LECTURE 12: GENE THERAPY

Gene therapy was conceptualized in 1972, by authors who urged caution before commencing human gene therapy studies.

The first attempt, albeit an unsuccessful one, at gene therapy (as well as the first case of medical transfer of foreign genes into humans not counting organ transplantation) was performed by Martin Cline on 10 July 1980.[5][6] Cline claimed that one of the genes in his patients was active six months later, though he never published this data or had it verified[7] and even if he is correct, it's unlikely it produced any significant beneficial effects treating beta-thalassemia.[8]

After extensive research on animals throughout the 1980s and a 1989 bacterial gene tagging trial on humans, the first gene therapy widely accepted as a success was demonstrated in a trial that started on September 14, 1990, when AshiDeSilva was treated for ADA-SCID.[9]

The first somatic treatment that produced a permanent genetic change was performed in 1993.[10]

This procedure was referred to sensationally and somewhat inaccurately in the media as a "three parent baby", though mtDNA is not the primary human genome and has little effect on an organism's individual characteristics beyond powering their cells.

Gene therapy is a way to fix a genetic problem at its source. The polymers are either translated into proteins, interfere with target gene expression, or possibly correct genetic mutations.

The most common form uses DNA that encodes a functional, therapeutic gene to replace a mutated gene. The polymer molecule is packaged within a "vector", which carries the molecule inside cells.

Early clinical failures led to dismissals of gene therapy. Clinical successes since 2006 regained researchers' attention, although as of 2014, it was still largely an experimental technique.[11] These include treatment ofretinal diseases Leber's congenital amaurosis[12][13][14][15] and Choroideremia,[16] X-linked SCID,[17] ADA-SCID,[18][19] adrenoleukodystrophy,[20] chronic lymphocytic leukemia (CLL),[21] acute lymphocytic leukemia(ALL),[22] multiple myeloma,[23] haemophilia[19] and Parkinson's disease.[24] Between 2013 and April 2014, US companies invested over $600 million in the field.[25]

The first commercial gene therapy, Gendicine, was approved in China in 2003 for the treatment of certain cancers.[26] In 2011 Neovasculgen was registered in Russia as the first-in-class gene-therapy drug for treatment of peripheral artery disease, including critical limb ischemia.[27] In 2012 Glybera, a treatment for a rare inherited disorder, became the first treatment to be approved for clinical use in either Europe or the United States after its endorsement by the European Commission.[11][28]

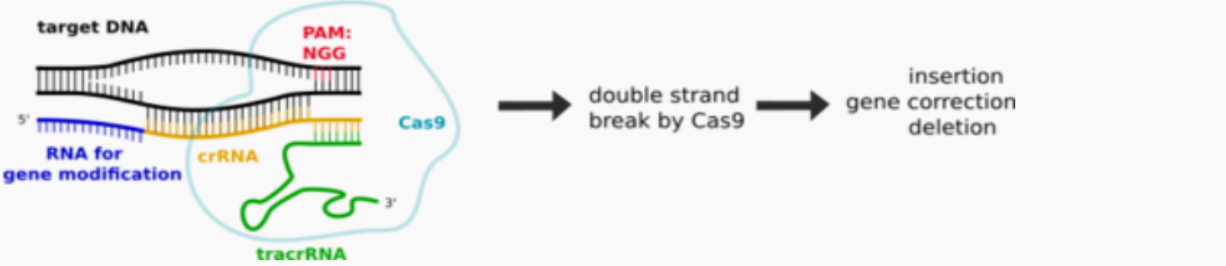
Approaches

Following early advances in genetic engineering of bacteria, cells and small animals, scientists started considering how to apply it to medicine. Two main approaches were considered – replacing or disrupting defective genes.[29] Scientists focused on diseases caused by single-gene defects, such as cystic fibrosis, haemophilia, muscular dystrophy, thalassemia and sickle cell anemia. Glybera treats one such disease, caused by a defect in lipoprotein lipase.[28]

DNA must be administered, reach the damaged cells, enter the cell and express/disrupt a protein.[30] Multiple delivery techniques have been explored. The initial approach incorporated DNA into an engineered virusto deliver the DNA into a chromosome.[31][32] Naked DNA approaches have also been explored, especially in the context of vaccine development.[33]

Generally, efforts focused on administering a gene that causes a needed protein to be expressed. More recently, increased understanding of nuclease function has led to more direct DNA editing, using techniques such as zinc finger nucleases and CRISPR. The vector incorporates genes into chromosomes. The expressed nucleases then knock out and replace genes in the chromosome. As of 2014 these approaches involve removing cells from patients, editing a chromosome and returning the transformed cells to patients.[34]

Future of CRISPR-Cas 9



A duplex of crRNA and tracrRNA acts as guide RNA to introduce a specifically located gene modification based on the RNA 5' upstream of the crRNA. Cas9 binds the tracrRNA and needs a DNA binding sequence (5'NGG3'), which is called protospacer adjacent motif (PAM). After binding, Cas9 introduces a DNA double strand break, which is then followed by gene modification via homologous recombination (HDR) or non-homologous end joining (NHEJ).

Gene editing has been a potential therapy for many genetic diseases. Targeted genome editing using nucleases provides a general method for inducing deletions or insertion. An earlier method for targeting relies on protein-DNA interactions; however, the most recent one, using CRISPR – associated protein 9 (Cas9), provides better specificity, simplicity, speed and pricing. The CRISPR System was first identified in single cell archaea (Prokaryotes). It is now widely used in different cell types and organisms including human cells (HEK293T,

HeLa, iPSC), mouse, fruit fly, rice, wheat etc. CRISPR –Cas9 genome editing has potential applications in human gene therapy, screening drug targets, synthetic biology, agriculture, programmable RNA targeting, and viral gene disruption.

