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"Transformative Approaches to Alzheimer Disease Treatment: Current Progress and Future Direction"

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Abstract:

Alzheimer's disease (AD) is a neurological condition that worsens over time and is characterised by tau tangles and amyloid plaques. Current medications mostly address symptoms, despite significant research advancement, leaving a demand for disease-modifying therapy unfulfilled. New treatment approaches target the genetic and molecular causes of AD by focussing on gene editing, specifically the CRISPR-Cas9 system. By specifically editing genes linked to disease, including APP, PSEN1, PSEN2, and APOE4, CRISPR-Cas9 aims to improve neuronal protection and lessen tau and amyloid-beta pathology. Base and prime editing are two recent developments in CRISPR technology that have increased therapeutic potential, decreased off-target effects, and improved editing specificity. In cellular and animal models, preclinical research shows that it is effective in repairing mutations, modifying gene expression, and lowering the synthesis of neurotoxic proteins. Adeno-associated viruses (AAVs) and lipid nanoparticles are two delivery systems that have been created to get across the blood-brain barrier's obstacles. Clinical use is nevertheless hampered by concerns about long-term safety, distribution efficiency, and ethical issues, despite encouraging results. CRISPR-Cas9's combination with immunotherapy and artificial intelligence has created new opportunities for precision medicine by providing specialised methods to address the pathological and genetic characteristics of AD. With its potential to shift AD treatment from symptomatic management to curative approaches, this review focusses on the advancements, difficulties, and prospects of CRISPR-Cas9 in AD therapies. To advance CRISPR-based medicines into clinical practice, ongoing innovation in gene-editing technologies, delivery methods, and ethical frameworks will be essential.

Keywords: Alzheimer's Disease, Gene Editing, Amyloid-β, Nanoparticle Delivery, Off-target Effects, CRISPR-Cas9.

1. INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative condition primarily affecting individuals over 65. Early-onset AD, occurring before this age, accounts for less than 5% of cases

and is often familial, driven by inherited genetic mutations [1]. In contrast, late-onset AD, usually sporadic, lacks identifiable genetic mutations. Familial AD is linked to mutations in the amyloid precursor protein (APP) gene and the presenilin (PSEN) genes, PSEN1 and PSEN2, which play critical roles in amyloid-beta (Aβ) production via gamma-secretase activity. A hallmark of AD includes the formation of extracellular Aβ plaques and hyperphosphorylated tau protein aggregates, leading to cognitive decline, motor dysfunction, and impaired daily living [2]. AD progression is categorized into stages, beginning with mild mental decline, advancing to mild cognitive impairment, and culminating in severe symptoms like hallucinations, anxiety, and sleep disorders. Beyond genetic factors, oxidative stress and neuroinflammation also play significant roles in AD pathogenesis, complicating treatment efforts. Current clinical approaches focus on slowing symptom progression and cognitive decline, but no cure exists, and patients often progress to advanced stages. The heterogeneity of AD presentations highlights the need for personalized treatments, emphasizing the urgency of innovative research targeting disease-modifying therapies [3]. Alzheimer's disease (AD) research has made significant strides, particularly through clinical trials targeting its complex pathology. Amyloid-centric therapies, such as Aducanumab by Biogen, Lecanemab by Eisai and Biogen, Gantenerumab by Roche, and Donanemab by Eli Lilly, have focused on reducing amyloid plaques to slow cognitive decline [4].While promising, these approaches have faced challenges, as seen in the failures of Semagacestat, a gamma-secretase inhibitor, and BACE inhibitors like Elenbecestat and Atabecestat. Tau-targeting therapies, such as LMTX by TauRx Pharmaceuticals, aim to mitigate tau tangle progression, while alternative approaches like ANAVEX 2–73, targeting the sigma-1 receptor, and ALZT-OP1, which combines anti-inflammatory and anti-amyloid strategies, offer innovative solutions [5]. Among these, Lecanemab, a monoclonal antibody targeting amyloid-beta protofibrils, has emerged as a promising therapy [6]. Clinical trials have shown it reduces amyloid levels and moderately slows cognitive decline in patients with mild cognitive impairment or mild AD dementia, leading to its approval. However, challenges remain, including its high cost, moderate efficacy, potential side effects such as amyloid-related imaging abnormalities (ARIA), and the need for further investigation into its long-term effects. Lecanemab represents a critical step forward in precision medicine for AD, highlighting the importance of targeted therapies while emphasizing the need for ongoing research to optimize treatment strategies and explore combination therapies for improved outcomes [7].

