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Genetic therapies for Alzheimer's Disease: A scoping review

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Abstract

Effective, disease modifying therapies for Alzheimer's disease (AD) remain a quandary, following a panoply of expensive failures in human clinical trials. Given the stagnation in therapeutics, alternative approaches are needed. Recent successes of genetic therapies in other neurodegenerative diseases may highlight the way forward. This scoping review explores suggested targets of genetic therapy in AD, with a focus on vector-based approaches in pre-clinical and clinical trials. Putative targets of genetic therapies tested in pre-clinical trials include amyloid pathway intermediates and enzymes modulation, tau protein downregulation, *APOE4* downregulation and *APOE2* upregulation, neurotrophin expression (Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF)), and inflammatory cytokine alteration amongst several other approaches. There have been three completed human clinical trials for genetic therapy in AD patients, all of which upregulated NGF in AD patients, showing some mixed evidence of benefit. Several impediments remain to be surpassed before genetic therapies can be successfully applied to AD, including the challenge of delivering monogenic genetic therapies for complex polygenic disorders, risks in the dominant delivery method (intracranial injection), stability of genetic therapies in-vivo, poor translatability of pre-clinical AD models and the expense of genetic therapy production. Genetic therapies represent an exciting opportunity within the world of AD therapeutics but clinical applications likely remain a long term, rather than short term, possibility.

Introduction

Alzheimer's disease (AD) is the leading cause of dementia and is considered one of the foremost public health challenges in the world, affecting over 44 million individuals globally in 2020 [1,2]. Currently available therapies focus on symptom management and include acetylcholinesterase inhibitors (donepezil, galantamine, rivastigmine) and an NMDA receptor antagonist (memantine). These pharmacological agents have been shown to only delay progression of cognitive decline for 3 – 6 months in mild-moderate AD [3]. A considerable number of disease modifying pharmacological and biological therapies have shown promise in pre-clinical models only to fail at great expense in human trials [4]. Several decades of trials using passive [5] and active [6] immunisation against amyloid and tau targets have been shown to engage the target in the brain but have largely failed to show cognitive benefit (e.g. solanezumab [7], gantenerumab [8], bapineuzumab [9], gosuranemab [10], semorinemab [11] and CAD106 [12]). The recent approval of aducanumab by the Federal Drug Administration (FDA) has kept the hope of the potential benefit of this strategy alive, although convincing clinical benefit of aducanumab remains to be demonstrated [13,14].

Considering the stagnation in therapeutics, more diverse approaches to AD therapy are required, and recent successes of genetic therapies in other neurodegenerative diseases may pave a way forward. The first genetic therapy for a neurological disease approved by the FDA was nusinersen for spino-muscular atrophy (SMA), an antisense oligonucleotide (ASO), given intrathecally monthly from birth [15]. This was followed by approvals for therapy for Duchenne muscular dystrophy (DMD), and familial amyloid polyneuropathy (FAP) [16]. There have been a number of human trials examining application of ASOs for Huntington's Disease (HD), with some showing promise [17] and others failing recently in trials [18].

This review will explore the suggested targets of genetic therapy in AD, the preclinical and clinical trials to date, and examine the key hurdles that remain to be surpassed. This review focuses on animal and human trials rather than cell-based models of AD. There are two main modalities of gene therapy: vector based and genetically modified cell replacement. Cell replacement therapies are comprehensively reviewed elsewhere [19] and thus this review will focus on vector based therapies.

What is a genetic therapy?

Clinically, genetic therapies are defined as therapeutic interventions that direct genetic material (DNA or RNA) to an individual's cells to rectify defective genes or gene products [20]. In-vivo delivery of genetic material via a viral vector or nanoparticles and ex-vivo genetically altered stem cells are the two central interventions for gene therapy [21]. There are a number of different approaches to gene therapies (see table 1) that fall into the categories of either inserting/deleting genes themselves [22] or modifying their expression [23,24].

