Gene Therapy for Neurologic Disease: A Neurosurgical Review

Frederick L. Hitti[1], Pedro Gonzalez-Alegre[2], Timothy H. Lucas[1]

Key words
- Clinical trials
- Gene therapy
- Neurosurgical gene delivery
- Review
- Viral vector

Abbreviations and Acronyms
AADC: Aromatic *L*-amino acid decarboxylase
AAV: Adeno-associated virus
AD: Alzheimer disease
ALS: Amyotrophic lateral sclerosis
CRISPR: Clustered regularly interspaced short palindromic repeat
DBS: Deep brain stimulation
HSV: Herpes simplex virus
IM: Intramuscular
IV: Intravenous
MLV: Murine leukemia virus
MRI: Magnetic resonance imaging
NGF: Nerve growth factor
PD: Parkinson disease
SGSH: *N*-sulfoglycosamine sulfohydrolase
STN: Subthalamic nucleus
TK: Thymidine kinase

From the Departments of [1]Neurosurgery and [2]Neurology, University of Pennsylvania, Philadelphia, Pennsylvania, USA

To whom correspondence should be addressed: Frederick L. Hitti, M.D., Ph.D. [E-mail: Frederick.Hitti@uphs.upenn.edu]

Citation: World Neurosurg. (2019) 121:261-273. https://doi.org/10.1016/j.wneu.2018.09.097

Journal homepage: www.journals.elsevier.com/world-neurosurgery

Available online: www.sciencedirect.com

Recent approval of unprecedented gene therapies has signaled the beginning of a new era in modern medicine. This rapidly changing landscape underscores the importance of neurosurgeons developing a fundamental understanding of gene therapy, because many neurologic disorders are caused by derangements in gene expression. Gene therapy, the transfer of genetic material to alter endogenous gene expression or introduce exogenous genes, has emerged as a novel therapeutic approach to treat a wide range of diseases. There are several methods to transfer genetic material to the nervous system through viral transduction. Here, we synthesize the latest developments in gene therapy for neurologic disorders and the neurosurgical approaches to treat them.

INTRODUCTION

Gene therapy involves the transfer of genetic material to treat disease. Currently available techniques allow for expression of exogenous or suppression of endogenous genes, making gene therapy a versatile treatment modality. In the United States, recent advances have led to approval by the U.S. Food and Drug Administration of several gene therapy agents, including Spinraza (nusinersen), Kymriah (tisagenlecleucel), and Luxturna (voretigene neparvovec).[1-4] Spinraza is an antisense oligonucleotide approved for the treatment of spinal muscular atrophy type 1. It has been shown to improve motor function in both infantile-onset and later-onset disease in recent phase 3 trials. In addition, survival is improved with treatment of infantile-onset disease.[1,3] Kymriah is a form of ex vivo gene therapy (CAR T-cell therapy) developed for the treatment of leukemia that is highly effective (81% remission rate within 3 months).[2] Subretinal injections of Luxturna, a virus encoding RPE65, has been shown to improve visual function in patients with RPE65-mediated Leber congenital amaurosis.[4] For neurologic disorders that are the result of gene mutations rendering a gene nonfunctional (loss of function), gene therapy may be used as a means of restoring function by providing a functional version of the gene. For disorders that involve neurodegeneration, genes that enhance neuronal survival may be delivered. Disorders caused by gene overexpression, toxic gain of function, or a dominant-negative effect may be ameliorated by gene knockdown. Neuronal circuit dysfunction may be treated by delivering genes that enhance or inhibit activity in key regions of the brain. Oncologic disease, including brain tumors, may be treated by delivering genes that render tumor cells susceptible to cytotoxic drugs, delivering genes that enhance or spur immune system recognition of tumor cells, or by delivering cytotoxic genes selectively to tumor cells. We give an overview of methods for gene transfer and clinical trials conducted or being conducted using gene therapy to treat neurologic disease. Throughout, we address neurosurgical considerations for this treatment modality.

METHODS FOR GENE TRANSFER

Multiple methods have been used to transfer genetic material to human cells. These methods include viral transduction and plasmid transfection. Viral transduction techniques involve engineering of viruses to deliver intended genetic sequences without pathogenicity. Plasmid transfection techniques involve delivery of circular DNA constructs often with the aid of liposomes, spherical lipid complexes, and more recently, nanoparticles.