The advent of gene-editing technologies, particularly CRISPR-Cas9, offers new hope in addressing AD's complexities. CRISPR-Cas9 has shown significant promise in treating genetic disorders in animal models, suggesting its potential for developing AD therapies. This review explores the evolution of CRISPR-Cas9, compares it with other gene-editing tools, and summarizes its current applications in advancing AD treatment strategies[8].CRISPR-Cas9, a bacterial immune system component, defends against mobile genetic elements like plasmids and viruses. Initially identified by Ishino in 1987, it gained laboratory application through Doudna and Charpentier's efforts [9]. The system's core components are the Cas9 enzyme, functioning as a molecular scissor, and single-guide RNA (sgRNA), which targets specific DNA sequences. CRISPR-Cas9 operates via two DNA repair pathways: Non-Homologous End Joining (NHEJ), which induces insertions or deletions, and Homology-Directed Repair (HDR), which enables precise sequence replacement [10].CRISPR-Cas9's genome-editing capabilities hold promise for treating Alzheimer's disease (AD) [11].

Delivery methods include viral vectors like adeno-associated viruses (AAVs), which efficiently reduce amyloid-beta (Aβ) production, and non-viral techniques, such as nanocomplexes, offering lower immunogenicity and targeted delivery [12]. For instance, nanocomplexes targeting the BACE1 gene effectively reduced β-cleavage products in AD models [13].

Gene-editing strategies have addressed various AD-associated genetic mutations. Targeting APP mutations and enhancing protective pathways has shown success in reducing Aβ production. CRISPR-Cas9 has also been used to delete toxic forms of tau, implicated in neurodegeneration, and to modulate genes like CysLT1R, which contributes to inflammation, and Mt1, enhancing neuroprotection through melatonin receptors[14]. Studies demonstrate CRISPR-Cas9's potential in familial AD by correcting mutations in genes like PSEN2 and improving neuronal resilience by editing APOE4 alleles in induced pluripotent stem cells (iPSCs). These advancements provide insights into AD mechanisms and suggest a path toward innovative therapeutic strategies [15].

2. CURRENT LANDSCAPE OF ALZHEIMER'S THERAPEUTICS

The FDA approval of amyloid-targeting therapies significantly impacts the design and conduct of Alzheimer's disease (AD) clinical trials, influencing study design, statistical analysis, safety monitoring, and participant recruitment and retention. A key question is whether a placebo arm remains appropriate for trials involving amyloid-targeting therapies or combination interventions[16]. A potential alternative, commonly used in psychiatry trials for conditions like major depressive disorder or schizophrenia, is a factorial or multi-arm design with both placebo and approved-drug control groups. However, such designs increase sample size requirements, complicate statistical analysis, and escalate costs and trial duration[16],[17].Safety monitoring in trials using amyloid-targeting therapies poses unique challenges. Amyloid-related imaging abnormalities (ARIA), the main adverse events associated with plaque-lowering monoclonal antibodies, require close monitoring through surveillance MRIs. ARIA can manifest as edema/effusion (ARIA-E) or hemorrhage (ARIA-H) and is often asymptomatic. Additional safety considerations arise when distinguishing adverse events caused by experimental drugs versus approved therapies[18].The availability of FDA-approved amyloid-targeting treatments also affects recruitment and retention for future AD trials. Barriers such as cost, limited access, and stringent eligibility criteria reduce patient availability. Furthermore, participants from early amyloid-targeting trials may no longer meet biomarker criteria due to reduced amyloid burden, shrinking the pool of eligible candidates. To address these challenges, decentralized clinical trials (DCTs) offer a promising solution. DCTs can enhance recruitment by reaching diverse populations, providing greater flexibility, and allowing home-based screening and administration of therapies. Expanding geographic accessibility for trials can further improve participation. However, global variations in regulatory approvals and access to treatments may complicate trial design and limit patient diversity, underscoring the need for innovative strategies to overcome these barriers[19].

While FDA-approved amyloid-targeting therapies have shown clinically meaningful benefits in trials, implementing them as standard care poses significant challenges. These include healthcare system infrastructure, clinician training, screening and monitoring methods, and limited real-world data. Clinicians must carefully weigh the risks and burdens of these treatments against their

potential benefits and engage patients and caregivers in shared decision-making processes[20]. The eligible patient population for amyloid-targeting therapies is diverse, including those with prior exposure to plaque-lowering treatments, varying amyloid burdens, and altered biomarkers. These subpopulations require tailored approaches. Furthermore, barriers such as inequitable access, financial burden, safety risks, comorbidities, and the invasive nature of IV infusions which involve high doses due to limited blood-brain barrier penetration may lead some patients to prefer enrolling in clinical trials over starting approved treatments [21]. For individuals with unique conditions like early symptomatic AD, DIAD, or Down syndrome, the risks and benefits of amyloid-targeting therapies remain uncertain. Offering multiple treatment options, including clinical trials, supports patient autonomy and ensures equitable care [22].