Other technologies employ antisense, small interfering RNA and other DNA. To the extent that these technologies do not alter DNA, but instead directly interact with molecules such as RNA, they are not considered "gene therapy" per se.

Cell types

Gene therapy may be classified into two types:

Somatic cell

In somatic cell gene therapy (SCGT), the therapeutic genes are transferred into any cell other than a gamete, germ cell, gametocyte or undifferentiated stem cell. Any such modifications affect the individual patient only, and are not inherited by offspring. Somatic gene therapy represents mainstream basic and clinical research, in which therapeutic DNA (either integrated in the genome or as an external episome or plasmid) is used to treat disease.

Over 600 clinical trials utilizing SCGT are underway in the US. Most focus on severe genetic disorders, including immunodeficiencies, haemophilia, thalassaemia and cystic fibrosis. Such single gene disorders are good candidates for somatic cell therapy. The complete correction of a genetic disorder or the replacement of multiple genes is not yet possible. Only a few of the trials are in the advanced stages.[35]

Germline

In germline gene therapy (GGT), germ cells (sperm or eggs) are modified by the introduction of functional genes into their genomes. Modifying a germ cell causes all the organism's cells to contain the modified gene. The change is therefore heritable and passed on to later generations. Australia, Canada, Germany, Israel, Switzerland and the Netherlands[36] prohibit GGT for application in human beings, for technical and ethical reasons, including insufficient knowledge about possible risks to future generations[36] and higher risks versus SCGT.[37] The US has no federal controls specifically addressing human genetic modification (beyond FDA regulations for therapies in general).[36][38][39][40]

Vectors

The delivery of DNA into cells can be accomplished by multiple methods. The two major classes are recombinant viruses (sometimes called biological nanoparticles or viral vectors) and naked DNA or DNA complexes (non-viral methods).

Viruses

In order to replicate, viruses introduce their genetic material into the host cell, tricking the host's cellular machinery into using it as blueprints for viral proteins. Scientists exploit this by substituting a virus's genetic material with therapeutic DNA. (The term 'DNA' may be an oversimplification, as some viruses contain RNA, and gene therapy could take this form as

well.) A number of viruses have been used for human gene therapy, including retrovirus, adenovirus, lentivirus, herpes simplex, vaccinia and adeno-associated virus.[3] Like the genetic material (DNA or RNA) in viruses, therapeutic DNA can be designed to simply serve as a temporary blueprint that is degraded naturally or (at least theoretically) to enter the host's genome, becoming a permanent part of the host's DNA in infected cells.

Non-viral

Non-viral methods present certain advantages over viral methods, such as large scale production and low host immunogenicity. However, non-viral methods initially produced lower levels of transfection and gene expression, and thus lower therapeutic efficacy. Later technology remedied this deficiency

Methods for non-viral gene therapy include the injection of naked DNA, electroporation, the gene gun, sonoporation, magnetofection, the use of oligonucleotides, lipoplexes, dendrimers, and inorganic nanoparticles

Gene therapy for Parkinson's disease

A number of proteins have already been used for gene therapy for Parkinson's disease. The choice depends on the **treatment strategy**. For example one strategy is to improve the delivery of dopamine to the relevant brain regions in Parkinson's disease. Other strategies have tried to provide growth factor support to brain regions with the expectation that this might help damaged nerve cells to recover and thus slow Parkinson's disease progression or reverse it.

Most gene therapy studies in Parkinson's disease have used AAV-2 as the vector. Lentiviruses have also been studied extensively. Because of their larger capacity, lentivirus is the vector when more than one gene is used.
Once a gene and vector have been selected, the treatment must be administered to the relevant area of the brain.

The studies performed thus far have been directed to particular regions of the **basal ganglia**. The basal ganglia are number of interconnected deep brain regions that are involved in movement control. A major pathway connects the **substantianigra** to the **putamen** (where dopamine is normally released) and then to the **globuspallidus** directly or by way of the **subthalamic nucleus**.

To date, gene therapy for Parkinson's disease has been administered by drilling a hole in each side of the skull and then injecting the selected dose of the viral vector (containing the gene) into the desired brain region (either putamen or subthalamic nucleus) using image-guided surgical techniques. These treatments are performed either in a standard operating room or in a specialized radiology suite. Recovery from these procedures is usually quite rapid, with most patients being discharged home 1 or 2 days after gene therapy.

In the descriptions below, reference will be made to the clinical studies conducted thus far in humans.

Phase 1 studies refer to small studies, usually 10-15 patients at a single institution. These studies are designed to determine the safety and possible benefit of a particular treatment and no untreated comparison group is recruited. If a phase 1 study shows that a treatment is well tolerated and provides some evidence of benefit, a phase 2 study may be performed.

Phase 2 studiesare larger (typically 30-60 patients), are conducted at a number of medical institutions, and a control or placebo group is included for comparison to the group treated with the gene therapy. Humans in research studies often obtain substantial improvements that are unrelated to the specific treatment they receive The improvement may be due to the expectation of benefit from treatment. This phenomenon is called the **placebo effect** and can be quite substantial in patients with Parkinson's disease. Therefore using a "control" or untreated group is considered crucial in determining whether a treatment offers true benefit, beyond the placebo effect. Because gene therapy involves surgical treatment, a simulated or **"sham"**surgical procedure is necessary in gene therapy studies. These sham surgical procedures usually involve drilling small holes in the skull but not injecting the brain with the gene therapy under study. In the studies, investigators who perform subsequent evaluations of the patients are also unaware of whether the patient underwent the gene therapy or the sham procedure. This is called a **double-blind** study since neither the subject nor the investigator who performs the routine visits knows the treatment status of the patient. Double-blind studies are considered fundamental in determining whether a treatment offers a true benefit. If a study treatment shows safety and benefit in a Phase 2 study, a

Phase 3 study may then be performed. This study is similar to a Phase 2 study but is larger study (usually involving hundreds of patients) and is designed to confirm the treatment effectiveness, monitor side effects, and collect information that will allow the treatment to be

used safely. Information from a successful Phase 3 studies (along with other information about the study treatment) is then used by the United States Food and Drug Administration (FDA) to determine whether a new treatment is approved for routine treatment of a medical disorder.