Various types of viruses have been used for gene therapy, each with its advantages and disadvantages (**Table 1**, **Figures 1** and **2**). These types include adenovirus, adeno-associated virus (AAV), herpes simplex virus (HSV), and various retroviruses. For nontumor applications, the most popular of these vectors has been AAV. This virus has several properties that make it ideal for gene therapy. It is classified as a dependovirus (it cannot replicate on its own) and it is nonpathogenic in humans. Because it is replication deficient and nonpathogenic, administration of AAV does not result in superinfection or generate large immune reactions. Furthermore, the virus can be engineered to contain nearly no viral genetic material (leaving only short packaging sequences) so that only the intended gene is

Table 1. Viral Vectors Used for Gene Therapy

Virus	Type	Carrying Capacity	Immunogenic	Duration of Expression	Integration into Genome
Adeno-associated virus	Single-strand DNA	<5 kB	No	Years	No
Adenovirus	Double-strand DNA	8–9 kB	Yes	Days to weeks	No
Herpes simplex virus	Double-strand DNA	40 kB	Yes	Days to weeks	No
Retrovirus	Single-strand RNA	8–9 kB	No	Years	Yes

transcribed and no viral proteins are generated. Once the virus delivers its genetic material, it remains episomal (i.e., extrachromosomal). This property is advantageous because unintended insertional mutagenesis can occur after other viruses integrate their genetic material into the host genome. Despite remaining extrachromosomal, AAV allows for long-term gene expression of years.[5] In addition, there are many different serotypes of AAV (various coat proteins) that allow for modifications of its cell-type targeting and spread.[6] AAV is an extremely small virus, which enables it to spread through tissue well; however, its small size limits its carrying capacity. Hence, despite all the advantages, if the intended genetic material is larger than 5 kB, a different vector must generally be used.[7] Retroviruses, adenovirus, and HSV are larger viruses with greater carrying capacities (Figures 1 and 2). Retroviruses, such as lentivirus, and adenovirus can carry 8-kB to 9-kB constructs, whereas HSV can carry up to 40 kB.[8] However, their larger size also results in more restricted tissue spread. Retroviruses are unique among the vectors used for gene therapy in that they integrate the genetic material into the host genome. Integration results in long-term gene expression but also carries the potential for insertional mutagenesis. Several groups have developed integrase-defective lentiviruses to prevent integration.[9] However, long-term stability of gene expression in this system has not been established. Both retroviruses and AAV result in long-lasting gene expression, whereas HSV and adenovirus do not. Furthermore, adenovirus and HSV are pathogenic in humans and can instigate an immune response. However, an advantage of HSV is rapid gene expression (within hours), whereas the other vectors take days for therapeutic gene expression. For tumor applications, HSV, adenovirus, and retroviruses have been largely used. For tumor treatment, long-lasting gene expression is not necessary and immune

