. For cancer, to enhance immunotherapy approaches, modification of immune cells have been utilized to improve their anti-tumor efficacy. Additionally, the review also investigates the role of CRISPR-Cas9 in targeting senescent cells, which are implicated in both aging-related disorders and cancer progression. While challenges remain in introducing delivery methods and specificity, CRISPR-Cas9 represents a significant advancement in developing targeted, personalized treatments for these challenging health issues.

Keywords

CRISPR-Cas9, Cancer Immunotherapy, Alzheimer's Disease, Biomedical, Genetics

1. Introduction

Globally cancer causes over 9.7 million deaths annually, with more than 6.7 million affected due to Alzheimer's and age-related diseases worldwide on the rise, linked to the accumulation of senescent cells. This poses an urgent need for innovative gene modification technologies in precision medicine.Alzheimer's is currently ranked as the seventh leading cause of death in the United States and is the most common cause of dementia among older adults [1] .Clustered regularly interspaced short palindromic repeat (CRISPR) and their associated protein (Cas-9) combine to form an efficient, fast, and cost effective technique for achieving knock-out genes in the cell for gene editing. CRISPR-Cas9 system refurbishes the

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targeted genome editing approach more expediently and competently, thus facilitating proficient genome editing through embattled double-strand breaks in approximately any organism and cell type. The off-target effects of the CRISPR Cas system have been circumnavigated by using paired nickases. Moreover, CRISPR-Cas9 has been used effectively for numerous purposes, like knock-out of a gene, regulation of endogenous gene expression, live-cell labeling of chromosomal loci, addition of single-stranded RNA, and high-throughput gene screening. The execution of the CRISPR-Cas9 system has amplified the number of accessible scientific substitutes for studying gene function, thus enabling the generation of CRISPR-based disease models [2].

CRISPR-Cas9 offers flexibility in usage of the two-part CRISPR-ribonucleic acid (crRNA) trans-activating CRISPR RNA (tracr-RNA) system for some gene editing applications while using single guide RNA (sgRNA) in others. Editing multiple genes simultaneously by deploying various sgRNAs to target different genetic loci makes CRISPR-Cas9 a scalable approach with multiplexing capabilities. Finally, when compared to other gene editing approaches such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), which require protein engineering, CRISPR-Cas9 is cost effective and simple [3].

Gene editing's rapid advancement has increased its medical and clinical value over the last decade. Due to the specificity and efficiency, CRISPR-Cas9 can accurately and swiftly screen the entire genome. This simplifies disease-specific gene therapy. To study tumor origins, development, and metastasis, CRISPR-Cas9 can alter genetic material. Recently, tumor treatment research has increasingly employed this method. CRISPR-Cas9 can treat cancer by removing genes which have mutated or correcting mutations, treating tumors at the gene-level. CRISPR-Cas9-based personalized and targeted medicines may shape tumor treatment [4]. The rise of CRISPR-Cas9 technology has marked a revolutionary period in cancer immunotherapy, providing an exceptional tool for accurate and precise genetic editing. This novel method allows for precise alterations in the genome and the correction or removal of mutations that promote cancer initiation and development [5].

Chronological age represents the single greatest risk factor for human disease. One plausible explanation for this correlation is that mechanisms that drive aging might also promote age-related diseases. Cellular senescence, which is a permanent state of cell cycle arrest induced by cellular stress, has recently emerged as a fundamental aging mechanism that also contributes to diseases of late life, including cancer, atherosclerosis and osteoarthritis. Therapeutic strategies that safely interfere with the detrimental effects of cellular senescence, such as the selective elimination of senescent cells (SNCs) or the disruption of the SNC secretome, are gaining significant attention, with several programmes now nearing human clinical studies [6].

