Research Article

Gene therapy – an effective, novel and safe treatment modality for neurodegenerative diseases.

Prashant Upadhyay*, Opiya Halder[1].

1. MSc. Biotechnology, Himalayan University, Itanagar, Arunachal Pradesh, India.

***Corresponding Author: Prashant Upadhyay,** MBBS Final Year student at Government Medical College Jalaun (UP), India.

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Abstract

The Central Nervous System (CNS) faces unique difficulties in attaining permanent therapy for neurodegenerative disorders. Genomic-level forms of therapy have gained wide interest in the recent decade. Various clinical trials for genetic therapy of neurodegenerative diseases have been completed and some are ongoing.

The results of these clinical trials on animal models, humans have given the proof of efficacy and safety of gene therapy. The overall number of clinical trials worldwide using Adeno-associated virus (AAV), Herpes Simplex virus (HSV), Lentivirus (LV) viral vectors for human disease has gone from 5 in 1994 to 465 in 2009. Gene therapy has given novel, effective ways to treat diseases like Spinal muscular atrophy (SMA), Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD) which were once thought to be non-curable.

Advancements in the method of precisely using AAV, HSV vectors, ex-vivo gene transfer, gene editing using antisense oligonucleotides (ASO), increased effectiveness of CRISPR/Cas9 binding to the on-site thus, reducing the off-target site unwanted effects had changed the field of gene therapy tremendously.

Keywords: Gene therapy, Neurodegenerative diseases, CRISPR/Cas9, Adeno-associated virus (AAV), CNS viral vectors.

Introduction

Gene therapy has provided treatment options for diseases that are beyond the reach of traditional approaches. **(**1**)** Gene therapy for neurological diseases holds great promise for future treatment by either modifying genetic deficits or delivering therapeutic proteins. **(**2**)** There are two major methods first, in-vivo gene therapy that involves the direct introduction of a new therapeutic gene (transgene) directly into the patient using viral vectors or plasmid DNA, CRISPR technology. Second, ex-vivo gene therapy based on genetic modification of cells in vitro followed by transplantation of these modified cells back into the patient body. **(**1-3**)**

The first adeno – associated virus (AAV) vector gene therapy for genetic disease, alipogene tiparvovec (Glybera) was approved in 2012 for recurrent or severe pancreatitis. **(**1**)** Neurodegenerative diseases are a common cause of morbidity and cognitive impairment in older adults. **(**4,5**)** Most common are Alzheimer's disease (AD), Parkinson's disease (PD), Spinal muscular atrophy (SMA), Amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), Spinocerebellar Ataxia (SCA), Multiple sclerosis (MS), Frontotemporal dementia (FTD). Neuronal damage and neuronal loss are a pathological hallmark of AD, PD, ALS, SCA and MS. **(**7,8**)** This neuronal damage is frequently associated with chronic activation of an innate immune response in the CNS. **(**9**)** Dysfunction of the blood-brain barrier (BBB), blood spinal cord barrier, blood-cerebrospinal fluid barrier (BCSFB) and an arachnoid barrier is well known to occur in MS, PD, AD, stroke, epilepsy and TBI (Traumatic Brain Injury) which explains increased susceptibility to neuroinflammation and neurodegenerative disorders in elderly.

High-capacity adenoviruses (HC –Advs), "gutless" vectors provide sustained very long-term transgene expression, up to 1 year in the brain even in animals pre-immunized against adenovirus. **(**10**)**

If the vector however reaches the brain ventricles it will generate systemic immune response. **(**11,12**)** Careful brain vector delivery is thus necessary. (10**)**

Retroviral vectors derived from Maloney murine leukaemia virus (MoMLV) were the first vectors to be used for FDA-approved clinical trials. Retrovirus vectors are generally not useful for neurological applications as they cannot be used for gene transfer to non-dividing cells such as neurons. Lentiviral vectors derived from HIV-1 mediate gene transfer to both dividing and non-dividing cells. **(**13**)**

Adenoviral (Ad) Vectors have a high transgenic package capacity 36kb in (HC-Adv) vectors and they do not integrate into the host genome, instead remain episomal. **(**14**)** AAV vectors are close to the ideal CNS gene therapy vector as they can mediate gene transfer to both mitotic and post-mitotic cells, are neurotropic, can exist stably in an episomal state, with no pathogenicity or cytotoxicity. **(**3,14-16**)** AAV1,

AAV 8 and AAV9 and AAVrh10 are primarily neurotropic, AAV4 preferentially infects astrocytes and ependymal cells and AAVS transduces both astrocytes and neurons. **(**14**)** Use of pressurized convection enhanced delivery (CED) and MRI (Magnetic Resonance Imaging) guided infusions can lead to significantly better distribution. **(**17**)**