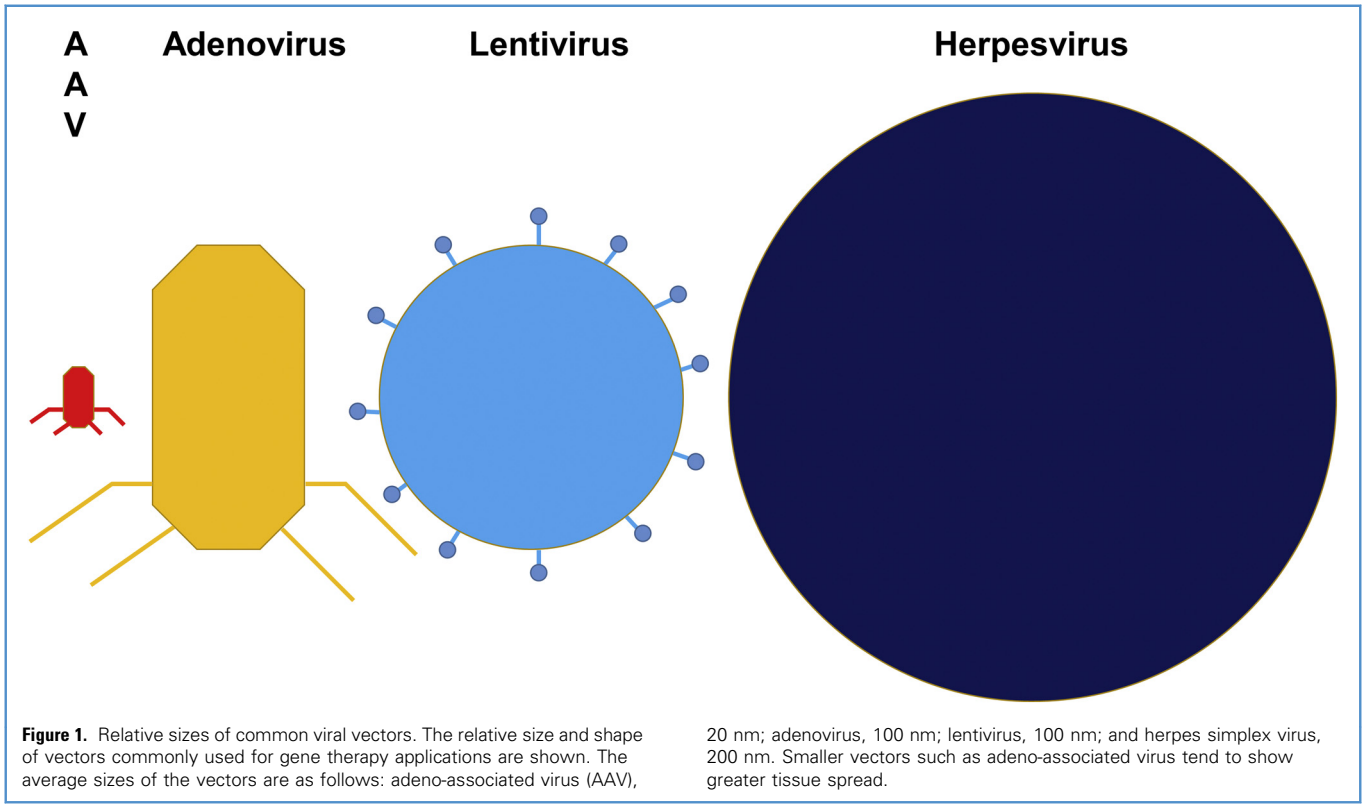

Figure 1. Relative sizes of common viral vectors. The relative size and shape of vectors commonly used for gene therapy applications are shown. The average sizes of the vectors are as follows: adeno-associated virus (AAV), 20 nm; adenovirus, 100 nm; lentivirus, 100 nm; and herpes simplex virus, 200 nm. Smaller vectors such as adeno-associated virus tend to show greater tissue spread.

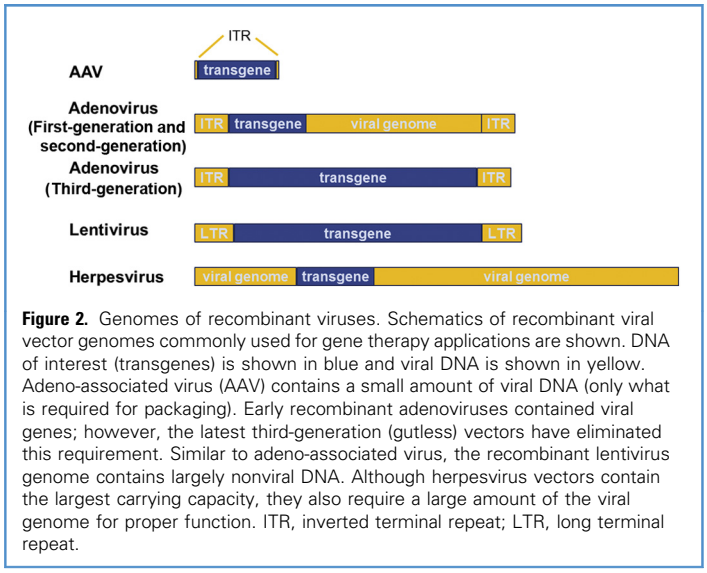

Figure 2. Genomes of recombinant viruses. Schematics of recombinant viral vector genomes commonly used for gene therapy applications are shown. DNA of interest (transgenes) is shown in blue and viral DNA is shown in yellow. Adeno-associated virus (AAV) contains a small amount of viral DNA (only what is required for packaging). Early recombinant adenoviruses contained viral genes; however, the latest third-generation (gutless) vectors have eliminated this requirement. Similar to adeno-associated virus, the recombinant lentivirus genome contains largely nonviral DNA. Although herpesvirus vectors contain the largest carrying capacity, they also require a large amount of the viral genome for proper function. ITR, inverted terminal repeat; LTR, long terminal repeat.

activation may be beneficial, making adenovirus and HSV good choices for this application.