Approach	Mechanism	Gene effect location	Approved for human therapeutics
CRISPR/Cas9	Insertion and/or deletion of genes	Gene editing	No
Direct Gene Delivery	Expression of alternative gene	Gene addition	Yes

Antisense oligonucleotides (ASOs)	Gene suppression or splicing regulation	Gene expression	Yes
Interfering RNA (e.g. siRNA)	Gene suppression	Gene expression	Yes
Engineered zinc fingers	Transcriptional activation	Gene expression	No
Spliceosome-mediated RNA trans-splicing (SMaRT)	Gene suppression or splicing regulation	Gene expression	No
Micro-RNA regulation	Gene suppression or splicing regulation	Gene expression	No
Antagomirs	miRNA suppression/Upregulation of miR targets/Splicing regulation	Gene expression	No

Table 1: Approaches to gene therapeutics in AD. CRISPR – Clustered Regularly Interspaced Short Palindromic Repeats, siRNA – small interfering RNA, miRNA – micro RNA. Table adapted from Loera Valencia et al (2018) [19].

Vectors and Delivery methods

The most common vector in pre-clinical trials of AD model mice has been adeno-associated virus (AAV), a single-strand DNA (ssDNA) parvovirus. It has the advantages of low immunogenicity, low pathogenicity and ability to infect a wide range of cells (both dividing and non-dividing). The key issues with AAV is that it has a small packaging capacity and may have off-target effects, given the range of cells it can infect [25]. The Lentivirus vector, a ssRNA HIV-like retrovirus, has also been frequently used. Rather than remaining episomal, genetic material is incorporated into the host cell genome, meaning that the therapy lasts longer but possesses the potential for oncogenicity [26]. The final commonly used viral vector in preclinical AD models is Herpes Simplex Virus (HSV), a double strand DNA (dsDNA) herpesvirus. HSV has the benefits of being targeted to neurons and having a large packaging capacity but has high immunogenicity and is more likely to stimulate an antibody response [27]. In saying this, all viral vectors (see table 2) face the challenge of neutralising antibodies [28]. This is partly why the dominant delivery method in preclinical AD trials continues to be direct stereotactic injection into the CNS, where there is diminished immune patrol and a lower likelihood of an antibody response [28]. Given there has been at least one fatality caused by direct CNS stereotactic injection of gene therapies [29] into AD patients, it is unlikely to become a viable, scalable, human delivery method, representing a considerable conundrum for the use of viral vectors.

Nanoparticles appear increasingly promising as vehicles for clinical gene therapy given their customisable size, shape, targeting and biological activity [30]. No nanoparticle vectored AD gene therapy has been trialed in humans yet, but liposomal and polymeric nanoparticles have been attempted in preclinical models [31,32]. They have demonstrated an ability to pass

through the blood brain barrier (BBB) and target subtypes of CNS cells, meaning they can be administered intravenously. Some challenges in utilising nanoparticle approaches include stability of nanoparticles, degradation while in circulation, endocytosis by target cells and delivery efficiency [33].