Many neurodegenerative diseases including Alzheimer's Disease (AD) have been attributed to non-canonical DNA secondary structures by affecting the neuron activity through controlling the gene expression, transcription,replication and recombination. Gene therapy has emerged as a promising therapeutic strategy for various conditions, including blood disorders, ocular disease, cancer, and nervous system disorders. CRISPR-Cas-based gene editing stands out because of its ability to introduce heritable genome changes by designing short guide RNAs. This simple yet powerful tool for editing genes showed the huge potential to correct unwanted mutations in AD-associated genes such as APP, PSEN1, and PSEN2. So, it has opened a new door for the development of empirical AD models, diagnostic approaches, and therapeutic lines in studying the complexity of the nervous system ranging from different cell types (in vitro) to animals (in vivo) [7]. The focus is on the application of the CRISPR-Cas9 technique in the fields of AD research and gene therapy and the application of CRISPR-Cas9 in the aspects of AD model construction, screening of pathogenic genes, and target therapy [8]. The advancements of the CRISPR system has now created multiple avenues to treat neurological disorders by means of varying the expression or suppression of genes and proteins. In this review, we uncover the gene editing strategies offered by CRISPR-Cas9 towards AD, cancer immunotherapy and SNCs focussing on recent developments with on-market drugs.

2. Discussion

2.1. CRISPR-Cas9 Principle of Operation

Clustered regularly interspaced short palindromic repeat (CRISPR) and their associated protein (Cas-9) is the most effective, efficient, and accurate method of genome editing tool in all living cells and is utilized in many applied disciplines. Guide RNA (gRNA) and CRISPR-associated (Cas-9) proteins are the two essential components in the CRISPR-Cas-9 system. The mechanism of CRISPR-Cas-9

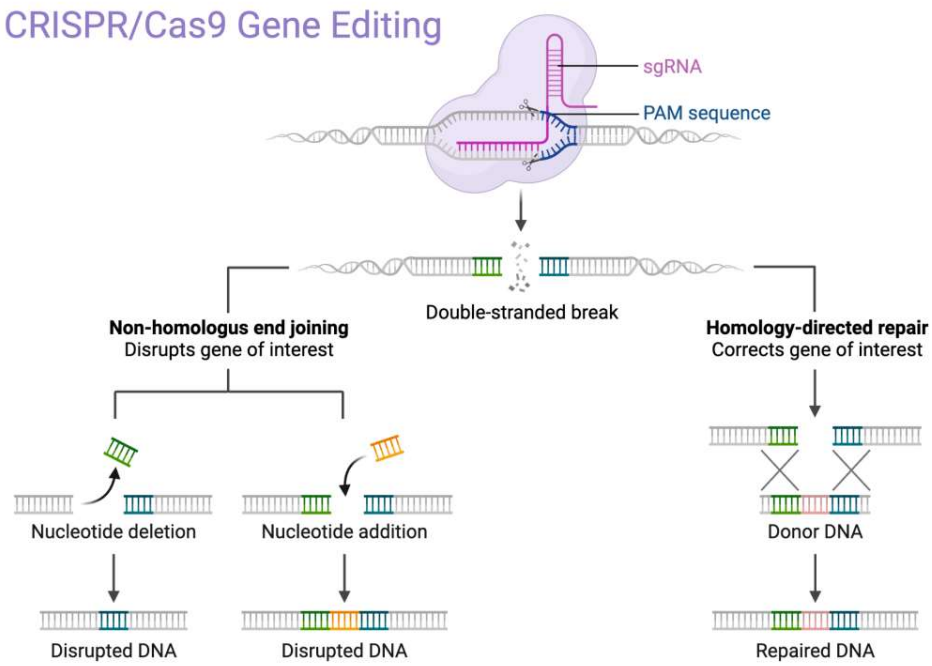

Figure 1. *Principle of operation CRISPR-Cas9. Adapted from "CRISPR-Cas9 Gene Editing", by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-template*