Compared with viral transduction, nonviral approaches have the advantage of being largely nonimmunogenic. Furthermore, production of the vector is simpler. However, plasmid/liposomal gene therapy is a less effective means of delivering genetic material to cells. These techniques also have the disadvantage of short-term gene expression. On the other hand, some applications such as gene editing may require only transient expression. Thus, there is increasing interest in the development of nanoparticles, lipid-based delivery, and other nonviral approaches.

Once a vector is designed, it may be delivered through a variety of routes. Clinical trials have used intracerebral, intraventricular, intravenous (IV), intramuscular (IM), intrathecal, and subcutaneous routes of delivery. Intracerebral injections allow for specific targeting of affected brain areas and bypass of the blood-brain barrier. Intraventricular injections may be used if expression throughout the brain is desired. Although IV injections are less invasive, their limitations include expression in organs other than the brain and difficulty bypassing the blood-brain barrier. Some of these limitations have been mitigated by development of novel AAV serotypes.[10] Other less invasive delivery routes include IM and subcutaneous. These routes of delivery may be used for diseases that involve the peripheral nervous system.

GENE THERAPY APPROACHES TO TREAT NEUROLOGIC DISEASE

Gene therapy is a versatile technique that can be used to treat many neurologic diseases (Table 2). The most intuitive application of gene therapy is for the treatment of Mendelian diseases (i.e., those resulting from single gene mutations). Gene therapy in these cases is directed at replacement of the missing/nonfunctional gene product. Most work has been undertaken on the treatment of diseases in which enzyme deficiencies lead to accumulation of toxic metabolites and resultant leukodystrophy and/or neurodegeneration.

Canavan disease results from mutations in the aspartoacylase gene (ASPA), leading to accumulation of N-acetylaspartate and leukodystrophy. Patients with this disease show developmental delay, macrocephaly, and death before their third decade of life.[12,13] Leone et al. have shown the safety and possible efficacy of both intraventricular plasmid injections and intracerebral AAV injections of vectors carrying the ASPA gene.[12,13] Several phase 1/2 gene therapy trials are under way for the treatment of late infantile neuronal ceroid lipofuscinosis, a lysosomal storage disease that results from a mutation of the CLN2 gene and is fatal by ages 8–12.[15] These trials are using intracerebral injections of AAV to deliver a normal copy of the CLN2 gene. A phase 1/2 trial is under way for the treatment of variant late infantile neuronal ceroid lipofuscinosis, a similar disease in which the CLN6 gene is mutated. In this trial, intrathecal injections of AAV are used to deliver a normal copy of the CLN6 gene (Table 2). Another approach to achieving widespread expression of a gene in the brain is intraventricular injection. Batten disease is caused by deficiency of the lysosomal enzyme tripeptidyl peptidase 1 (TPP1). Katz et al.[23] have shown efficacy of intraventricular AAV2-TPP1 injections in a TPP1-deficient canine model.

Phase 1/2 trials of gene replacement therapy by intracerebral or intrathecal AAV injections are also under way for aromatic L-amino acid decarboxylase (AADC) deficiency, giant axonal neuropathy, and metachromatic leukodystrophy (Table 2).

Sanfilippo type A syndrome (mucopolysaccharidosis type IIIA) is a lysosomal storage disease caused by a mutation in the N-sulfoglycosamine sulfohydrolase (SGSH) gene. A multicenter phase 1/2 trial has shown that intracerebral injections of AAV carrying the SGSH and sulfatase-modifying factor (SUMF1) genes are safe and possibly effective for the treatment of Sanfilippo type A syndrome.[16] This trial is unique among this group of studies, because the vector used carries the genetic sequences for 2 genes. This bicistronic expression (expression of 2 proteins with 1 construct) is achieved by the insertion of an internal ribosome entry site sequence.[24] In mammalian systems, 1 gene normally produces 1 protein (and its splice variants). Using a viral internal ribosome entry site sequence allows 1 gene promoter to drive expression of 2 separate protein products as a result of initiation of translation at 2 sites in the same messenger RNA. There is also a phase 1/2 trial for the treatment of Sanfilippo type B syndrome, a deficiency of α -N-acetylglucosaminidase (NAGLU) function, in which intracerebral injections of AAV are being used to deliver a functional copy of the NAGLU gene (Table 2).