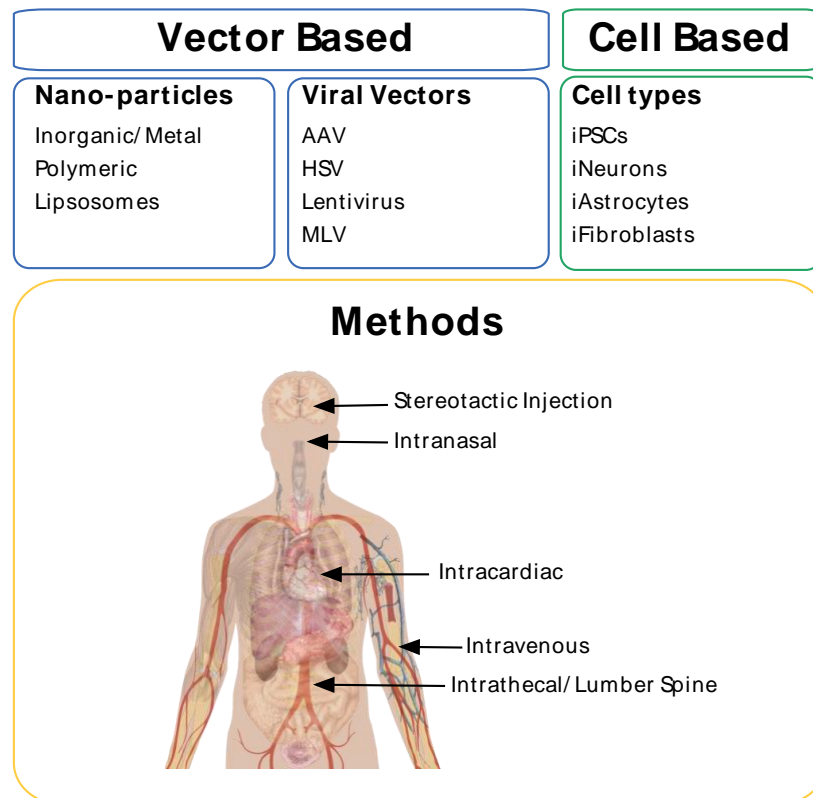


Figure 1 – Summary of vector types and delivery methods applied in genetic therapies. AAV; Adeno Associated Virus, HSV; Herpes Simplex Virus, MLV; Moloney Leukaemia Virus, iPSCs; Induced Pluripotent Stem Cells, i; induced.

Vector type	Virus type	Immunogenicity	Tropism	Maximum gene size	Transgene location
AAV	Parvovirus (ssDNA)	Low	Dividing and non-dividing cells	4.5 kB	Episomes
Adenovirus	Adenovirus (dsDNA)	High	Dividing and non-dividing cells	7 kB	Episomes
HSV	Herpesvirus (dsDNA)	High	High in Neurons	50 kB	Episomes
Lentivirus	Retrovirus (ssRNA)	Low	Dividing and non-dividing cells	10 kB	Integration into chromosomes
MLV	Alphavirus (ssRNA)	Low	Dividing cells only	7 kB	Integration into chromosomes

Table 2. Summary of key viral vectors used for AD gene therapy. AAV; Adeno-associated virus, HSV; Herpes simplex virus. MLV; Moloney leukaemia virus, ss; single strand, ds; double strand.

Gene therapy targets in Alzheimer's Disease

Amyloid pathway

A β /APP

Increases in extracellular soluble A β oligomers and insoluble A β plaques are the pathological hallmarks of both familial and sporadic AD, and have long been thought to play a critical role in pathogenesis [34]. Over the last few decades there have been a number of immunotherapy clinical trials aimed at reducing amyloid load. While some of them effectively diminished cerebral amyloid burden [35], none of them ameliorated patient clinical symptoms, with the possible exception of aducanumab [35]. These multiple failures have variously been attributed to poor cohort design, intervention at too late a stage, and poor passage through the BBB, although some have argued that these failures point away from the amyloid hypothesis of AD.

As a way of addressing these issues, Elmer et al. (2020) [36] performed intrahippocampal injections of an AAV vector encoding a transcript for anti-A β IgG into 2-month-old APP mutation mice, just prior to the time of A β plaque deposition in this model. They found that CNS expression of anti-A β IgG was maintained at levels higher than peak concentration achieved with IV injection and that it resulted in reduced cortical and hippocampal A β load. Similarly, in an experiment using direct hippocampal injection of a HSV vector containing a short hairpin RNA that reduced translation of APP RNA, there was a considerable reduction in A β accumulation [37]. While reduction in excessive accumulation of APP or A β may be helpful, pre-clinical attempts to completely knockdown the APP gene have generated pathological phenotypes including locomotor and behavioural dysfunction in mice [38]. Indeed, Fol et al (2016) [39] found that injecting an AAV2 containing the *APPs α* gene (a subcomponent of APP) into the hippocampus of APP/PS1 Δ E9 AD model mice resulted in a recovery of dendritic spine density deficits, improvements in spatial working memory and reduction in soluble and insoluble A β levels. Another approach used by Park et al (2021) [40] has been to inject AAV encoding A β variants (F20P and F19D/L34P) that inhibit A β aggregation and reduce oligomer toxicity. They found that after intraventricular injection, mice expressing F20P, but not F19D/L34P, showed decreased A β levels, plaque burden, and plaque-associated neuroinflammation [40].