Gene Therapy for Alzheimer's disease

Alzheimer's disease (AD) is a progressive unremitting, neurodegenerative disorder characterized by brain atrophy caused by neuronal loss. **(**8,18**)** In 90% of cases AD remains an idiopathic form of unknown origin and affects wide areas of the cerebral cortex and hippocampus. Amyloid plaques consist of beta-amyloid protein Aβ-42, a breakdown product of amyloid precursor protein (APP) is considered to be neurotoxic. An increase in Aβ42/Aβ40 ratio has been identified as an important indicator of the ongoing pathogenesis of alzheimer's disease. **(**8,18**)** Neurofibrillary tangles (NFT's) consists of micro tubular proteins called tau that initially accumulates inside neurons, becomes extracellular after neuronal death. **(**18**)** APP, PSEN1 and PSEN2 (encoding for presenilin-1 and 2 respectively) mutations have seen in familial forms of AD (fAD). **(**19-22**)** Studies have shown that normalization of electrophysiology and Aβ secretion is possible through CRISPR genome editing of familial mutation in PSEN1 and PSEN2. **(**13**)** Currently APP, APOE and tau are three elements that have substantial evidence as contributors of AD. **(**18**)** APOE on chromosome 19 is the strongest genetic risk factor for AD, mostly due to APOE-4 whereas APOE-2 decreases the risk of AD. **(**18-23**)** Early pioneering ex vivo gene therapies for AD involved the use of genetically modified fibroblasts. **(**2**)** A direct gene therapy using an AAV2 encoding neuron growth factor (NGF) is more preferred. **(**24,25**)** One study demonstrated to improve synaptic density and ameliorate pathology in AD triple and thy-1 – APP transgenic mouse models by using neural stem cells (NSC's) genetically modified to release Aβ degrading enzyme, neprilysin. **(**26**)**

Gene Therapy for Parkinson's Disease

Parkinson's Disease is the second most common neurodegenerative disease and is estimated to afflict up to 5.8 million people worldwide. **(**27**)** It is characterized by progressive loss of dopaminergic neurons and decline of striatal dopamine leading to disinhibition of glutamatergic neurons. **(**28**)** Typical clinical symptoms of parkinson's disease like bradykinesia, rigidity become evident when approximately 70% of dopaminergic neurons in the substantia nigra of the mid brain are damaged. Most common genetic cause of sporadic and familial PD is LRRK-2 (Leucine-Rich Receptor Kinase-2) present on chromosome 12, mutation that induces toxicity in dopaminergic neurons. **(**29) Mutations of parkin gene on chromosome 6 and alpha synuclein gene on chromosome 4 are also associated with familial PD, with

the early onset PD in perkin gene mutation. Several rodent studies found striatal vector-mediated AAPC overexpression to be well tolerated and able to improve parkinsonian symptoms. **(**27**)**

In another study findings it supported that delivery of AAV GAD (Glutamic Acid Decarboxylase) to STN (subthalamic nucleus) modulates the excitatory output, changing the overall neurotransmitter balance in basal ganglia. **(**28**)**

Also, studies investigating direct GDNF delivery to STN and striatum have shown that GDNF induces neurite sprouting and reduces parkinsonian behaviour in toxin models of PD. **(**27**)**

There are studies reporting behavioural improvement after the delivery of AAV- GAD into the STN of a standard rodent model of PD associated with evoked GABA release from the STN and reduction in nigral dopamine cell death. **(**28**)**

No adverse events were observed after STN gene therapy and is potentially effective in a primate model of PD. **(**28**)**

Gene Therapy for Spinal Muscular Atrophy

Spinal Muscular Atrophy (SMA) is an autosomal recessive neurodegenerative disease, mostly involves homozygous deletion of the SMN-1 (survival motor neuron) gene on chromosome 5q13. This causes a deficiency in SMN protein, critical for motor neuron development. **(**30,31**)** Characterized by progressive muscle atrophy and weakness, delayed milestone development, death or need of mechanical ventilation by two years of age. **(**32,33**)** Approximately 75% of individuals with two copies of the SMN-2 gene are predicted to develop type 1 SMA while 80% with three copies of SMN-2 gene are predicted to develop SMA type 2. **(**31**)**

A systemically administered AAV had shown success in the treatment of SMA (CT identifier: NCTO2122952). **(**1,2**)**

In December 2016 the FDA approved nusinersen, an ASO (antisense oligonucleotide) for treatment of all SMA types. **(**30**)** In a study, 80 infants had been enrolled for 6 months, 41% of those receiving nusinersen showed motor improvements based on scores on the Hammersmith Infant Neurological Examination (HINE). **(**30,32**)**

In the multi-site, open-label, single-arm NURTURE trial, all 25 participants achieved the ability to sit without support, 23 achieved walking with assistance and 22 achieved walking independently. Though 8 infants had adverse events considered possibly related to nusinersen by the study investigator. **(**31**)**

Gene Therapy for Amyotrophic Lateral Sclerosis

Amyotrophic Lateral Sclerosis (ALS) is a neurodegenerative disease caused by the death of motor neurons in the spinal cord and brainstem. **(**34**)** It is the most common form of fatal motor neuron disease (MND), also known as Lou Gehrig's disease with no cure. **(**35**)**