Regarding therapeutic strategies, 3 approaches have been developed thus far. These are as follows:

The first approach is to **increase dopamine production**in specific regions of the brain. One study using this approach approaches uses the gene for the enzyme **aromatic amino acid decarboxylase (AADC)**. This enzyme converts levodopa into dopamine, a neurotransmitter that is deficient in Parkinson's disease. Studies have shown that AADC is gradually lost in Parkinson's disease. The progressive loss of this enzyme is thought to contribute to the need to increase levodopa doses as time goes on. The rationale for this approach is that if a greater amount of AADC is present in the location where dopamine should be released, then a more reliable and perhaps a more robust response to levodopa will occur. Moreover, it is possible that a patient who no longer is obtaining a reliable benefit from levodopa therapy might regain responsiveness to this treatment after gene therapy with AADC. Inherent in this approach treatment is that the patient may alter the effect of his gene therapy by adjusting his daily dose of levodopa, since the effect of this therapy depends on continuing treatment with levodopa. A phase 1 study in which AADC was injected into the putamen has been completed at 2 different doses. In the 10 patients treated, clinical rating scales and diaries of motor function suggested benefit and specific imaging studies provided evidence of successful gene therapy.

A variation on this strategy uses 3 genes that produce the enzymes AADC, tyrosine hydroxylase (TH), and GTP-cyclohydrolase-1 (GCH-1). Together these 3 enzymes can generate dopamine independent of external levodopa. The advantage of this approach is that it may be possible for the patient to discontinue treatment with levodopa. Although this approach seems very attractive, there are concerns that its benefits relies on producing precisely the right amount of dopamine. For example, too high a dose of gene therapy might result in complications due to excessive production of dopamine. The results of the study should be published in the near future.

1. The second gene therapy strategy is to **adjust or modulate the excitatory and inhibitory pathways** of the brain. The rationale of this approach is that the nerve cells of the subthalamic nucleus are overactive and that release of an inhibitory neurotransmitter in this brain region might normalize these cells. The gene for the enzyme **glutamic acid decarboxylase (GAD),** which produces the inhibitory neurotransmitter called GABA, has been examined in a phase 2 study in which 45 subjects were randomized to either bilateral treatment with GAD or a sham or simulated surgical procedure. While both patient groups showed improvement at 6 months, the improvement was greater in the subjects who underwent GAD treatment. Overall this study provided support for both the efficacy and safety of this approach.
2. The third approach is using brain proteins, termed **growth factors** (because of their role in brain development), that might protect against progression of Parkinson's disease or possibly even reverse it by stimulating regrowth of injured nerve cells. A number of growth factors have been identified over the years. These include glial cell line-derived neurotrophic factor (GDNF) and Neurturin which is similar to GDNF and shares the ability to promote the survival of dopaminergic neurons. In models of Parkinson's disease, GDNF and Neurturin have been shown to promote the survival of dopaminergic neurons. Both a phase 1 and phase 2 study using Neurturin gene therapy targeted to the putamen have been performed. In the phase 2 study, 38 patients were randomized to Neurturin gene therapy or to sham surgery. Unfortunately, there was no significant difference in the main outcome measures at 12 months. While the lack of benefit in the main outcome measures was disappointing, a subgroup of patients followed for 18 months was slightly better in the Neurturin patient than the sham treatment group, suggesting that slightly longer period of observations might be necessary to see a benefit with this gene therapy. Because of this interesting result, a second phase 2 study is underway in which Neurturin gene therapy is also targeted to the substantianigra.

Other gene therapy strategies are being considered. One such study includes using human erythropoietin. This study in experimental animals showed protection against toxins that usually damage dopaminergic cells. Other therapies are using insights provided by improved understanding of genetic causes of Parkinson's disease. It is conceivable that, as our knowledge of specific genetic defects causing Parkinson's disease improves, specific gene therapies could be developed for each individual genetic defect.

Treatment strategy	Gene(s)	Vector	Completed studies	Ongoing or Enrolling studies	
Increase dopamine	AADC	AAV-2	Phase 1	Phase 1 to start in 2013	
	AADC, TH, & GCH-1	Lentivirus		Phase 1 & 2 in progress	
Alter excitatory activity	GAD	AAV-2	Phase 1 &2		
Growth factors	GDNF	AAV-2	-	Phase 1 to start in 2012	
	Neurturin	AAV-2	Phase 1 &2	Second Phase 2 in progress	