Beta-site amyloid precursor protein cleaving enzyme 1 (BACE1)

BACE1 cleaves A β at its N-terminal end from APP, and works in concert with gamma secretase to produce A β 40 – 42 peptides. Singer et al. (2005) [41] performed one of the first pre-clinical trials of BACE1 suppression in which they injected a Lentivirus expressing BACE1 suppressing siRNA into the hippocampi of 10 month old APP mice. There was significantly diminished amyloid load and neurodegeneration as well as improvements in behavioural deficits. In the last three years, there has been a number of interesting in-vivo demonstrations of BACE1 suppression using intravenously (IV) administered nanocomplexes. Wang et al. (2018) [42] used IV delivery of a PEGylated acylate polymer nanocomplex to deliver BACE1 suppressing siRNA to 8-month-old APP/PS1 mice. The nanocomplex was modified with both the Cingulin (CGN) peptide, which allows for blood-brain barrier (BBB) penetration, and the Tet1 peptide,

which facilitates neuron-specific binding. There was a reduction in amyloid plaques and p-tau, improved hippocampal neurogenesis, and better spatial working memory performance in transgenic mice, returning to the level of the wild-type controls. These results have been corroborated by other studies using IV injections of BACE1 suppressing gene therapies to similar effects [31,43]. Park et al. (2019) [44] took a different approach and, using 6-month-old 5XFAD transgenic mice, stereotactically injected into the CA3 region amphiphilic nanocomplexes containing CRISPR-Cas9 constructs designed to delete BACE1 gene. They found there was a 70% decrease in BACE1 expression in the CA3 region and there was no genome wide elevation in mutation rates. Associative learning and spatial working memory performance were improved compared to controls, demonstrating in-principle that targeted deletion of a gene may hold therapeutic potential. However, similar to APP, BACE1 serves an adaptive function and complete knockdown of the gene causes hypomyelination and seizures in mice, indicating that its therapeutic suppression must be carried out with caution [45].

A β Degrading Enzymes – Neprilysin and Endothelin Converting Enzyme (ECE)

Neprilysin is a membrane-bound metallo-endopeptidase and a critical A β -degrading enzyme [46,47]. One study found that knockdown of neprilysin in AD model mice doubles the degree of A β pathology [46]. Neprilysin levels decrease with age (in mice and humans) but have been found to be comparatively increased in brains of AD patients [48,49], indicating a possible adaptive response to more rapidly degrade accumulating A β . Genetic approaches to upregulate neprilysin were first trialled by Marr et al (2003) who injected a lentivirus encoding human neprilysin into the hippocampus of APP transgenic mice. They found that A β deposits were reduced by 50% and neurodegeneration was significantly diminished. Three similar trials [50–52] using hippocampally injected AAV or lentivirus vectors have since corroborated these results, finding additionally that unilateral injection diminished A β levels bilaterally. Furthermore, they found that neprilysin upregulation causes A β reduction in young and old mice and that there were significant improvements on murine memory tests. Guan et al. (2009) [53] took a different approach and performed a lentiviral gene transfer of neprilysin to mouse allogenic haemopoietic stem cells in 8-month-old 3 \times Tg-AD mice. They then gave the mice a partial bone marrow transplant and later found that treated mice showed a ~30% reduction in soluble brain A β peptide and 50-60% reduction in accumulation of A β plaques as well as recovery of previous spatial working memory deficits. This study highlights the importance of systemic A β peptide for AD progression and indicates that therapy may not need to penetrate the CNS. Endothelin Converting Enzyme (ECE) is another enzyme critical to the degradation of A β [54] and, similar to the studies of neprilysin, it was found that after ECE gene delivery there were decreases in A β plaques around the CNS injection sites [55].

Tau

Hyperphosphorylated tau aggregates are a pathological hallmark of AD; they may precede amyloid pathology, and are thought to be the dominant cause of AD neurodegeneration [56,57]. Mutations in the MAPT gene have been associated with a range of neurodegenerative diseases but interestingly not with AD [58–61]. Three studies [62–64] have examined the use of CNS injected AAVs encoding components of anti-tau antibodies in tau mice models. They achieved reductions of p-tau (of up to 90%) and considerable slowing of neurodegenerative processes. DeVos et al. (2017) [65] examined the use of CNS injected antisense-oligonucleotides (ASOs) in both tau model mice and non-human primates. They found that a 1-month long intracerebroventricular infusion of anti-tau ASOs in 6 – 9-month-old PS19 mice reversed p-tau pathology, prevented hippocampal volume loss and neurodegeneration and extended survival. Then, in 2–8-year-old Cynomolgus monkeys, they gave either one or two doses of an ASO targeting tau via lumbar puncture. They demonstrated that anti-tau ASOs moved through the entire CNS and effectively downregulated tau mRNA and protein in the brain and spinal cord. The studies examining effects of genetic therapy reducing tau were all in tau transgenic mice, and given the lack of genetic association between MAPT and AD, it is unlikely these experiments effectively model a viable therapeutic approach for AD.