genome editing contains three steps: recognition, cleavage, and repair. These steps involve complex biochemical processes that ensure precise targeting and modification of the DNA sequence. The designed sgRNA directs Cas-9 and recognizes the target sequence in the gene of interest through its 5′crRNA complementary base pair component. The Cas-9 protein remains inactive in the absence of sgRNA. The initial recognition step is crucial and involves the identification of the Protospacer Adjacent Motif (PAM) sequence, which is essential for target DNA binding. The PAM sequence acts as a molecular anchor, allowing Cas9 to identify its target site with high specificity. The Cas-9 nuclease makes double-stranded breaks (DSBs) at a site 3 base pairs upstream to PAM. PAM sequence is a short (2–5 base-pair length) conserved DNA sequence downstream to the cut site and its size varies depending on the bacterial species. The most commonly used nuclease in the genome-editing tool, Cas-9 protein recognizes the PAM sequence at 5′-NGG-3′ (N can be any nucleotide base). Once Cas-9 has found a target site with the appropriate PAM, it triggers local DNA melting followed by the formation of RNA-DNA hybrid, but the mechanism of how Cas-9 enzyme melts target DNA sequence is not clearly understood yet. Upon binding to the target DNA, Cas9 undergoes a significant conformational change which is critical as it activates the nuclease domains of Cas9, preparing it for DNA cleavage. Then, the Cas-9 protein is activated for DNA cleavage. The HNH domain will cleave the complementary strand, while the RuvC domain will cleave the non-complementary strand of target DNA to produce predominantly blunt-ended DSBs. The DSB is repaired by the host cellular machinery [9]. The complete principle of operation is illustrated in Figure 1.

Double-Stranded Break Repair Mechanisms Non-homologous end joining (NHEJ), and homology-directed repair (HDR) pathways are the two mechanisms to repair DSBs created by Cas-9 protein in the CRISPR-Cas-9 mechanism. NHEJ facilitates the repair of DSBs by joining DNA fragments through an enzymatic process in the absence of exogenous homologous DNA and is active in all phases of the cell cycle. It is the predominant and efficient cellular repair mechanism that is most active in the cells, but it is an error-prone mechanism that may result in small random insertion or deletion (indels) at the cleavage site leading to the generation of frameshift mutation or premature stop codon. HDR is extremely precise and requires the use of a homologous DNA template. It is most active in the late S and G2 phases of the cell cycle. In CRISPR-gene editing, HDR needs a large amount of donor (exogenous) DNA templates containing a

sequence of interest. HDR executes the precise gene insertion or replacement by adding a donor DNA template with sequence homology at the predicted DSB site. The CRISPR-Cas9 technology is mainly used in the fields of biotechnology, agriculture, and medicine [10].

2.2. Applications

CRISPR-Cas9 has shown promise as a gene editing tool which can be leveraged for T-cells, enhancing the function of natural killer (NK) cells, targeting immunosuppressive factors to name a few; all that show promise in cancer immunotherapy with an aim to optimize the use of CRISPR-Cas9 in cancer treatment [11]. Cellular senescence is a fundamental biological process and a major contributor to age-related disorders. Similarly, neurological disorders such as AD have utilized the molecular mechanisms of the CRISPR-Cas9 system targeting the APP gene, Mapt gene and Plcγ2-P522R variant in various in-vivo models to identify how known risk factors for AD contribute to disease pathogenesis [12] [13].