Gene therapy for Huntington's disease

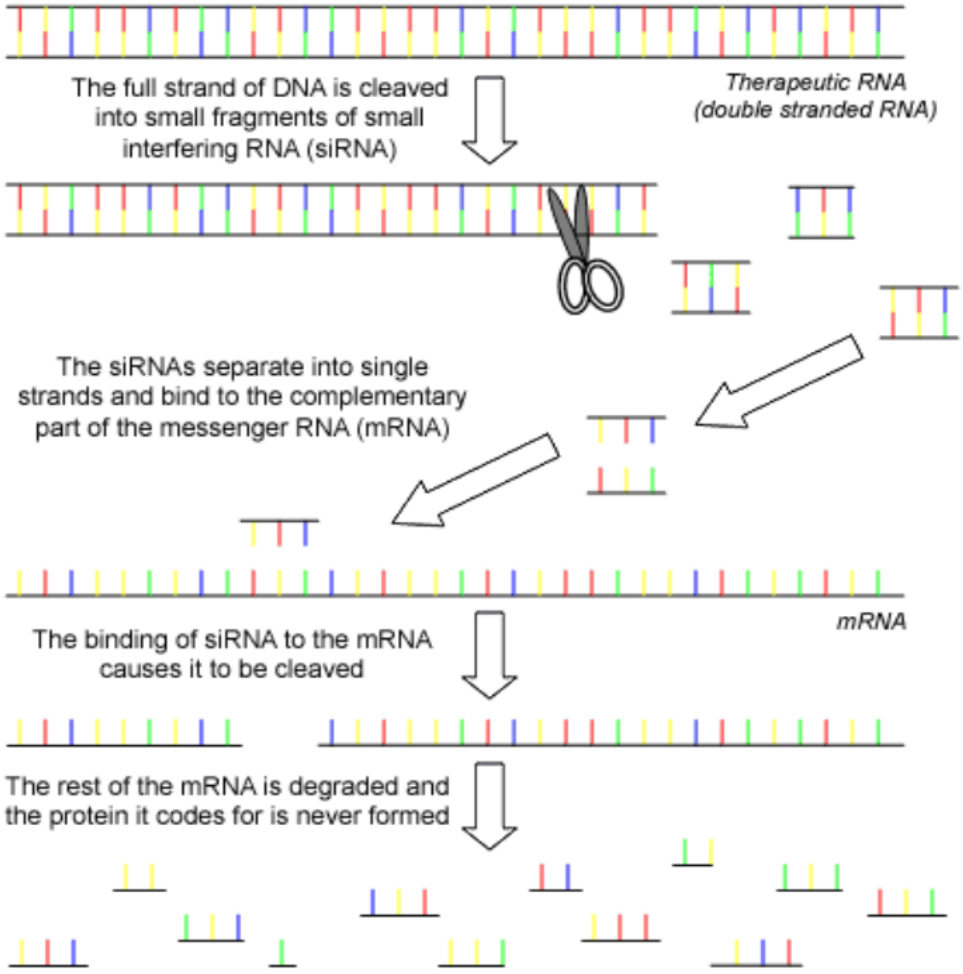
1. **RNA interference**

RNA interference (RNAi) is a way to "silence" genes by preventing the formation of the proteins that they code for. A type of gene therapy, it takes advantage of an intermediate step between DNA and protein.DNA acts as a blueprint for the final protein but it uses a kind of "middleman," called messenger RNA (mRNA), in order to get there. Going from gene to protein is a two step process. The first step in proteinsynthesis, transcription, takes place in a cell's nucleus, where the DNA template is used to make a single strand of mRNA. The mRNA then exits the nucleus and enters the cytoplasm, where now it serves as the template for making the protein. With the help of several different molecules, a string of amino acids forms according to the order of the mRNA bases, which are very similar to DNA bases. This process is called translation because the mRNA code is translated into the language of amino acids, the building blocks of proteins.

RNAi comes into play between the steps of transcription and translation. RNA is introduced into the celland binds to and destroys its mRNA target. Scientists can tailor make pieces

of RNA that arecomplementary (matched up) to a specific strand of mRNA. In some organisms, the whole strand ofcomplementary RNA can be introduced and an enzyme called *dicer* cuts it up into small fragments once it is inside the cell. Experiments have shown that introducing large strands of RNA into mammals does not work, so scientists were able to overcome this problem by making small interfering RNA (siRNA), also called short interference RNA. This is basically creating smaller chunks of double-stranded RNA (RNA is usually single stranded, except in some viruses) before injecting it into the cell. When these pieces of siRNA match up with the mRNA, they initiate a process that cuts up the mRNA into small fragments. Thecell recognizes these fragments as waste and degrades them, and the proteins never form. (See figure below for a representation of the RNAi mechanism.)

Figure H-2: RNA Interference - Simplified Mechanism



How can RNAi be used to treat HD?^

DNA serves as the template for mRNA. This means that the mRNA from the HD allele will be different from the mRNA from the non-HD allele in the same way that their DNA differs. Since the non-HD allele makes a functional protein, it is important that we only silence the disruptive mRNA from the HD allele (mouse studies have shown that shutting down both Huntington genes could be fatal). In order to do this we must first find a difference between the HD and non-HD mRNA. Unfortunately, targeting the most obvious difference between these two molecules, the extra CAG repeats, has proven ineffective .However, there are other differences within the HD allelethat are present in most of the people who have it. These differences can be as small as one basesubstitution (remember how the DNA "alphabet" consists of only four letters, A, C, G, and T? This would be like substituting an "A" for a "C" somewhere in the middle of the chain.). These small differences are called single nucleotide polymorphisms (SNPs). Scientists can create pieces of RNA that are only complementaryto the HD mRNA containing a SNP so that only the disruptive HD protein is prevented from being formed. This would allow the non-HD allele to continue to make the normal protein and prevent the harmfulprotein aggregations that form from the HD protein.