APOE

Apolipoprotein E (*APOE*) $\epsilon 4$ allele is the strongest genetic risk factor for AD [66], with one allele bringing a 2-3 fold increased risk and two alleles bringing a 12-15 fold risk as well as a reduction in age of onset [67]. The population frequencies of the predominant *APOE* alleles are $\epsilon 2$; 8.4%, $\epsilon 3$; 77.9% and $\epsilon 4$; 13.7% [68], with 58.5% of the AD population being at least heterozygous for *APOE* $\epsilon 4$ [69]. *APOE* is expressed in astrocytes and microglia and forms lipoprotein particles with cholesterol after it is lipidated by the ABCA1 transporter [70]. These *APOE* lipoprotein particles bind soluble A β , are taken up by cellular surface receptors (e.g. LRP1, LDLR, and HSPG [71]) and are degraded in lysosomes. *APOE* isoforms have different affinities to A β and to the aforementioned internalisation receptors ($\epsilon 2 > \epsilon 3 > \epsilon 4$) [70]. Considering this, several research groups have trialled various forms of *APOE* $\epsilon 2$ insertion or *APOE* $\epsilon 4$ suppression [72]. Hu et al (2015) [73] found in mice that after intracerebroventricular injection of AAV particles encoding *APOE* $\epsilon 4$ there was an increasingly poor lipidation of lipoprotein particles and an increase in endogenous A β levels, whereas injecting *APOE* $\epsilon 2$ had the reverse effects. Hyunh et al (2017) [74] applied a different approach and, using APP/PS1-21 mice homozygous for *APOE* $\epsilon 4$, they gave intracerebroventricular injections of anti-*APOE*- $\epsilon 4$ ASOs. They found that, when given at birth (prior to amyloid plaque development), this ASO treatment decreased A β plaque number by ~50%, whereas when given at 6 weeks (around the start of amyloid plaque development) it had no effect on plaque number but did increase the plaque size. By contrast, elevation of *APOE* $\epsilon 4$ using an AAV vector [75] in mouse models of tauopathy (rTg4510 and PS19 lines) caused no change in tau pathology. An *APOE* gene therapy trial in African Green monkeys (n = 11) [76] determined that intracisternal injection was the least invasive, effective delivery method. This work is currently being translated into a Phase 1, open label, dose ranging trial. Having started in 2018, Lexeo

Therapeutics (NCT03634007) [77] are examining the safety and maximum tolerable dose of intracisternally injected AAV10 encoding *APOE* ϵ 2 in A β positive patients with MCI or dementia who are *APOE* ϵ 4 homozygotes (n expected = 15). It is predicted to finish in January 2023. This trial represents the first iteration of a personalised genetic medicine approach to AD, that is treating *APOE* ϵ 4 homozygotes with *APOE* ϵ 2 replacement. It is precisely this approach of identifying and correcting specific monogenic abnormalities that have produced successes in genetic medicine in the past [15,16] and researchers are hopeful that this will be what brings new therapeutics to AD in the future.

Neurotrophins

Neurotrophins are a group of structurally similar peptides that influence plasticity, synaptogenesis, differentiation and longevity of neurons [78]. Studies examining the role of neurotrophins in AD have predominantly explored the role of Nerve Growth Factor (NGF) and Brain Derived Neurotrophic Factor (BDNF) in the cholinergic basal forebrain (CBF) and the hippocampus, and hence these have been the subjects of most of the pre-clinical and clinical studies.

NGF

CBF neurons require an ongoing supply of NGF for maintenance and survival through their lifespan. NGF is expressed by CBF neurons and are targeted to hippocampus and cerebral cortical neurons that express p75^{NTR} and TrkA [79]. In the hippocampus and cerebral cortex, NGF forms a complex with TrkA, supported by p75^{NTR}, and this NGF/TrkA construct is transferred back to the basal forebrain where it mediates signal transduction [80,81]. Transgenic mice expressing anti-NGF antibodies manifest a more rapid age-dependent loss of CBF neurons [82,83]. Thus, studies of genetic therapies in AD have attempted to increase NGF signalling using various techniques.