Trials of monoclonal antibodies targeting amyloid show these therapies reduce amyloid burden, as measured by amyloid-PET, and slow cognitive and functional decline. They also affect biomarkers such as soluble amyloid species, total and phosphorylated tau, and markers of neurodegeneration and neuroinflammation [23]. However, amyloid-PET imaging is costly and not widely accessible, highlighting the need for more affordable and practical biomarkers to assess clinical efficacy, safety (e.g., ARIA), and treatment risks as these therapies become more prevalent [24]. In combination therapy trials, attributing biomarker changes to specific treatments can be challenging [25]. Fluid biomarkers like cerebrospinal fluid (CSF) and plasma enable profiling of multiple markers from a single sample. Research suggests plasma biomarkers, particularly P-tau217, may outperform CSF in identifying amyloid-positive patients. Plasma P-tau217 also offers a cost-effective option for monitoring amyloid burden and eligibility screening for trials requiring amyloid positivity. This approach could streamline combination trials and expand accessibility as shown in table 1.

Table 1: Evidence and Biomarker Insights for Amyloid-Targeting Therapies in AD

ect	**ils**	**rences**
rapeutic Impact	oclonal antibodies targeting amyloid lower amyloid en (measured via amyloid-PET) and slow cognitive and ional decline. They also influence biomarkers like soluble oid species, tau proteins, and neurodegeneration esses.	[25]
tations of Amyloid-	High cost and limited accessibility. Need for less expensive, widely available biomarkers for uring clinical efficacy, risk (e.g., ARIA), and safety.	
l Biomarkers	CSF and plasma biomarkers allow for profiling multiple ers from a single sample. Plasma P-tau217 shows higher accuracy in identifying oid positivity compared to CSF biomarkers.	
ible Alternative points	CSF Aβ42/40 shows potential for determining amyloid tivity but with higher accuracy in CSF samples. Plasma P-tau217 could serve as a cost-effective native for eligibility screening and monitoring.	

ıbination Therapy lenges	Attributing biomarker changes to specific treatments in ɔination therapies is complex. Research is needed to refine the use of plasma amyloid ters for guiding treatment decisions in combination trials.	

3. PATHOPHYSIOLOGY OF ALZHEIMER'S DISEASE

The pathophysiology of AD is primarily characterized by the accumulation of amyloid plaques and neurofibrillary tangles, which are critical neuropathological features of the disorder. Amyloid β protein undergoes aberrant folding and aggregation as shown in (fig.1), leading to the formation of senile plaques, a hallmark of AD [27], [28]. These plaques result from the cleavage of APP by secretases, contributing to neuronal dysfunction and loss [28]. Additionally, tau protein plays a significant role in the disease's progression, as its abnormal aggregation forms neurofibrillary tangles, further disrupting synaptic integrity and neuronal health[29]. The convergence of these pathological processes, including mitochondrial damage and selective neuronal loss, underpins the cognitive decline observed in affected individuals[30]. Moreover, the interplay between genetic and environmental factors exacerbates the disease's progression, highlighting the complexity of its etiology[29]. Ultimately, the loss of synapses and the resultant cognitive impairment are central to the clinical manifestations of Alzheimer's disease, making the understanding of these molecular mechanisms crucial for developing effective therapies [29], [31].