ALS is diagnosed using an electro-myography (EMG) test characterized by loss of motor neurons and muscle weakness, which results in respiratory failure and death within (3-5) years of onset. **(**36**)** On the basis of onset of clinical symptoms, it is of two types (a) limb onset – symptoms in the upper arm or lower limb, (b) bulbar onset – weakness of muscles controlling the speech and swallowing. Two types of ALS familial (fALS 10%) and sporadic (sALS 90% cases). **(**34**)**

According to the amyotrophic lateral sclerosis online database, over 100 genes may be linked to ALS. **(**37-39**)** Mutant SOD1 (Superoxidase dismutase) was the first gene to be linked, located on chromosome 21q 22.1. **(**34**)**

A recent study conducted a targeted gene correction of the SOD1 A272C mutation in patient-derived iPSCS (induced pluripotent stem cells). **(**40**)** A similar study applied CRISPR/Cas9 gene correction strategy to the SOD1 E100G mutation. **(**41**)**

Mutation in the SOD1 gene was corrected by inserting wild type SOD1 with homologous arms, corrected cell line showed reversal of SOD1 associated pathological phenotype, increased motor neuron survival, degradation of misfolded SOD1 protein. **(**34**)**

Ku80 is an essential DNA repair protein, elevated ku80 in ALS causes neurodegeneration. Application of genome editing using two sgRNA's (small guide RNA) reduced ku80 levels with corrected repeat expansion. **(**42,43**)** Recently FDA approved zolgensma, an AAV-mediated treatment for another MND-the infant form of spinal muscular atrophy. Accelerated progress in gene therapy, is potentially a promising avenue to develop an efficient and safe cure for ALS. **(**35**)**

Gene Therapy for HD (Huntington's Disease)

HD is a progressive, fatal, neurodegenerative disorder caused by an expanded CAG repeat in the huntingtin gene, which encodes an abnormally long polyglutamine repeat in the huntingtin protein. **(**44**)** Characteristics included cognitive and behavioural disturbances, chorea, neuronal inclusions, striatal and cortical degeneration. **(**45**)**

It can be regarded as model neurodegenerative disorder. Huntingtin protein (Htt) is a very large protein of about 50 amino acids, termed HEAT repeats.

Till now, no drug has been proven to be efficacious in a RCT (randomised control trial) of disease-modifying therapy. Clinical trials are challenging because HD progresses slowly and there is clinical heterogeneity. **(**44,45**)**

Potential biomarkers for pre-manifest and early progression of HD (46-48**): -**

1. Blood: Creatinine kinase, branched-chain amino acids, cholesterol metabolites, Inflammatory proteins, brain-derived neurotrophic factor (BDNF), adenosine 2A receptors.
2. Brain imaging: - striatal volume
3. Subcortical white volume
4. Cortical thickness
5. PET (Positron emission tomography)

RNAi (RNA interference) has emerged as a potential therapeutic tool for treating dominant diseases by directly reducing disease gene expression. **(**45,49-50**)** "HTT" gene silencing improved behavioural and neuropathological abnormalities associated with HD. **(**51**)**

It has been shown that CRISPR/Cas 9 mediated inactivation of endogenous mutant HTT (mHTT) expression in the striatum of mHTT-expressing mice can reduce the production of mHTT effectively. **(**29,52**)**

Potential complications of Gene Therapy

As every therapy has its own advantages and disadvantages, gene therapy too has some potential complications such as gene silencing, genotoxicity, phenotoxicity, immunotoxicity, risks of vertical and horizontal transmission. But these complications have been effectively reduced due to the use of various

strategies like using vector for example AAV (that not integrate to the host genome), endogenous cellular promoters, carefully monitoring of T cell reactivity to the vector and transgene, monitoring of vector shedding.

Gene Therapy approaches for PD using AAV vector have been shown to be well tolerated in early phase clinical trials of mid-late stage patients. **(**7-12**)** rAAV2- Neurturin (LERE-120) gene transfer was also found to be safe in patient-with PD. **(**13**)**

These trials are adding to the growing evidences that AAV vector-mediated gene therapy to CNS can be administered safely and effectively.

Conclusion

Gene therapy is becoming a viable option for clinical intervention largely due to the success and safety of the current generation of virus-based vectors. **(**53**)** Gene therapy for CNS disease using AAV vectors was initiated nearly two decades ago using stereotactic intracerebral delivery of AAV2 vectors.

Various CNS gene therapy trials have demonstrated the efficacy and safety of the intervention. The trials are now opening new paths in treating these disorders, which at some point of time were considered incurable. European medicines agency (EMA) and the US FDA have approved six gene therapy products since 2016. **(**1**)**

Though the clinical trials numbers are increasing significantly across the world, we still need more of such trials to effectively apply these technologies from labs onto the patients with much more knowledge and understanding of its efficacy and safety.

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