Nagahara et al (2008) [84] using elderly rhesus monkeys (mean age = 23.9 ± 1.8 years, ntreated = 3, ncontrols = 8) injected a lentivirus encoding NGF into the CBF region. They found there were no identified adverse effects, that NGF expression was maintained for at least 1 year, and that cholinergic p75-labeled neuron quantity and size in aged monkeys recovered to the level of young monkeys. NGF has been trialled in three completed clinical trials with mixed results. Tuszynski et al (2005) [85] conducted a Phase 1 open label trial involving 8 patients with probable mild-moderate AD (mean age 67.2 ± 2.6 years, range 54 – 76). They injected autologous fibroblasts modified to express NGF into the basal forebrain adjacent to nucleus basalis of Meynert and measured disease progression using the Alzheimer Disease Assessment Scale-Cognitive subcomponent (ADAS-C). After a mean follow-up of 22 months, there were no adverse effects of NGF, significant slowing in the rate of cognitive decline after compared to before the intervention, and a significant increase in cortical 18-fluorodeoxyglucose uptake (i.e. brain metabolic activity) on serial PET scans. Regrettably, one of the participants died of an intracranial haemorrhage following the injection, highlighting the considerable risk of such invasive procedures, particularly for the elderly population. Eriksdotter-Jönhagen et al (2012) [86] performed a similar trial but instead used a modified human retinal epithelial cell as the vector for NGF and inserted them into the nucleus basalis of Meynert of 6 AD patients using a catheter like device over 12 months. They found that no

patients suffered adverse events related to the therapy, and in 2 of 6 patients, there were positive findings in cognition (as measured by ADAS-C and MMSE), EEG morphology, and nicotinic receptor binding. By contrast, Rafii et al (2018) [87] stereotactically injected AAV2–NGF into the nucleus basalis of Meynert and found that there were no differences in cognitive outcomes (ADAS-C) ($N_{\text{treated}} = 25$, $N_{\text{controls}} = 24$). However, post-mortem analysis of a subset of these patients ($n = 3$) found that NGF expression had not reached the cholinergic neurons because of poor spread and ineffectual targeting. They argued that their study did not demonstrate that NGF was an ineffective therapy for AD [88]. Taken together, the studies do not illustrate clear therapeutic potential of NGF but they do highlight the risks and operator dependency of the intracranial injection method. Thus, there have been recent attempts to move away from such delivery methods. Recent studies in APP/PS1 mice have illustrated that IV injections of nanoparticles encoding NGF can downregulate soluble A β peptides and increase CNS neuronal proliferation [32].

BDNF

BDNF is present throughout the brain but it is highly expressed in the cerebral cortex and hippocampus [89]. BDNF regulates cellular specification and synaptic plasticity, especially in dopaminergic and cholinergic neurons by acting on high-affinity receptor TrkB and its low-affinity receptor p75^{NTR} [90,91]. BDNF has a role in long-term potentiation (LTP), with animal studies showing that TrkB overexpression augments LTP in the hippocampus [92]. Within AD, diminished cortical concentrations of BDNF has been correlated to higher Braak stages [93]. Further, BDNF expression has been shown to be protective against A β neurotoxicity in rat cells, in-vivo and in-vitro [94,95], and ameliorate A β -induced LTP impairment [96,97].

One study found that in 6 month old APP transgenic mice injected with a Lentivirus encoding the BDNF gene there was amelioration of spatial memory deficits and a supranormal response to contextual fear conditioning. This was in combination with diminished neurodegeneration and reduced cell atrophy without changes in amyloid load [98,99]. The authors argued that BDNF represents a therapeutic approach independent of the amyloid cascade. Contrarily, Eremenko et al (2019) [100] argue that the therapy may be pleiotropic and at least part of the benefit is derived from amyloid cascade alterations. Using 12-month-old 5XFAD mice, they injected BDNF expressing A β -specific CD4 T cells intracerebroventricularly. They found that increased BDNF levels reduced levels of beta-secretase 1 (BACE1) and improved amyloid pathology/inflammation, suggesting that BDNF, when targeted to A β , promotes its clearance.