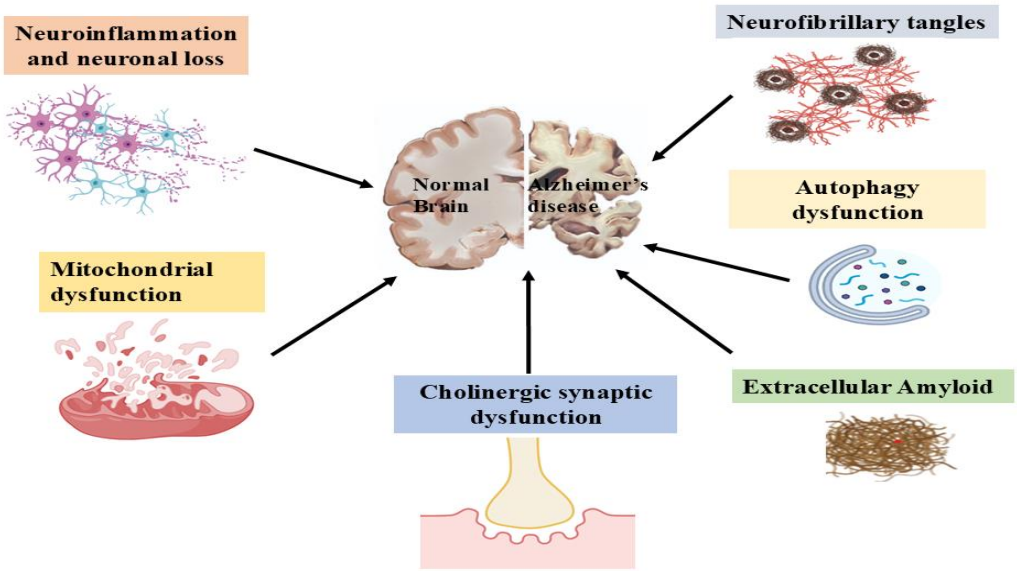

Fig.1: Pathogenesis of Alzheimer's Disease

4. ADVANCES IN CRISPER/CAS9 TECHNOLOGY FOR AD

The CRISPR-Cas9 system has emerged as a pivotal tool for genetic modification, offering promising therapeutic strategies for managing genetic diseases like Alzheimer's disease (AD). Scientists are leveraging CRISPR to target specific genes implicated in AD, such as amyloid

precursor protein (APP), presenilin (PSEN1, PSEN2), and apolipoprotein E (APOE). Recent advancements, including base editing and prime editing, have significantly improved the precision and specificity of gene editing, minimized off-target effects and enhanced the accuracy of targeting mutations associated with AD [32]. Preclinical studies have compared various CRISPR variants, such as SpCas9, SaCas9, and AsCas12a, revealing differing success rates in editing AD-related genes[33]. A 2019 study demonstrated the efficacy of CRISPR-Cas9 in reducing BACE1 expression, Aβ levels, and cognitive deficits in mouse models of AD, highlighting its therapeutic potential. Despite these advances, technical and ethical challenges persist[34]. Long-term effects on the brain, potential unintended genetic modifications, and ethical concerns regarding human genome editing must be thoroughly addressed before clinical application can proceed[35]

The APOE4 allele is strongly associated with Alzheimer's disease (AD) pathogenesis, significantly increasing the risk of developing AD by promoting tau protein hyperphosphorylation. Individuals with one APOE4 allele have approximately a threefold higher risk, while those with two alleles face up to a 15-fold increased risk. About 80% of AD patients carry at least one APOE4 allele. A single amino acid substitution can convert APOE4 into the protective APOE2 allele or the neutral APOE3 allele, providing a foundation for targeted therapies[36]. Recent advancements in CRISPR-Cas9-based "base editing" have demonstrated promising results for modifying APOE4. In mouse astrocytic cells, lentivirus-delivered CRISPR-Cas9 selectively reduced APOE4 protein production by 60%, sparing APOE3. Similarly, in HEK293T cells, the system fused with cytidine deaminase irreversibly converted APOE4 into APOE3, mitigating tau hyperphosphorylation and correcting mutations linked to AD[36].The APP gene is another critical target for sporadic AD. Lentiviral CRISPR-Cas9 selectively edits the C-terminus of the APP gene, reducing amyloidogenic pathway activity while preserving its physiological roles. This strategy has effectively reduced Aβ production and AD-like pathology in mouse models and cell lines, demonstrating its therapeutic potential[37].

CRISPR-Cas9 has transformed gene editing with its vast potential for clinical applications, but off-target effects remain a significant challenge. These effects occur when the Cas9 protein unintentionally cleaves DNA at unintended sites, potentially disrupting normal gene functions and compromising genomic stability. Precision in therapeutic applications, such as those for Alzheimer’s disease (AD), depends heavily on the accurate interaction between the single-guide RNA (sgRNA) and the target DNA sequence[38]. Factors influencing off-target effects include the length and composition of sgRNAs. Truncated sgRNAs shorter than 17 nucleotides show reduced activity compared to the standard 20-nucleotide sgRNAs, emphasizing the importance of maintaining full-length sequences to minimize mismatches. Additionally, the GC content of sgRNAs plays a critical role; extremes in GC content can increase the likelihood of off-target effects, with optimal performance achieved at a GC content of 40–60%.[39].To address these challenges, advanced Cas9 variants, such as SpCas9-HF1, Sniper-Cas9, eSpCas9 1.1, HypaCas9, and xCas9, have been developed to enhance DNA cleavage specificity while preserving on-target activity. Computational algorithms further improve precision by enabling the selection of highly specific sgRNA sequences, reducing unintended DNA cleavage. Ongoing innovations in Cas9 protein engineering and sgRNA design are critical to mitigating off-target effects, paving the way for the safe and effective clinical use of CRISPR-Cas9 in AD and other diseases [40].