2.2.1. Cancer Immunotherapy

Cancer is one of the world's leading causes of mortality. In 2023, the United States witnessed approximately 1,958,310 new cancer cases diagnosed with 609,82 cancer deaths [14]. While there have been techniques developed to rapidly evaluate the efficacy of anti-cancer drugs on tumor growth, microfluidic based biosensors to be used as confirmatory tests, biocompatibility of foreign substances has always remained a challenge [15] [16] [17]. Instead of attempting to solve a complex problem of chemotherapy, cancer immunotherapy has shifted the paradigm for cancer treatment altogether. This type of therapy aims to reduce adverse effects compared to chemotherapy which aims to kill cancer cells by utilizing the body's immune system to fight against cancer [18] [19]. Among immunotherapy classifications, the two popular methods include adoptive cell transfer (ACT) and immune checkpoint inhibitors (ICIs). These methods have obtained durable clinical responses, but with variable efficacy and response [20]. Although the first marketed immunotherapy treatment for cancer was approved by the US Food and Drug Administration (FDA) in 1986, the recombinant versions of the cytokine interferon-α (IFNα) used for immunotherapy were quickly replaced due to its short therapeutic duration. Recombinant Interleukin-2 (IL-2) was a pioneering step for cancer immunotherapy when it was approved for kidney cancer in 1992 and later for melanoma in 1998, however the success was short lived due to toxicity and expanded regulatory T-cells otherwise known as Tregs expansion. After a decade of failed attempts due to unsuccessful vaccine trials, a notable development of an immune checkpoint inhibitor, Pembrolizumab, gained FDA approval in 2014. Targeting and blocking the PD-1/PD-L1 pathway, it allowed the body's immune system to recognize and attack cancer cells effectively. This was challenged by patient safety during clinical trials. In February of 2024, the FDA approved lifileucel (Amtagvi), the first treatment for cancer that uses immune cells called tumor-infiltrating lymphocytes (TILs). The approval covered those who were previously treated with a PD-1 blocking antibody and showcased unresectable or metastatic melanomas. Of the 70 participants in the clinical trials, approximately one-third showcased reduction or complete removal of the tumors.

Despite the challenges that cancer immunotherapies have faced over the last few decades, they have showcased steady progress with efficacy and safety. This growth has the potential of being incorporated with new techniques of biosensors for therapeutic applications [25] [26] [27] [28] [29]. Novel approaches to cancer immunotherapy considering risk stratification includes utilizing Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)- Cas9 can modify TILs to improve the tumor-targeting abilities. This would improve accuracy for targeting and attacking cancer cells. Another exciting opportunity for intersection would be Optimizing Chimeric Antigen Receptor (CAR) T-cells to allow CRISPR-Cas9 recognize precise tumor antigens [30]. Another novel benefit of this technology is the use of CRISPR-Cas9 in cellular transplantation to correct major histocompatibility complex mismatches and aid in the replacement of large MHCs at native loci. Some studies demonstrated human primary CD4+ T cells were employed to knock out the B2M gene through CRISPR-Cas9, as a result of which the expression of the MHC-I surface was lost. Due to the advantage in the production of transferable T cells, this is portrayed to be advantageous to numerous cancer patients, despite the antigen genotypes of human leukocytes, important for tumor control [31]. The patients that have B cell malignancies were found to have some strong antileukemic function due to CAR19 T-cells. CRISPR-Cas9 mediated genome editing could be a useful tool in aiding to remove the genes that encode the inhibitory T-cell surface receptors. Recent studies suggest that CRISPR-Cas9 is currency undergoing various trials in order to examine the true potential use of the gene editing system in various cancer therapies. The advantages of the CRISPR-Cas9 technology might help cancer immunotherapy advance as currently immunotherapy has emerged as one of the most significant therapeutic modalities for some many diseases as depicted in Figure 2 [32].

2.2.2. Senescent cells

Senescence was first described in 1961 when Hayflick and Moorhead observed that fibroblasts in vitro showed a limited capacity for cell division before entering a phase characterized by irreversible growth arrest known as replicative senescence [33]. Biologically, senescence is the process by which a cell

ages and irrevocably stops dividing yet remains alive. This leads to the accumulation of huge

Year of Approval	Type	Cancer Treatment	Limitations	Reference
1986	Roferon-A- Recombinant Interferon-alpha 2b (IFNα2b)	Hairy cell leukemia, melanoma, follicular lymphoma and AIDS-related Kaposi sarcoma	Poor tolerance (frequent dosing) affecting short half-life and immunogenicity	[18]
1992-98	Aldesleukin- Recombinant interleukin-2 (IL-2)	metastatic renal cell carcinoma (mRCC) in 1992 and metastatic melanoma (mM) in 1998	Capillary leak syndrome (toxicity) and dampening anti-tumor immunity (Tregs expansion)	[21]
2014	Pembrolizumab- (PD-1 mAb)	Unresectable or Metastatic Melanoma (BRAF mutations), non-small cell lung cancer (NSCLC), liver, colorectal and Stage III-IVA Cervical Cancer	Adverse immune-mediated responses (pneumonitis, colitis), durability of response	[22]
2024	Amtagvi- lifileucel tumor-infiltrating lymphocytes (TILs)	Unresectable or metastatic melanoma (previously treated with PD-1 blocking antibody)	Severe cytopenia (low-blood counts), infections and unknown long-term effects	[23], [24]