Because these inborn errors of metabolism involve the entire brain, the trials discussed earlier have used either intraventricular/intrathecal injections or more

Table 2. Gene Therapy for Neurologic Disease

Disease	Disease Category	Vector	Injection Site	Mechanism	Institution/Company	Phase	Status	Outcome	Immunosuppression	Trial Number	Reference
AADC deficiency	Inherited/metabolic	AAV2-hAADC	Putamen	Gene replacement	National Taiwan University Hospital	1/2	Active	Pending	Unk	NCT01395641	
AADC deficiency	Inherited/metabolic	AAV2-hAADC	SNc and VTA	Gene replacement	UCSF	1	Active	Pending	Unk	NCT02852213	
AD	Neurodegenerative	AAV2-CAG-NGF	NBM	Growth factor	Sangamo/UCSD, Rush	1	Complete	Safe, biologically effective	No	NCT00087789	Tuszynski et al., 2015[11]
AD	Neurodegenerative	MLV transduced fibroblasts	NBM	Growth factor	UCSD	1	Complete	Safe, biologically effective	No	NCT00017940	Tuszynski et al., 2015[11]
AD	Neurodegenerative	AAV2-CAG-NGF	NBM	Growth factor	Sangamo, multicenter	2	Active, not recruiting	Pending	Unk	NCT00876863	Tuszynski et al., 2015[11]
ALS	Neurodegenerative	VEGFA TF plasmid	IM	Increase VEGF expression	Sangamo	2	Complete	Pending	Unk	NCT00748501	
ALS	Neurodegenerative	CMV-HGF plasmid	IM	Growth factor	ViroMed/Northwestern	1/2	Active, not recruiting	Pending	Unk	NCT02039401	
ALS	Neurodegenerative	Antisense oligo	Intrathecal	Gene knockdown	Ionis	1	Completed	Safe	No	NCT01041222	
Canavan disease	Inherited/metabolic	AAV2-NSE-ASPA	Intracerebral*	Gene replacement	Multicenter	1/2	Complete	Safe	No	n/a	Leone et al., 2012[12]
Canavan disease	Inherited/metabolic	Liposome plasmid CMV-ASPA	Intraventricular	Gene replacement	Thomas Jefferson University	n/a	Complete	Safe, possibly effective	No	n/a	Leone et al., 2000[13]
Chronic pain	Acquired	HSV1-CMV-PENK	Subcutaneous	Opioid expression	Diamyd	1	Complete	Safe, possibly effective	No	NCT00804076	Fink et al., 2011[14]
Chronic pain	Acquired	HSV1-CMV-PENK	Subcutaneous	Opioid expression	Diamyd	2	Complete	Pending	Unk	NCT01291901	
GAN	Inherited/metabolic	scAAV9-JeT-GAN	Intrathecal	Gene replacement	National Institute of Neurological Disorders and Stroke	1	Active	Pending	Unk	NCT02362438	
LINCL	Inherited/metabolic	AAV2-CAG-CLN2	Intracerebral*	Gene replacement	Cornell	1	Active, not recruiting	Pending	No	NCT00151216	Worgall et al., 2008[15]
LINCL	Inherited/metabolic	AAVrh10-CAG-CLN2	Intracerebral*	Gene replacement	Cornell	1/2	Active	Pending	Unk	NCT01414985	
LINCL	Inherited/metabolic	AAVrh10-CAG-CLN2	Intracerebral*	Gene replacement	Cornell	1	Active	Pending	Unk	NCT01161576	
MLD	Inherited/metabolic	AAVrh10-CAG-ARSA	Intracerebral*	Gene replacement	Institut National de la Santé et de la Recherche Médicale	1/2	Active	Pending	Unk	NCT01801709	