5. MECHANISM OF CRISPR CAS9 IN ALZHEIMER'S THERAPY

The adaptive immune system of prokaryotes-which are primarily descended from bacteria and archaea is where the CRISPR-Cas9 (Fig.2) system was discovered [41]. Enzyme Cas9, trans-activating crRNA and tracrRNA are the three primary components of the extensively used Type II CRISPR-Cas9 system. Among these three components, Cas9 is an enzyme that cleaves the target DNA through six domains: the bridge helix, the PAM-interacting domain, the Recombination Enhancer Cis-acting Element (REC I, II) the HNH, and the RuvC. The guide RNA's binding to the target sequence is facilitated by the REC I domain. The arginine rich bridge helix is necessary to initiate the cleavage activity, and the PAM-interacting domain is responsible for initiating the binding process with the target DNA once it has been bound. A chimeric single can be created by joining the crRNA-tracrRNA duplex, which will effectively progress genome engineering [42], [43].The synthetic RNA's 20-nucleotide guide sequence is complementary to the target site. Once the target sequence is identified, the sgRNA attaches by base-pairing via Watson-Crick method, which instructs Cas9 to cut the DNA strand and form a double strand break (DSB) at the intended site. The nuclease domains of HNH and RuvC cleave target DNA.

Non-homologous end joining (NHEJ) and homology-directed repair (HDR) are the two main techniques for mending the fractures (Fig.3) [44]. When DSBs are damaged for gene alteration, the HDR repair mechanism correctly repairs them using a donor DNA template, while the NHEJ repair mechanism often causes genomic insertions or deletions (indels) for gene disruption, which is more efficient. Normally, the NHEJ is prone to errors and has the ability to join break sequences directly. It can also introduce arbitrary insertions or deletions (indels) at the DSB site[45]. When sgRNA and Cas9 are expressed together, high-efficiency cleavage of any target sequence is readily accomplished [46]. sgRNA attaches to a complementary genomic DNA fragment through an RNA–DNA complex after Cas9 locates its DNA-binding sites. Cas9 endonucleases cause a double-strand break that can be repaired via the HRR route or the error-prone NHEJ, both of which can cause DNA mutagenesis. Frameshift mutations can result from insertion or deletion (indel) mutations, which cause NHEJ repair. On the other hand, in the presence of a homologous DNA template, the HDR process can be utilized to induce precise genetic alterations.

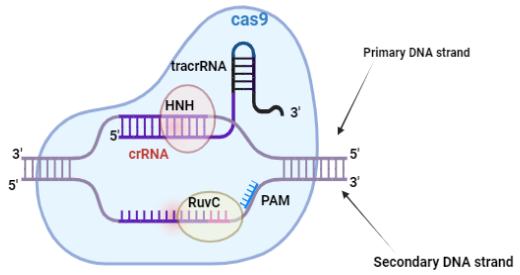

Fig.2: An outline of the Cas9 endonuclease.

Single guide RNA (sgRNA) is the name given to this combined RNA molecule, which is connected to the 3′ end of crRNA and functions as an anchor to the Cas9 endonuclease. Cas9 looks for the proper protospacer adjacent motif (PAM) in possible targets' DNA. Upon locating the PAM, the protein undergoes a confirmation alteration that causes the DNA to unravel, hence facilitating

communication between the crRNA and DNA. If complementary binding takes place between the guide and seed area, the RuvC and HNH domains subsequently cut the target DNA.