Table 1. Approval timelines of cancer immunotherapies

numbers of old or senescent cells over time in tissues of the body. These cells remain active, capable of releasing harmful substances that might cause inflammation and damage surrounding healthy cells even leading to fatal diseases like cancer [34]. Ultimately, cellular senescence is a type of process that results from various stresses, leading to a state of irreversible growth arrest that, under certain conditions, contributes to tumor suppression. There is emerging evidence, however, that senescent cells exert harmful effects in vivo, driving tissue remodeling and contributing to organismal aging and a wide range of age-related diseases. Senescent cells can negatively affect their surrounding niche/microenvironment by losing their specific function and by secreting a pro-inflammatory cocktail of molecules known as Senescence-Associated Secretory Phenotype (SASP). The composition of the SASP varies depending on the senescence trigger and the cell type, but it includes proinflammatory chemokines, cytokines and growth factors that induce neuroinflammatory response. Some of these secreted proteins can promote degenerative or proliferative changes in neighboring cells. In early stages senescent cells secrete cytokines that induce the migration and infiltration of effector immune cells to the affected tissue area, as well as growth factors and proteases that support tissue repair and remodeling. Nonetheless, persistent signaling leads to chronic inflammation, a hallmark of aging and a major contributor to age-related disorders. SASP factors also have an autocrine role, reinforcing the senescent phenotype, and a paracrine role driving senescence in adjacent cells, inflammation and tumorigenesis. The senescent cells exhibit molecular features and morphological features that make them distinguishable from normal working cells [35].

Strategies targeting these cells for chemotherapeutic purposes that are currently in use include conventional seno-therapeutics, prodrugs, protein degraders, nanocarriers, and immunotherapies. These therapies target the elimination of senescent cell functions in three ways, (i) use of senolytics, (ii) SASP inhibition and (iii) improvement of immune system functions against senescent cells, immuno-surveillance [36]. Certain anti-tumor therapies are based on the induction of senescence in tumor cells. However, these senescent-like cancer cells are later cleared to avoid a chronic pro-tumorigenic state. Another strategy to inhibit the functions of senescent cells by specific silencing of SASP, a complex mixture of soluble factors like cytokines, chemokines, growth factors, proteases, and angiogenic factors that mediate paracrine and autocrine functions of senescent cells. By targeting SASP components using CRISPR-Cas9, interventions could be developed to alleviate senescence-associated pathologies without damaging regular cellular functions. During this last decade, pro senescence therapies development has become an attractive strategy because cellular senescence acts as a barrier against tumor progression [37]. CDK4/6 inhibitors trigger senescence and block tumor growth in breast cancer patients. These inhibitors tend to arrest cancer cells, limiting their growth. However, despite considering that the cancer cells get arrested by the treatment given with a CDK4/6 inhibitor, genes regulating senescence in such a context are still unknown; hence, their anti-

tumor activity is proved to be limited. Therefore, in a bid to endow genes involved in proliferation arrest by CDK4/6 inhibitors, a functional genome-wide CRISPR-Cas9 genetic screen can be used. Downregulation by sgRNA and shRNA of F9 abrogates the palbociclib-induced cell cycle arrest and senescent-like phenotype in MCF7 breast tumor cells. Another breast cancer cell line, T47D, and an alternative CDK4/6 inhibitor Abemaciclib further supported these results, then tested on a panel of 22 cancer cells. While F9 knockout prevents the induction of senescence, treatment with a recombinant F9 protein was sufficient to induce a cell cycle arrest and a senescence-like state in MCF7 tumor cells. Endogenous F9 is upregulated during senescence in different human primary cell cultures. Finally, bioinformatic analysis of cancer datasets suggests that F9 may play a key role in human tumors. This data collectively put forth the key genes involved in the response to CDK4/6 inhibitors, which may be useful in designing new therapeutic strategies for increasing their efficiency and stratifying patients in personalized medicine to avoid drug resistance [38].