What are some of the challenges of RNAi?^

RNAi is a very promising new tool for treating many kinds of genetic disorders, but much more research and testing need to be done before it can be put to use. One of the main challenges right now is finding avector, or delivery system, to bring the therapeutic RNA into the nerve cells in the brain. Some researchers have successfully used certain modified viruses for this purpose. This is done by first removing the virus' own genetic material, thus removing its potential to cause harm, and then replacing it with the therapeuticRNA. One of the first successful RNAi tests was done on mice with a disease similar to HD called spinocerebellar ataxia type 1. (For more information on spinocerebellar ataxia type 1, click here). After injecting the mice with a modified virus, their condition improved. Mice receiving RNAi treatment stopped producing the mutant protein. With the disruptive protein out of the way, the mice no longer experience physical symptoms and are able to move around more easily.

While these are very promising results, we must remember that the testing was done on mice with a similar but different disease than human HD. Much work still needs to be done before the lessons learned from mouse experiments can be safely adapted for use in humans with

HD. For instance, further testing has shown that the virus used in the mouse experiment will not work on humans, so another virus must be used. So far, researchers have had success with treating human cells in the laboratory using a virussimilar to HIV that normally infects cats, not humans. It is also very important that the siRNA only silence the mRNA from the HD allele. As mentioned before, this requires finding a difference between the two alleles other than the extra CAG repeats. One problem with this in developing an effective treatment is to find a difference that is present in most or all people with HD. In the testing done on mice, the difference was a SNP that was present in 70% of mice with spinocerebellar ataxia. This begs the question, what about the other 30%? Scientists will need to find SNPs present in all people with HD so that they can eventually treat 100% of the population.

Many other important questions about RNAi have yet to be answered. At what age should people start to receive treatment? Would this be before or after they start showing symptoms of HD? Researchers must also run trials to see how much and how often patients should receive treatment. Right now we have no idea if RNAi therapy is long-term or only temporary. In addition, researchers need to continue their work on finding a vector that is both safe and effective. RNAi holds great promise for future treatment of HD but several more years of research and clinical trials need to be done before it can be widely available to the HD community.

2. Increased Trophic Support

It has been well established that reductions in pro-cell survival neurotrophic factors (NTFs), such as brain derived neurotrophic factor (BDNF), is dramatically reduced in the HD brain [9,10]. The BDNF protein, produced in the cerebral cortex and transported via corticostriatal tracts to MSNs [11], is necessary for MSN survival [12]. To further understand the significance of BDNF in the striatum of the HD brain, transgenic HD mice were crossed with heterozygote BDNF knockout mice, which accelerated the progression of the disease phenotype [11,13]. Conversely, crossing HD transgenic mice with mice that overexpress the BDNF gene delayed the progression of the disease and protected against neuropathological dysfunction [14,15]. Although, it should be noted that when the HD-BDNF transgenic cross reached 16 months, the animals began to show epileptic-like seizure activity, most likely because the level of BDNF was not tolerated [15]. Delivery of therapeutically significant amounts of NTFs is challenging. Although repeated peripheral administrations of fibroblast growth factor-2 into mouse models of HD have shown some efficacy [16], delivery of many other NTFs, including BDNF, into the HD brain remains a challenge, due to the fact that

many NTFs are large, polarized proteins that do not readily cross the blood-brain-barrier [17], relegating the delivery of NTFs directly into the central nervous system. 2.1. Overexpression in Endogenous Cells With the knowledge that BDNF is neuroprotective, researchers have attempted to increase BDNF production from endogenous cells within the striatum via adenoviral injections [18] or through an adeno-associated virus (AAV) vector injections into the striatum [19,20]. Increases in the numbers of cells expressing dopamine- and cAMP-regulated phosphoprotein of 32 kDa (DARPP32; specific to MSNs) were observed in animals injected with an AAV-BDNF, following quinolinic acid (QA) lesions, relative to lesioned AAV-control animals [19]. Similarly, Goldman and colleagues have developed an AAV which overexpress BDNF and noggin, the latter to inhibit glial differentiation, which has shown to increase recruitment of MSNs to the striatum [20]. Injection of these AAVs has been shown to reduce motoric deficits and prolong lifespan in the transgenic R6/2 mouse model of HD [21–23]. Interestingly, an increase of parvalbumin expressing interneurons was observed following an injection of an AAV vector, which also increased the release of glial derived neurotrophic factor (GDNF) [19]. These findings suggest that multiple NTFs are more efficacious in protecting multiple cell types.

2.1 Mesenchymal Stem Cells

Originally documented by Friedenstein and colleagues [24], MSCs are multipotent stromal cells, which have since been defined by three main characteristics: (1) plastic adherence; (2) ability to differentiate into a diverse set of tissue within the mesoderm lineage; and (3) self-renewal [25]. MSCs can be derived from various sources (including bone marrow, umbilical cord blood, adipose tissue, etc.), and can be easily isolated and expanded in vitro. Transplantation of bone-marrow derived MSCs into rodent models of HD has been shown to reduce motor and cognitive deficits [26–29]. In 2003, Lescaudron and colleagues transplanted autologous, whole bone marrow into the brain of rats that were treated with QA [26]. Reductions in working memory errors were reported, however no neuronal differentiation of transplanted cells was observed, suggesting that beneficial effects were the result of a mechanism other than cell replacement. It was later observed that MSCs transplanted into a 3-nitropropionic acid (3-NP) rat model of HD improved latency to fall on the rotarod as well as reduced the size of the lesion to the striatum [28], which was hypothesized to be a result of NTF release from the cellsThe intrinsic characteristics of MSCs, such as immunomodulation and NTF release, have made these cells a target for cell-based

therapies in HD. The ability of MSCs to modulate the local immune environment comes from cell-to-cell contact and through the release of interleukins and cytokines that interact with a wide range of immune cells that, in turn, help to protect transplanted MSCs from acuteand prolonged immune responses [30–33]. In vitro characterizations of MSCs have revealed the presence of many NTFs, including BDNF, GDNF, and nerve growth factor (NGF) [28,34]. Recent advances in stem cell characterization techniques have allowed researchers to explore the secretome of MSCs, with the aim of identifying all secreted molecules which, in turn, may provide insight into the mechanisms of MSC benefits (for review [35]). The direct correlation between intrastriatal levels of BDNF led one group to induce MSCs to overexpress BDNF and GDNF through changes in culture conditions of the MSCs in vitro. When transplanted prior to QA injection, these NTF cells were able to reduce lesion size, relative to non-transplanted controls [36]. Similarly, R6/2 mice transplanted with human MSCs induced to secrete NTFs displayed transient reductions in rotarod deficits, which were dependent upon age at which R6/2 mice were transplanted [37].