The Tuszynski group [98] also found that that BDNF gene therapy reduced neuronal cell loss in rhesus monkeys with entorhinal lesions (14.6% in treated vs 45.9% in untreated monkeys) and, when given to aged mice, led to significant improvements in a visuospatial discrimination task. Recruitment for a three-year human clinical trial [101] that will consist of 24 participants ($N_{\text{AD}} = 12$, $N_{\text{controls}} = 12$) stereotactically injected with AAV2-BDNF began in 2021 with the hope that this therapy may ameliorate AD symptoms. Such treatment strategies that avoid directly treating pathology raise interesting possibilities of their use in improving healthy individuals rather than just mitigating disease.

Inflammatory cytokines

A number of recent genome wide studies have emphasised the importance of immune-inflammatory pathways in late-onset, spontaneous AD (e.g. *TREM2*, *CD33*) [102,103]. Recent evidence has suggested that prior to amyloid or tau deposition, immune-inflammatory activity is manifested in astrogliosis and pro-inflammatory cytokine upregulation [104,105]. Whether components of this inflammatory response are adaptive or harmful is still contested [106]. Regardless, inflammatory pathways seem to play a critical role in AD and thus targeted genetic therapies have been attempted in preclinical models, albeit with mixed results.

CD33

CD33 is an anti-inflammatory immune signalling protein involved in cell adhesion and endocytosis [107]. Griciuc et al (2021) [108] used APP/PS1 mice and injected an AAV construct intracerebroventricularly encoding miRNA designed to downregulate CD33. They found there were significant decreases in CD33 mRNA, proinflammatory cytokines (e.g. *Tlr4*, *Ccl2* and *Tnfα*), soluble Aβ₄₀₋₄₂ and insoluble Aβ plaque, with treatment at 2 months being more effective than at 8 months.

TREM2

Triggering receptor expressed on myeloid cell 2 (*TREM2*) expression is elevated in microglia surrounding amyloid plaques of APP mice [109]. In 7-month-old APP^{swe}/PS1^{dE9} mice [110], after stereotactic injection of lentiviral particles encoding *TREM2* into the cerebral cortex and hippocampus, there were reductions in Aβ deposition, neuroinflammation, and neuronal and synaptic losses as well as improvements in spatial working memory. The same intervention in 18-month-old APP^{swe}/PS1^{dE9} mice produced no change in neuropathology or behaviour, indicating that *TREM2* upregulation is unlikely to mollify firmly established disease.

IL-2

IL-2 is expressed by antigen-stimulated T cells and induces T-cell proliferation, B-cell growth and antibody production amongst various other roles [111]. Alves et al (2017) [112] took a different approach to most gene therapy deliveries, injecting an AAV8 encoding IL-2 intraperitoneally into APP/PS1 mice. They found that there was an increase in systemic and brain T cell expansion and activation as well as diminished amyloid-β_{42/40} ratio, reduced amyloid plaque load and improved spatial working memory.

IL-4

IL-4 is secreted by CD4⁺ T cells, is broadly anti-inflammatory and is involved in Ig class switching recombination in B cells [113]. Two similar studies [114,115] that involved injecting an AAV-IL4 construct into the hippocampi of 3-month-old APP+PS1 mice found that it resulted in diminished astro/microgliosis, Aβ deposition and one found improved behavioural outcomes. But contrarily Chakrabarty et al (2012) [116], in an almost analogous experiment using AAV-IL4 in 4-month-old APP mice, found there was worsened Aβ deposition, increased microglial “M2 phenotype” and reduced soluble Aβ₄₀ uptake by microglia.

IL-10

IL-10 is another anti-inflammatory cytokine that suppresses the synthesis of a collection of pro-inflammatory cytokines including IFN-gamma, IL-2, IL-3, TNF, and GM-CSF [117]. As in studies in IL-4, there are several experiments in this area, that despite similar designs, have directly contrary results. Kiyota et al (2012) [118] used 3-month-old APP/PS1 mice and found that hippocampal injection of AAV2/1-IL-10 resulted in sustained increases in CNS IL-10 expression, diminished astro/microgliosis, improved neurogenesis and spatial learning. By contrast, Chakrabarty et al (2015) [105] injected AAV2/1-IL-10 into the cerebral ventricles of neonatal APP mice and found that upregulation of IL-10 caused elevated A β accumulation, increased *APOE* expression and impaired spatial working memory.