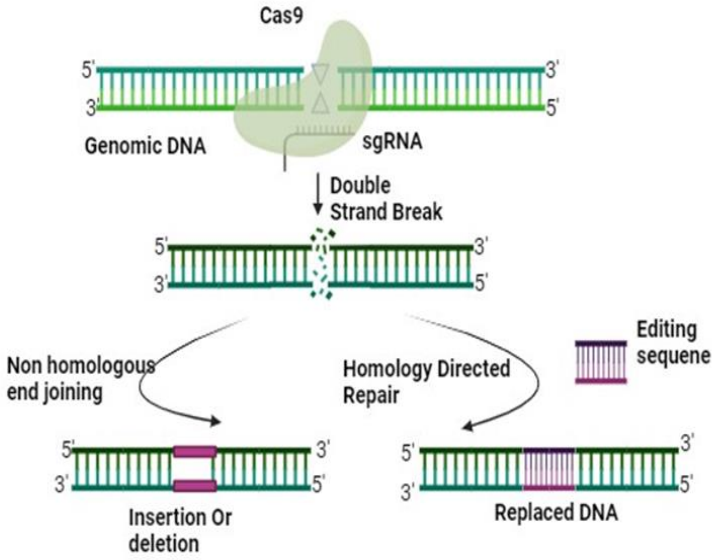

Fig.3: The CRISPR-Cas9 mechanism for genome editing

6. PROPOSED CRISPR-CAS9 DELIVERY METHOD FOR ALZHEIMER'S DISEASE

The delivery of CRISPR/Cas9 systems for applications in AD can be approached through various methods, each with its advantages and challenges. Adenoviral vectors (AdVs) are recognized as effective delivery vehicles for introducing RNA-guided nucleases into human somatic cells, making them suitable for targeting neuronal cells in AD research [47]. These viral vectors can package genetic material efficiently, broadening the applicability of CRISPR/Cas9 to different cell types, including those that are quiescent or dividing [48]. However, safety concerns associated with viral vectors necessitate the exploration of nonviral drug delivery systems. Recent advancements in lipid- or polymer-based nanocarriers present promising alternatives for in vivo delivery of CRISPR/Cas9 components, addressing the challenges faced in effectively targeting the brain [49]. The combination of these delivery methods could enhance the precision and safety of CRISPR/Cas9 applications in AD, ultimately contributing to more effective gene editing strategies in therapeutic contexts [50], [51]. Thus, a multifaceted approach utilizing both viral and nonviral systems may be essential for optimizing CRISPR/Cas9 delivery in the treatment of Alzheimer's Disease. Lipid nanoparticles and polymeric nanoparticles also hold potential as CRISPR-Cas9 delivery tools, having been extensively used in cancer and viral disease treatments[52]. However, their application in AD management remains largely unexplored. Gold nanoparticles represent another innovative approach. Wang et al. demonstrated that CRISPR-Gold targeting the CXCR4 gene achieved 3-4% HDR efficiency in various human cell types. A single local infusion of CRISPR-Gold into the muscles of mdx mice corrected the mutated dystrophin gene responsible for Duchenne muscular dystrophy, without significantly altering the inflammatory cytokine profile, indicating low toxicity and good tolerability [53].

Recently, macrovesicles have emerged as a novel method for delivering CRISPR-Cas9 therapeutics. In this approach, a producer cell line is transfected with sgRNA, Cas9 protein, and a micro vesicle-inducing protein (RAB proteins). The cells then produce macrovesicles containing the Cas9-sgRNA complex, which are harvested and used to deliver the gene-editing payload to target cells as shown in (fig.4) [54], [55]. This method shows promise for future applications in gene therapy, including the treatment of Alzheimer's disease.

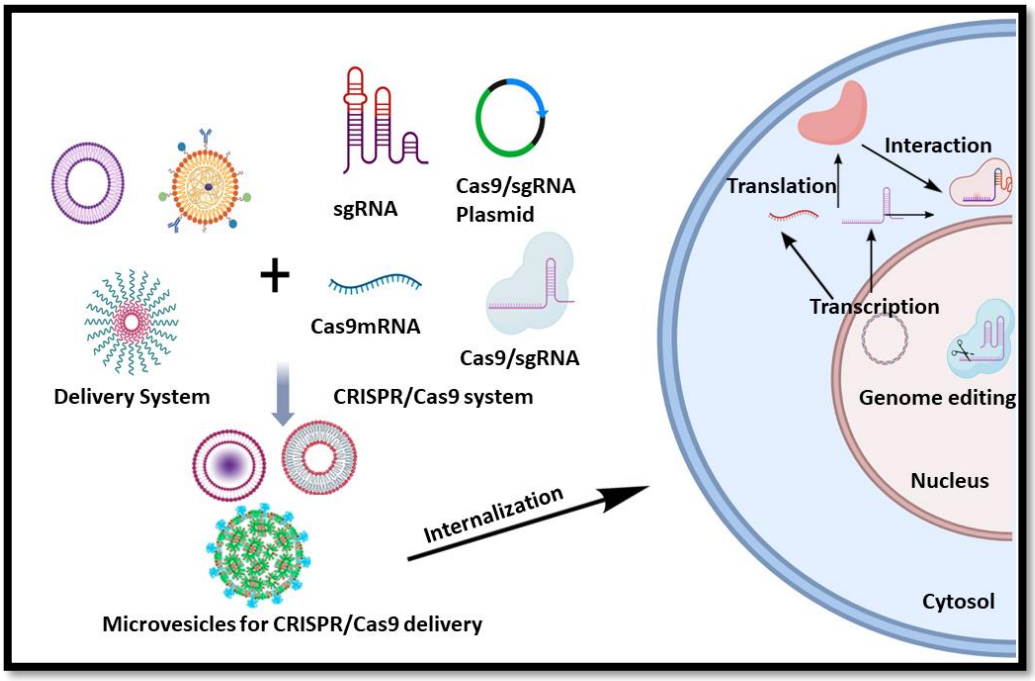

Fig.4 Schematic illustration of CRISPR/Cas9 delivery systems using various vectors. Different delivery systems such as liposomes, nanoparticles, and microvesicles are used to transport CRISPR/Cas9 components, including Cas9/sgRNA plasmid, Cas9 mRNA, and Cas9/sgRNA ribonucleoprotein (RNP). Following internalization, the plasmid DNA is transcribed into mRNA in the nucleus, which is then translated into the Cas9 protein in the cytosol. The Cas9 protein interacts with sgRNA to form the Cas9 RNP complex, facilitating genome editing within the nucleus.