2.2. Genetic Engineering of Transplantable Cells To facilitate a greater release of NTFs for an extended period, transplantable cells have been engineered to secrete greater than physiological levels of NTFs. In 2000, a group tested the ability of rat fibroblasts, individually engineered to overexpress a variety of NTFs, including BDNF, NT-3, or NT4/5, to protect against QA lesion [38]. The engineered cells were first transplanted into the rat striatum, followed by a QA injection 24 h after transplantation. It was observed that all rats transplanted with engineered cells reduced TUNEL labeling when observed 7 days after inducing a lesion. Furthermore, all engineered cell types differentially protected against the death of neurons expressing glutamate- decarboxylase-, preproenkephalin-, and preprotachykinin-, but not dynorphin-expressing cells. As a whole, it was observed that transplants of fibroblasts overexpressing BDNF were the most efficient in protecting against neuronal death. Similarly, a second group of researchers transplanted astrocytes which overexpressed BDNF [39]. To this end, a transgenic mouse was created to overexpress BDNF via control of the glial fibrillary acidic protein promoter [39]. Astrocytes were isolated and expanded from the transgenic animals, then transplanted into wild-type mice, followed by QA injections at 30 days post-transplantation. Similar to previous results, the transplanted BDNF-expressing astrocytes gave protection against the toxic lesion, increasing the total number of DARPP32 and parvalbumin expressing neurons, relative to non-engineered astrocytes. Animals transplanted with BDNF-expressing astrocytes also showed a reduction in apomorphine-induced rotations. Both fibroblasts and astrocytes, that were engineered to

overexpress BDNF, were efficient in protecting against QA induced HD-like pathology, but were not tested in a transgenic animal model of HD. Given that MSCs are easy to isolate and expand, our lab utilized bone-marrow derived MSCs, and then retrovirally transfected to overexpress BDNF, NGF, or a combination of BDNF and NGF [40]. These cells were then transplanted into the YAC128 transgenic mouse model of HD, and observed for 9 months. All YAC128 mice that were transplanted with the engineered MSCs had reductions in disease pathology, with an increased latency to fall from the rotarod and reduced clasping scores. Compared to all engineered cell lines, BDNF-expressing MSCs showed the highest amount of behavioral protection. Neuropathologically, YAC128 mice transplanted with BDNF-expressing MSCsdisplayed a normalization of total neurons within the striatum, and protected against significant losses of DARPP32 neurons

These transplantation studies, along with the results viral-vector-mediated increases in BDNF levels have shown that treatments of this nature are protecting endogenous neurons against cell death. With the promising results from the BDNF expressing MSCs [40] and lentiviral safety [41], the California Institute for Regenerative Medicine (CIRM) granted the University of California at Davis to take lentivirally engineered MSCs to overexpress BDNF to the clinic, with the goal of creating an FDA-approved cellular therapy for HD. It is generally accepted that MSCs do not differentiate into neurons following transplantation. Thus, the use of stem-cell mediated transfer of NTFs into the striatum of HD patients will provide the most benefit early in disease progression, when high numbers of MSNs are still present in the striatum.

Gene Therapy for Alzheimer's Disease

Inhibiting secretase activity to decrease amyloid pathway

Anti-BACE1: Given the pivotal role BACE1 plays in Aβ production, it is an obvious therapeutic target. Small-molecule inhibitors of BACE1 have been in development, and many are now at various stages of clinical trials. Lowering BACE1 levels using lentiviral vectors expressing siRNAs reduced amyloid production and the neurodegenerative and behavioral deficits in AD mice. However, recent results showed unexpected and undesirable effects of BACE1 inhibition on synaptic function and cognition. In addition to the well-documented neurotoxic effects of Aβ, there is evidence that Aβ has neuroprotective properties and positive role on mechanisms underlying cognition at low or physiologic levels, raising the

possibility that inhibiting BACE1 in wild-type animals may reduce endogenous Aβ production to such levels that its neurotrophic and synaptotrophic properties are lost.[20–22]

Inhibiting gamma secretase activity

γ-Secretase is a membrane protease carrying out cleavage of more than 100 single transmembrane-spanning proteins, including APP, Notch, and N-cadherin. Presenilin subunit is the catalytic component: mutations in the presenelin gene are a major cause of early onset familial AD (FAD)[8] because they lead to an increase in the production of the highly amyloidogenic Aβ42 isoform. Drugs aimed at γ-secretase are considered to be promising therapeutic targets for AD. However, inhibition of γ-secretase can cause severe adverse events, particularly because of the blockage of other pathways particularly the Notch signaling process.[23,24]

Increasing amyloid degrading enzymes: ECE, IDE, and NEP

Several proteases, including neprilysin (NEP), endothelin converting enzyme (ECE), and insulin degrading enzyme (IDE), have been shown to cleave Aβ.[25] AAV5-ECE-1 administration was evaluated in APP/PS1 transgenic mice. Strong expression was obtained in areas surrounding the injection sites, allowing Aβ and plaques decrease in the anterior cortex and hippocampus.[26]