TNF α

Tumor necrosis factor- alpha (TNF α) is a pro-inflammatory cytokine expressed by a range of cells including macrophages, endothelial cells [119], neurons [120] and glia [121]. TNF α and its receptors are elevated in mouse models of AD [122] as well as human AD patients [123]. Two studies examining TNF-alpha genetic therapies in AD have also produced directly contradictory results. Janelins et al (2008) [124] used 2-month-old 3xTg-AD mice and injected an AAV vector encoding murine TNF α into the hippocampus. They found in treated mice there was increased A β , p-tau, and microglial activation. Chakrabarty et al (2011) [125] used the same design, but used 4-month-old APP mice (rather than 2-month-olds) and they found rather in mice treated with TNF α there was a significant reduction in A β plaque burden.

The contradictory findings between studies of inflammatory cytokine gene therapy in AD, may reflect small differences in timing, dosage and delivery in the studies. These contradictions highlight that both excessive pro- and anti-inflammatory activity are likely to worsen AD pathology and thus careful titration of the dosage and inflammatory response is a critical, albeit complex, therapeutic priority. The evidence taken together supports the idea that modulating neuroinflammation is a promising therapeutic target but a better understanding of the inflammatory pathogenesis of AD is needed before its fruitful manipulation can be managed.

Opportunities and Challenges ahead for Genetic therapies in AD

Up to this point, successful genetic therapies have almost universally been applied to monogenic disorders with a relatively simple deficit to correct [15]. By contrast, AD is a complex multigenic disorder with considerable environmental influences [126]. In saying this, there seems to be final common pathways in all AD cases that present themselves as hopeful targets. Furthermore, given that genetic material (particularly compared to proteins) is compact, some groups have been trialling delivery of multiple therapies for AD in single vectors [31] with encouraging results. This multi-targeted approach to AD particularly makes sense given that excessive downregulation of common therapy targets (e.g. APP and BACE1) is dangerous and harmful, and thus a more titrated multi-pronged approach may produce best outcomes.

As discussed previously, all human clinical trials and most animal trials have used direct intracranial injection as the delivery method, partly because this is what is needed for viral vectors to reach their targets with appropriate concentrations and specificity. Not only is this dangerous in the elderly, it is also costly and unlikely to be scalable to any reasonable proportion of the AD population. Intravenously injected nanoparticles can be modified to pass through the BBB and be targeted to specific subpopulations of neurons. These techniques have been used in the last few years with great success in several pre-clinical trials and may pave the way away from viral vectors and intracranial injections. Viruses have other specific risks, including oncogenesis, that make them less favourable as a therapeutic vector [127]. In a trial of 20 patients with Severe Combined Immuno-Deficiency (SCID), when using a retroviral vector to insert a working gene, 3 patients developed leukaemia secondary to insertional mutagenesis. Other high-profile deaths in clinical trials of genetic therapies have historically generated a general scepticism and fear of trialling and implementing them in the future.

Finally, the cost of genetic therapies is a considerable impediment to development. Zolgensma, the AAV9-based gene therapy for spinal muscular atrophy, in 2020 costed £1.79m per dose [128]. Incredibly, this has been publicly funded in several developed countries, but funding would not be feasible if SMA was not so rare. Even if the cost of genetic therapies for AD were a fraction of this, there would be no health system in the world that could afford such a therapy for all AD sufferers. Thus, development of an effective but costly genetic therapy could generate further ethical and clinical conundrums, raising questions of who gets treated, when and why. Economies of scale are likely to reduce cost of genetic therapies, but it seems that further technological innovation is needed before such therapies become widely available.

Conclusion

Clinical trials for disease modifying Alzheimer's treatments have had a sobering history, and genetic therapies are a promising avenue of treatment. Vector based genetic therapies targeting the amyloid pathway, tau, *APOE*, neurotrophins and inflammatory cytokines have all shown exciting results in mouse models, but convincing evidence of their benefit is yet to be illustrated in a human clinical trials. Critical challenges, including the risks of vectors and delivery methods, as well as the considerable cost of therapy and need for multiple drug targets, must to be addressed before clinical applications can be realised.

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