7. Synergies between CRISPR/Cas9 & Immunotherapies

The combination of immunotherapy and CRISPR/Cas9 gene editing technology offers novel approaches to the treatment of Alzheimer's disease (AD). In order to fight the illness, these tactics seek to combine the accuracy of genome editing with the strength of immune responses.

7.1 CRISPR/Cas9 and Immunotherapy Synergistic Mechanisms

The amyloid precursor protein (APP) and presenilin (PSEN) genes are two examples of genes linked to Alzheimer's disease that may be directly edited using CRISPR/Cas9. These specific changes can

lessen the development of neurofibrillary tangles and amyloid-beta plaques, two characteristics of AD. CRISPR may improve the effectiveness of immunotherapy strategies that depend on the immune system's capacity to identify and eradicate these abnormal aggregates by changing the genetic composition[56].

Additionally, the overall efficacy of immunotherapy can be increased by using CRISPR/Cas9 gene editing to modify immune cell activity. For example, improving the brain's innate immune cells' (microglia) response can result in improved amyloid plaque removal[57]. By enabling genetic changes that increase microglia's phagocytic activity, CRISPR can help them play a part in the immune response against Alzheimer's disease. This two-pronged strategy could potentially address some of the drawbacks of immunotherapies alone, which might be less successful when specific genetic variables are present[58].

8. Challenges in CRISPR Therapy for Alzheimer Disease

Although site-specific gene editing and other applications have shown significant promise with the CRISPR/Cas9 system, there are a number of aspects that affect its effectiveness that need to be taken into consideration, particularly if it is to be utilized for in vivo human gene therapy. These elements include Cas9 activity, off-target cutting, sgRNA design, incidence/efficiency of HDR vs. NHEJ, target DNA site selection, and delivery strategy.

8.1 Delivery to the Brain:

The blood-brain barrier (BBB) is a protective layer of cells that prevents harmful substances from entering the brain while also limiting the delivery of therapeutic agents, including CRISPR components. This makes it difficult to effectively deliver CRISPR-based treatments for Alzheimer's disease (AD) because the CRISPR machinery (such as the Cas9 protein and guide RNA) needs to cross the BBB to reach the brain cells. The BBB’s selective permeability means that most drugs or therapies struggle to reach their targets in the brain, which is a major hurdle in developing effective gene-editing therapies for AD[59].

8.2 Target DNA site selection

Choosing appropriate target DNA locations for CRISPR-Cas9 application in Alzheimer's disease (AD) is difficult for a number of reasons. Initially, the requirement for a Protospacer Adjacent Motif (PAM) sequence (usually "NGG") restricts the target locations that are available inside pertinent genes. Given the intricate genetic makeup of AD, which includes several genes and interactions, this restriction is especially important. Furthermore, there is a risk of off-target consequences as comparable genome sequences may result in accidental changes that could impair vital neural activities[60]. Since epigenetic alterations such DNA methylation are common in AD pathophysiology, they make site selection even more difficult by affecting target regions' accessibility[61].

8.3 Off Targeting

In Alzheimer's disease (AD), the brain's complex genetic landscape increases the risk of these off-target modifications, which can disrupt critical genes and potentially worsen disease symptoms or cause new issues. Ensuring precise gene editing is vital, as even small, unintended changes in the DNA can have significant consequences in sensitive brain cells, making it crucial to improve the accuracy of CRISPR systems for safe and effective treatments[62].

8.4 Efficient Delivery Vehicles

Efficient delivery of CRISPR components to target cells is a major challenge, particularly in the context of Alzheimer's disease (AD). Viral vectors, such as adeno-associated viruses (AAVs) and lentiviruses, are commonly used for gene delivery but have limitations. One issue is the size constraint: the Cas9 protein and guide RNA are often too large to fit within the packaging capacity of AAVs. Additionally, these viral vectors can trigger immune responses in the body, which not only reduces their effectiveness but can also pose safety risks. Non-viral vectors, like lipid nanoparticles (LNPs) and electroporation, are being explored as alternatives. While LNPs are more versatile, they may have low efficiency in delivering CRISPR components to specific cell types, particularly in the brain. Electroporation, on the other hand, is invasive and unsuitable for systemic delivery, limiting its use in treating widespread conditions like AD[63].

9. Applications of CRISPR/Cas9:

Early-onset Alzheimer’s disease (AD) is primarily caused by over 200 genetic mutations. The CRISPR-Cas9 system enables precise establishment of these mutations in cellular and animal models, effectively mimicking AD pathogenesis. This technology facilitates the study of dynamic biological processes, explores synergistic gene interactions through multi-gene editing, and surpasses traditional methods in speed, cost-efficiency, and reliability. By rapidly generating numerous AD models, CRISPR-Cas9 provides a transformative platform for drug screening, treatment development, and advancing our understanding of AD pathophysiology, significantly accelerating basic research in the field.