Another study compared AAV-induced increased expression of NEP and IDE. AAV vectors expressed either native forms of NEP (NEP-n) or IDE (IDE-n), or engineered secreted forms of NEP (NEP-s) or IDE (IDE-s). In a six-week study, total Aβ and plaques were decreased in animals receiving the NEP-n and NEP-s but not for IDE-n or IDE-s in either the hippocampus or cortex. Thus, NEP, but not IDE, may be a good candidate for AD gene therapy.[27]

Delivering anti-amyloid antibodies

Monoclonal antibodies or polyclonal immunoglobulins targeting Aβ have been used to promote its clearance. Results from animal studies have shown that anti-Aβ antibodies can prevent oligomer formation and reduce brain amyloid load with improvement in cognitive functions. Several studies are ongoing to evaluate the tolerance and the efficacy of these strategies in human patients.[19,28]

A gene therapy strategy was also used based on the delivery of AAV encoding anti-Aβ single chain antibody (scFv) injected into the corticohippocampal regions of AD mouse models. One year after injection, expression of scFv was detectable in the neurons of the hippocampus. Amyloid deposits were strongly decreased at the injection sites with no sign of neurotoxicity.[29]

Increasing physiological APP pathway

The proteolytic processing of APP can be achieved following two competing pathways: the amyloidogenic and the nonamyloidogenic pathways[4] (Fig. 1). The last one not only prevents the production of amyloid toxic forms, but also enables the release of the soluble APPsα. APP has important physiological functions, many of which are thought to be mediated via the secretion of the APPsα.[30] *In vivo* the administration of APPsα enhances memory performance and long-term potentiation (LTP) in rodents, and displays crucial physiological properties for synaptic plasticity and hippocampal function.[31] In APP-KO mice, APPsα knock-in completely rescued spatial learning.[32]

The idea emerged that, beside the well-established neurotoxic and synaptotoxic properties of β-CTF and soluble and oligomeric Aβ, loss of the neuroprotective APPsα also contributes considerably to the development of AD. APPsα levels are decreased in the cerebrospinal fluid of AD patients, in both genetic and sporadic forms, which is correlated with poor memory performance.[33,34] APPsα was also shown to inhibit tau phosphorylation through GSK3β modulation.[35] This suggests that overexpression of APPsα could be of great interest to alleviate AD-related symptoms. One way to do this would consist of overexpressing the α-secretase (ADAM10). However, ADAM10 was shown to have many substrates and its upregulation was implicated in tumorigenesis, thereby precluding a human clinical trial.[36] APPsα was overexpressed by means of AAV virus in hippocampal neurons of an aged AD mouse model (APP/PS1).[37] Two months after gene therapy, APPsα partially rescued spatial memory defects, restored synaptic plasticity and spine density, and decreased soluble Aβ and amyloid plaques. This was associated with microglial activation and phagocytosis (increase of IDE and TREM2) around amyloid plaques.

Increase neurotroprotection

Nerve growth factor

Nervous system growth factors prevent neuronal death in various correlative animal models of AD.[38] Specifically, nerve growth factor (NGF) stimulates and prevents the death of the function of basal forebrain cholinergic neurons that undergo early and prominent degeneration in AD.[39]

Degeneration of cholinergic neurons is an early and prominent contributor to cell loss and cognitive decline in AD. Indeed, NGF levels in the basal forebrain region decline in AD. Previous studies in animal models of AD have shown that NGF can stimulate cholinergic neurons that are necessary for maintenance of cognitive function, and undergo atrophy in early AD and prevent their death.[40] However, growth factors can cause off-target adverse effects, necessitating a targeted delivery strategy to control their localization and spread in the brain.[41]

In a first phase I trial, NGF was delivered through transplantation of autologous fibroblasts transduced with a Moloneyleukemia viral vector to express human NGF into the basal forebrain region containing cholinergic cell bodies that send their projections throughout the cortex and hippocampus. The clinical findings of the phase I *ex vivo* trial suggested possible beneficial effects over a 2-year observation period compared with pretreatment rates of cognitive decline. A second phase I clinical trial included 10 patients who received AAV2-NGF into the basal forebrain region. An escalation dose protocol was used (1.2×10^{10} to 1.2×10^{11} vector particles[42]). The brains of all 8 patients in the first phase I trial were examined. All patients exhibited a trophic response to NGF in the form of axonal sprouting toward the NGF source. Cholinergic neuronal hypertrophy occurred on the NGF-treated side. Activation of cellular signaling and functional markers was present in the 2 patients who underwent AAV2-NGF gene transfer. An overall lower rate of cognitive decline and increased cortical glucose uptake were reported; two individuals had subcortical hemorrhage during implantation. No other adverse pathological effects related to NGF were observed.

A phase II multicenter, sham-surgery-controlled trial of NGF in AD is ongoing in 49 patients with mild to moderate AD based on a single administration of AAV-NGF vector that encodes the gene for NGF (CERE-110) or an appropriate sham (placebo) surgery control treatment. Data are expected soon.[43]

Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF) is expressed in multiple cortical regions, including the entorhinal cortex and hippocampus.[44] BDNF levels decline in AD.[45] Administration of BDNF using a lentiviral vector (under the control of the CAG promoter) to the entorhinal cortex in an AD mouse model (APP transgenic mouse line J20) improved learning and memory, enhanced expression of the synaptic protein synaptophysin,[38] and prevented neuronal loss with early life BDNF treatment. The lentiviral-mediated *BDNF* gene was delivered into the entorhinal cortices of mice at age 2 months, and mice were examined 5 months later. BDNF revealed neuroprotective properties. Interestingly, this beneficial effect was not accompanied by a decrease of amyloid plaques.