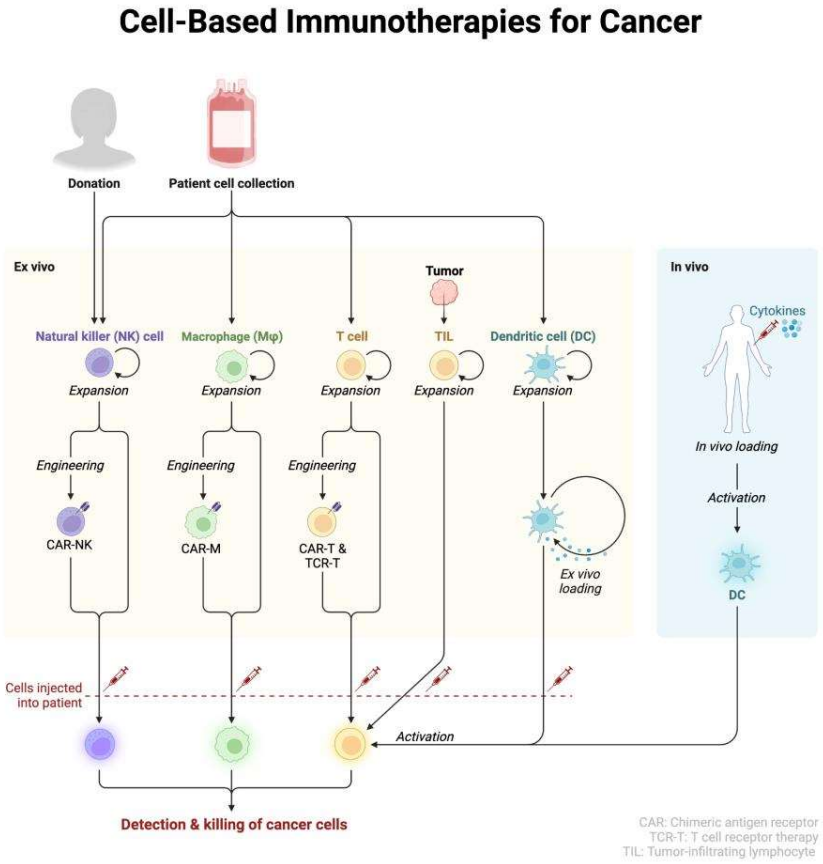

Figure 2. *Illustration of various cell-based immunotherapies, including natural killer (NK) cells, macrophages, T-cells, tumor-infiltrating lymphocytes (TIL), dendritic cells, and in-vivo loading techniques. Adapted from "Cell-Based Immunotherapies for Cancer", by BioRender.com (2024). Retrieved from https://app.biorender.com/biorender-templates*

2.2.3. Alzheimer's Disease

Alzheimer's, a neurodegenerative disease, is primarily characterized by two hallmark pathologies: β-amyloid plaque deposition and neurofibrillary tangles of hyperphosphorylated tau. Diagnosis is based upon clinical presentation fulfilling several criteria as well as fluid and imaging biomarkers. Treatment is currently targeted toward symptomatic therapy, although trials are underway that aim to reduce the production and overall burden of pathology within the brain [39]. AD being a highly complex neurodegenerative disorder with current treatment strategies ineffective, calls for a need for significant advancements in the field of molecular medicine. Repeatedly, early promising preclinical and clinical results have catapulted into devastating setbacks leading to multi-billion dollar losses not only to the top pharmaceutical companies but also to the AD patients and their families. We have made sincere efforts to feature the ongoing progress especially in the field of AD gene therapy and stem cell-based regenerative medicine [40].