MPS 2IA	Inherited/metabolic	AAVrh10-PGK-SGSH-IRES-SUMF1	Intracerebral*	Gene replacement	Lysogene	1/2	Complete	Safe, possibly effective	Yes	NCT01474343	Tardieu et al., 2014[16]
MPS 2IA	Inherited/metabolic	scAAV9-U1a-hSGSH	IV, peripheral	Gene replacement	Abeona Therapeutics/ Nationwide Children's Hospital	1/2	Active	Pending	Unk	NCT02716246	
MPS 2IB	Inherited/metabolic	AAV5-PGK-NAGLU	Intracerebral*	Gene replacement	Hôpital Bicêtre	1/2	Complete	Pending	Yes	ISRCTN19853672	
PD	Neurodegenerative	AAV2-CAG-GAD	STN	Neuromodulation	Neurologix	1	Complete	Safe	No	NCT00195143	Kaplitt et al., 2007[17]
PD	Neurodegenerative	AAV2-CAG-GAD	STN	Neuromodulation	Neurologix	2	Complete	Safe, effective	No	NCT00643890	LeWitt et al., 2011[18]
PD	Neurodegenerative	AAV2-CMV-hAADC-2	Striatum	Gene replacement	Genzyme	1	Complete	Safe, possibly effective	No	NCT00229736	Christine et al., 2009[19]
PD	Neurodegenerative	AAV2-CAG-NTN	Putamen	Growth factor	Sangamo/UCSD, Rush	1	Complete	Safe, possibly effective	No	NCT00252850	Marks Jr. et al., 2008[20]
PD	Neurodegenerative	AAV2-CAG-NTN	Putamen	Growth factor	Sangamo/multicenter	2	Complete	Not effective	No	NCT00400634	Marks Jr. et al., 2010[21]
PD	Neurodegenerative	AAV2-CMV-GDNF	Putamen	Growth factor	National Institute of Neurological Disorders and Stroke	1	Active	Pending	Unk	NCT01621581	
PD	Neurodegenerative	AAV2-CMV-hAADC	Putamen	Gene replacement	Voyager Therapeutics/ UCSF, University of Pittsburgh	1	Active	Pending	Unk	NCT01973543	
PD	Neurodegenerative	AAV-hAADC-2	Putamen	Gene replacement	Jichi Medical University	1/2	Active	Pending	Unk	NCT02418598	
PD	Neurodegenerative	AAV2-CAG-NTN	Substantia nigra and putamen	Growth factor	Sangamo, multicenter	1/2	Active, not recruiting	Pending	Unk	NCT00985517	
PD	Neurodegenerative	AAV2-CMV-hAADC-2	Putamen	Gene replacement	Jichi Medical University	1	Complete	Safe, possibly effective	No	n/a	Muramatsu et al., 2010[22]
SMA	Neurodegenerative	scAAV9-CAG-SMN	IV, peripheral	Gene replacement	AveXis/Nationwide Children's Hospital	1	Active, not recruiting	Pending	Unk	NCT02122952	
VLINCL	Inherited/metabolic	scAVV9-CAG-CLN6	Intrathecal	Gene replacement	Nationwide Children's Hospital	1/2	Active	Pending	Unk	NCT02725580	

Intracerebral injections were performed bilaterally for all trials listed. Vectors are listed as virus-promoter (if information was available)-gene.

AADC, aromatic *L*-amino acid decarboxylase; AAV, adeno-associated virus; Unk, unknown; SNc, substantia nigra pars compacta; VTA, ventral tegmental area; UCSF, University of California at San Francisco; AD, Alzheimer disease; CAG, chicken β-actin; NGF, nerve growth factor; NBM, nucleus basalis of Meynert; MLV, murine leukemia virus; ALS, amyotrophic lateral sclerosis; TF, transcription factor; IM, intramuscular; VEGF, vascular endothelial growth factor; HGF, hepatocyte growth factor; n/a, not applicable; CMV, cytomegalovirus; HSV, herpes simplex virus; GAN, giant axonal neuropathy; LINCL, late infantile neuronal ceroid lipofuscinosis; MLD, metachromatic leukodystrophy; MPS, mucopolysaccharidosis; SGSH, *N*-sulfoglycosamine sulfohydrolase; IRES, internal ribosome entry site; IV, intravenous; PD, Parkinson disease; GAD, glutamate decarboxylase; STN, subthalamic nucleus; NTN, neurturin; SMA, Spinal muscular atrophy; SMN, survival of motor neuron; vLINCL, variant late infantile neuronal ceroid lipofuscinosis; hAADC, human AADC; VEGFA, Vascular endothelial growth factor A; NSE, neuron specific enolase; ASPA, aspartoacylase; PENK, proenkephalin; scAAV, self-complementary AAV; JeT, synthetic promoter; AAVrh10, AAV derived from rhesus macaque; ARSA, Arylsulfatase A; PGK, Phosphoglycerate kinase; SUMF1, sulfatase-modifying factor; U1a, murine small nuclear RNA promoter; hSHSH, human N-sulfoglucosamine sulfohydrolase; GDNF, Glial cell-derived neurotrophic factor; n/a, not available.