Glial cell-derived neurotrophic factor

Glial cell-derived neurotrophic factor (GDNF) is emerging as a potent neurotrophic factor with therapeutic potential against a range of neurodegenerative conditions, including AD. Lentiviral vectors were used to overexpress the*GDNF* gene in hippocampal astrocytes of 3xTg-AD mice *in vivo*. After 6 months of GDNF overexpression, 10-month-old 3xTg-AD mice showed preserved learning and memory. GDNF therapy did not significantly reduce amyloid and tau pathology, but upregulated the expression of BDNF and induced neuroprotection.[46]

IGF1 and IGF2

Insulin-like growth factor 2 (IGF2) plays a critical role in memory consolidation in rats and mice, and IGF2 expression decreases in the hippocampus of patients with AD. AAV-IGF2 administration in the hippocampus of aged wild-type mice enhances memory and promotes dendritic spine formation. AAV-IGF2 or AAV-IGF1 injection into the hippocampus of APP Tg2576 mice rescues behavioral deficits, promotes dendritic spine formation, and restores synaptic transmission. IGF2, but not IGF1, injection allows significant reduction in amyloid levels. Results demonstrate that IGF2/IGF2R is involved in the extracellular Aβ degradation mechanism, and suggest that IGF2R may act as an Aβ scavenger.[47] Another study showed that intracerebroventricular infusion of IGF2 in APP/PS1 mice that express the green fluorescent protein in cholinergic neurons (APP.PS1/CHGFP) and control littermates, at 6 months, reduced the number of hippocampal amyloid plaques and increased the level of hippocampal protein ACh-synthesizing enzyme.[48]

Nrf2 antioxidant pathway activation

Oxidative injury is thought to be central in the pathogenesis of AD. Binding of the transcription factor nuclear factor E2-related factor 2 (NRF2) to the antioxidant response element (ARE) enhancer sequence is an endogenous defense system against oxidative stress, triggering the simultaneous expression of numerous protective enzymes and scavengers.

A lentiviral vector was used to deliver NRF2 bilaterally into the hippocampus of 9-month-old transgenic AD mice (APP/PS1 mice). Reduction in spatial learning deficits of aged APP/PS1 mice was achieved. Six months after injection, *NRF2* gene transfer was associated with a reduction in astrocytic, but not microglial activation, and induction of NRF2 target gene hemeoxygenase 1 in hippocampal neurons.[49]

Boosting autophagy-mediated pathways

Autophagy, the major cellular pathway for degradation of long-lived proteins and protein turnover, has been implicated in AD. Evidence suggests that increasing the levels of autopagy-related proteins may have potential for therapy. Lentiviral-mediated overexpression of beclin-1 in the hippocampus and cortex of APP transgenic mice reduced both intracellular Aβ as well as extracellular β-pleated Aβ depostis.[50] Thus, restoring beclin-1 and enhancing autophagy may be a novel approach to treat AD.

Targeting inflammatory pathway

Interleukin-4

Increasing data demonstrate the role of inflammation in AD. Anti-inflammatory cytokine signaling may play an emerging role as neurotransmitters, neuromodulators, and neurohormones in the brain. IL-4 has been characterized as a potential modulator of neuronal activities in the brain. IL-4 receptors are expressed in the hippocampus, and downregulation of IL-4 causes aging-related deficits of hippocampal LTP.[51] Moreover, IL-4 stimulation of human macrophages or microglia enhances Aβ degradation. AAV-mediated expression of the mouse IL-4 gene in APP/PS1 mice attenuates AD. Vector encoding IL-4 injection into the hippocampus resulted in sustained expression of IL-4, reduced astro/microgliosis, Aβ oligomerization and deposition, enhanced neurogenesis, improved spatial learning, and promoted phosphorylation of N-methyl-D-aspartate receptor subunit 2B.[52]

Gene Therapy in Multiple Sclerosis

The key issues for any successful gene therapy approach is the nature of the vector and definition of targets. Gene therapy has not yet been attempted in MS, but there have been a number of studies in EAE that have invariably shown some level of efficacy at inhibiting the disease (Table 1), although in many cases this has only been an amelioration rather than elimination of disease.[32, 33, 34, 35, 36,37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61,62, 63, 64] As the majority of the CNS is postmitotic, this puts constraints on the nature of the vector that can be used, and to date administration of plasmid DNA,[32,33,34,35,36,37,38,39,40,41] viral infection,[42,43,44,45,46,47,48,49,50,51,52,53] and retrovirally transduced cell (RVC)-carriers[47,54,55,56,57,58,59,60,61,62,63] have been investigated in EAE (Table 1). These have largely focused on inhibition of the immune response either applied centrally to target the local pathological events within the CNS or peripherally administered to inhibit: initial sensitization, the activities of circulating cells or perivascular events in areas of local BBB breakdown. In addition, some studies have attempted to promote repair or inhibition of the demyelination process.[40,44,49,57,61] Cytokines are dynamically expressed as lesions evolve and resolve[12,13,14,15,16] and are of major importance in the development and control of autoimmunity.[12] Many studies in EAE focus on the use of knockout mice.[22,24,65] However, in these mice there is cytokine redundancy, compensation and sometimes lethality due to developmental effects. Exogenous gene delivery provides a useful tool to probe the biology of disease in 'physiologically normal' adult animals. Importantly, it also provides a route for therapy, particularly as gene delivery of cytokines can be shown to be more efficacious than bolus protein delivery.