9.1 CRISPR/Cas9 in Precision Therapy

CRISPR/Cas9 technology is a promising tool for targeted therapy in Alzheimer’s disease (AD). It allows precise editing of genes associated with AD pathogenesis, such as *APP*, *PSEN1*, and *PSEN2*. By correcting pathogenic mutations or silencing harmful gene expressions, CRISPR/Cas9 can potentially reduce the production of toxic amyloid-beta plaques and tau tangles, the hallmarks of AD. Additionally, it enables the introduction of protective genetic variants or the regulation of pathways involved in neuroinflammation and neuronal survival, paving the way for personalized and effective treatments for AD[64]**.**

9.2 CRISPR/Cas9 in the Development of Alzheimer's Disease Models

Three essential amino acids—G676R, F681Y, and R684H—that distinguish the human and rat APP sequences were shown to differ by researchers using computer modelling. These variations affected APP’s binding affinity for β-secretase BACE1, influencing Aβ synthesis. Using CRISPR-Cas9 technology, they created a humanized APP rodent model (Apphu/hu) by introducing these amino acid substitutions into the mouse genome. Additionally, they developed a double knock-in

Apphu/hu; Psen1M139T AD model by incorporating the M139T mutation, associated with early-onset familial Alzheimer's disease, into the PSEN1 gene of mice. Both the humanized APP mouse and rat models produced about three times more Aβ than wild type, confirming the computer model's predictions. This increased Aβ production was due to the enhanced cleavage efficiency of BACE1 by the humanized APP sequence. These new humanized APP rodent models more accurately replicate human AD Aβ pathology, offering greater physiological relevance compared to previous transgenic overexpression models [65].

9.3 CRISPR/Cas9 in Screening Alzheimer's Disease Pathogenic Genes

A study used CRISPR-Cas9 technology to delete the 3' untranslated region (3'-UTR) of the App gene in an App knock-in mouse model with three FAD mutations and a humanized Aβ sequence. The researchers found that greater deletion of the App 3'-UTR led to reduced Aβ deposition in the brain, due to lower transcriptional and translational levels of APP. Further analysis identified a critical 34 bp deletion near the conserved 52 bp sequence in the 3'-UTR, significantly reducing Aβ pathology[66]. CRISPR-Cas9 enhances AD gene screening by enabling precise gene editing, improving experimental accuracy, and facilitating gene expression regulation. It also offers a tool for correcting mutations in genes associated with early-onset familial AD, such as the APP gene, to reduce Aβ accumulation and prevent disease onset[67].

10. Future Prospect

The integration of CRISPR/Cas9 gene-editing technology and artificial intelligence (AI) is driving innovative approaches in the treatment and understanding of Alzheimer's disease (AD). CRISPR enables precise genetic modifications, offering hope for correcting mutations linked to early-onset AD and creating more accurate disease models. AI enhances this process by analysing vast datasets to identify drug targets, predict treatment outcomes, and optimize patient selection and personalized treatment plans[68]. AI tools like Deep CRISPR and CRISOT further refine sgRNA design and predict off-target effects by analysing genomic features and using machine learning models to predict potential cleavage sites[69]. However, challenges such as off-target effects, data heterogeneity, and the limited availability of labelled data remain. Future advancements will focus on improving CRISPR delivery methods, refining AI algorithms, and addressing ethical concerns to fully realize the potential of these integrated technologies in Alzheimer's treatment and gene editing therapies.

Conclusion

The therapeutic landscape is constantly being shaped by developments in Alzheimer's disease research, which give rise to fresh optimism for disease-modifying therapies. Measurable progress has been made with amyloid- and tau-targeting treatments as well as precision medicine strategies like lecanemab, while issues with accessibility and effectiveness still exist. With its ability to precisely target genetic variables connected to AD pathogenesis, the ground-breaking CRISPR-Cas9 gene-editing technology is an example of the future frontier. But there are challenges including brain delivery, off-target effects, and ethical issues that need to be addressed. Combining these developments with enhanced clinical trial designs and artificial intelligence has the potential to

speed up research and improve treatment results. Future research should concentrate on improving these approaches to give people with AD individualized, efficient, and equitable care.

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Author Contributions

Vandana Bhatia and Dr. Swati Rana conceptualized the review topic, designed the structure of the manuscript, and led the writing process. Shagun Thakur and Aditya rattan conducted the primary literature search and data collection. Anjali Chandel & Yavnika Minhas conducted an extensive literature review, contributed to the writing of the various section in manuscript, and assisted in refining the manuscript's framework. Dr. Vir Vikram Sharma provided critical revisions and feedback, ensuring the accuracy and scientific integrity of the content. All authors participated in reviewing and editing the manuscript, approved the final version, and agreed to be accountable for all aspects of the work.

Conflicts of interest

“The authors declare that they have no conflicts of interest.”.

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