The CRISPR-Cas9 system has been significantly used for genetic modifications in various species. Leveraging the CRISPR-Cas9 system, scientists have the capability to establish a suitable and useful treatment method for the management of genetic diseases that have undergone mutations. The treatment methods are classified into three groups: anti-tau, anti-amyloid, and anti- apolipoprotein E (APOE) regimens. Editing specific genes historically associated with AD such as the amyloid precursor protein (APP) or presenilin located at the β-secretase cleavage site [41]. However, further studies are required to analyze the allele disruption needed to reduce AD progression.

Recent advancements in CRISPR-Cas9 technology have significantly improved the precision and specificity of gene editing strategies for AD. Base editors and prime editing techniques have been developed to allow for more precise modifications at target sites, reducing the likelihood of off-target effects. These techniques show promise for editing genes associated with AD, such as APP, PSEN1, and PSEN2, with higher accuracy.

Comparative analyses of different gene editing approaches have shown varying efficacy in preclinical models of AD. For instance, studies comparing SpCas9, SaCas9, and AsCas12a have demonstrated different success rates in editing specific AD-associated genes. These studies provide valuable insights into the most effective CRISPR variants for targeting AD-related mutations.

A study in 2019 used CRISPR-Cas9 to target BACE1 in two mouse models, (i) 5xFAD (expressing human APP and PSEN1 transgenes with a total of five AD-linked mutations) and (ii) App knock-in. The study used sgRNA and the Cas9 protein in combination with the R7L10 peptide in the hippocampus of mice. Genome sequencing after 8-12 weeks showed a significant reduction in BACE1 expression, Aβ levels, and cognitive deficits in the mice treated with the Cas9 complex [42]. The study demonstrated a strong baseline indicative of therapeutic approaches for AD management, however clinical trials are required to comprehensively understand the long term effects of such a mechanism.

While these advancements show great potential, it's important to note that technical and ethical considerations still need to be addressed before clinical application can be achieved. The long-term effects of gene editing in the brain, potential off-target effects, and the ethical implications of modifying human genetic material remain significant challenges in the field.

2.3. Limitations and Challenges

Although CRISPR-Cas9 as a gene editing tool has shown promise in various applications, the lack of long term effects on a large sample size is concerning. The risk of off-target mutations in the event of unwanted genetic material is a scenario that may have adverse effects [43]. Furthermore, T-cell-based immunotherapy may face limitations related to T-cell exhaustion and senescence, long term effects of both yet to be explored with extensive clinical trials [44]. Gene editing tools for AD and PD have shown promise in recent years, however optimizing the delivery and specificity of CRISPR-Cas9 in clinical studies are yet to be done [45]. Recent studies have also demonstrated a promising role of nanoliposomes and exosomes as smart drug delivery systems able to penetrate the blood-brain barrier and target brain tissues [46].

The translation of CRISPR-Cas9 from laboratory research to clinical practice faces several hurdles such as off-target effects and challenges in delivery methods. Methods such as viral and non-viral vectors are being explored, each with its own set of risks and limitations. Viral vectors, while efficient, may trigger immune responses, while non-viral methods often suffer from lower efficiency.

Recent advancements in CRISPR-Cas9 technology have aimed to address some of these limitations. For instance, a study by Allemailem et al 2023 has developed high-fidelity Cas9 variants that significantly reduce off-target effects. These engineered Cas9 proteins, such as eSpCas9 and SpCas9-HF1, show enhanced specificity while maintaining on-target efficiency. Such improvements provide a more comprehensive view of the current state of the technology and its

potential for safer applications in various fields, including cancer immunotherapy and neurodegenerative disease treatment.

Technical developments have made it possible to measure biomarkers (amyloid-β: Aβ42, total tau: T-tau, and phosphorylated tau: P-tau) using fully automated assays with high precision and stability. Standardization efforts have given certified reference materials for CSF Aβ42, with the aim to harmonize results between assay formats that would allow for uniform global reference limits and cut-off values. These encouraging developments have led to the core AD CSF biomarkers having a central position in the novel diagnostic criteria for the disease and in the recent National Institute on Aging and Alzheimer's Association biological definition of AD. Ultrasensitive immunoassays and novel mass spectrometry techniques offer scope of biomarkers to monitor brain amyloidosis (the Aβ42/40 or APP669-711/Aβ42 ratios) and neurodegeneration (tau and neurofilament light proteins) in plasma samples, but future studies are warranted to validate these promising results further [47]. Moreover, as the mechanisms of bidirectional communication between the brain and microbiota are better understood, it is expected that these pathways will be harnessed to provide novel methods to enhance health and treat AD [48].

Additionally, ongoing research is focusing on optimizing delivery methods for CRISPR-Cas9 components for in vivo applications. This includes the development of novel nanoparticle-based delivery systems and the use of engineered viral vectors that can more efficiently target specific cell types or tissues. These advancements aim to improve the precision and effectiveness of CRISPR-Cas9 gene editing in clinical settings.

Ethical considerations surrounding gene editing in humans are also crucial. While CRISPR-Cas9 offers potential for treating genetic diseases, concerns about unintended consequences and the possibility of germline modifications necessitate careful regulation and oversight.

Overall, CRISPR-Cas9 is a potential therapeutic tool that has been pivotal in generating animal models and cell-based models that elucidate clinical AD phenotypes to decipher the intricate pathology and facilitate the investigation and manipulation of specific gene sequences.The application of CRISPR-Cas9 genome editing for the treatment of AD has run into several challenges such as lack of an efficient delivery mechanism could further lead to an unfavorable immune response in AD patients as well as fatal outcomes. On the other hand, future advances that can potentially increase the effectiveness of CRISPR-Cas9 in AD therapies are anticipated on a number of fronts. AD models created via the CRISPR-Cas9 system have a more realistic phenotype and reveal the processes of pathogenesis; following the screening of defective genes and ultimately establishing treatments [49].

Despite these challenges, several clinical trials using CRISPR-Cas9 are underway, particularly in cancer therapy and genetic disorders. These trials may provide valuable insights into the real-world applicability of CRISPR-Cas9 technology and help address translational challenges

3. Conclusion

CRISPR-Cas9 system has emerged as a promising tool in the field of precision medicine, its ability to modify genes offer pathways towards highly targeted therapies. In the field of cancer immunotherapy, CRISPR-Cas9 has demonstrated potential in enhancing T-cell efficacy and optimizing CAR-T cell therapy, both aimed at modifying T-cells potential to kill and effectively target cancer cells. Additionally, the system can also be used as a tool to target and modify senescent cells thus serving as one of the new therapeutic approaches for treating age-related disorders in the future. By targeting genes implicated in the pathogenesis of Alzheimer's, such as APP, PSEN1, and PSEN2, this technology can open new avenues to understand disease mechanisms and develop targeted therapies.

Despite these emerging advancements, the gene editing technology still faces significant challenges.The large size of the protein (Cas9) and its encoding gene (~4kb) make it difficult to use traditional delivery methods like adeno-associated viruses (AAVs) which have limited cargo capacity. Non-viral delivery methods like liquid nanoparticles have shown great promise however they face challenges in reaching the target tissues. CRISPR-Cas9 can also inadvertently edit off-target sites in the genome which is a concern for many therapeutic applications due to mutations. To address these challenges, researchers are developing improved delivery mechanisms and introducing Cas9 variants with higher specificity to limit off-target effects. Over the next few years, addressing such challenges will prove to play a crucial role in leveraging CRISPR-Cas9 technology at a clinical scale.

Author Contributions

KD, SV: conceptualization. KD, SV, PG: resources, formal analysis, writing – original draft, writing – review & editing.

Conflicts of Interest

The authors declare no competing financial interests or conflicts of interest.

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