*Injections were given at multiple sites, usually within white matter tracts.

Table 3. Gene Therapy for Treatment of Oncologic Disease

Disease	Vector	Injection Site	Mechanism	Institution	Phase	Status	Outcome	Trial Number	Reference
GBM/AMO	AdV-hIFN-β	Resection cavity	Antitumoral cytokine	Ohio State University Medical Center	1	Completed	Safe	n/a	Chiocca et al., 2008[34]
GBM	Fibroblasts producing RV-hsvTk	Resection cavity	Render tumor susceptible to drug	Genetic Therapy	3	Completed	Safe, not effective	n/a	Rainov, 2000[35]
GBM	Fibroblasts producing RV-hsvTk	Intratumoral	Render tumor susceptible to drug	Universidad Autónoma de Madrid	1	Completed	Safe	n/a	Izquierdo et al., 1996[36]
GBM	AdV-HSVtk	Resection cavity	Render tumor susceptible to drug	Advantagene	1	Completed	Safe, possibly effective	NCT00751270	Chiocca et al., 2011[37]
GBM	Fibroblasts producing RV-hsvTk	Resection cavity	Render tumor susceptible to drug	GLI328 European-Canadian Study Group	1/2	Completed	Safe	n/a	Shand et al., 1999[38]
GBM	Fibroblasts producing RV-hsvTk	Resection cavity	Render tumor susceptible to drug	Study Group On Gene Therapy For Glioblastoma	1/2	Completed	Safe, possibly effective	n/a	Klatzmann et al., 1998[39]
GBM	Fibroblasts producing RV-hsvTk	Resection cavity	Render tumor susceptible to drug	Novartis	1/2	Completed	53% adverse events	n/a	Prados et al., 2003[40]
GBM/AA	AdV-HSVtk	Resection cavity	Render tumor susceptible to drug	University of Kuopio	2	Completed	Safe, effective	n/a	Immonen et al., 2004[41]
GBM/AA/GS	AdV-HSVtk	Intratumoral	Render tumor susceptible to drug	Baylor College of Medicine	1	Completed	Safe, possibly effective	n/a	Trask et al., 2000[42]
GBM	AdV-HSVtk	Resection cavity	Render tumor susceptible to drug	Mount Sinai School of Medicine	1	Completed	Safe, possibly effective	n/a	Germano et al., 2003[43]
GBM/AO/AMO	AdV-HSVtk	Resection cavity	Render tumor susceptible to drug	Erasmus University Medical Center	1	Completed	Safe	n/a	Smitt et al., 2003[44]
GBM/AA/AO	Fibroblasts producing RV-hsvTk and AdV-HSVtk	Resection cavity	Render tumor susceptible to drug	University of Kuopio	2	Completed	Safe, possibly effective	n/a	Sandmair et al., 2000[45]
GBM/AA	Adv-HSVtk	Resection cavity	Render tumor susceptible to drug	Advantagene, Inc.	2	Active, not recruiting	Safe, effective	NCT00589875	Wheeler et al., 2016[46]
GBM/AA	RV-CD	Resection cavity	Render tumor susceptible to drug	Tocagen Inc.	2/3	Active	Pending	NCT02414165	
GBM/AA	Adv-HSVtk	Resection cavity	Render tumor susceptible to drug	University of Pennsylvania	1	Completed	Safe	NCT00002824	Alavi and Eck, 2001[47]
GBM	Fibroblasts producing RV-hsvTk	Resection cavity	Render tumor susceptible to drug	National Institute of Neurological Disorders and Stroke	1	Completed	Safe	NCT00001328	Ram et al., 1997[48]
GBM	Fibroblasts producing RV-hsvTk-IRES-IL-2	Intratumoral/ resection cavity	Render tumor susceptible to drug and stimulate immune system	University of Padova	1	Completed	Safe	n/a	Palù et al., 1999[49]
GBM/AA/AO/AE	AdV-hCMV-HSVtk and AdV-hCMV-Flt3L	Resection cavity	Render tumor susceptible to drug and stimulate immune system	University of Michigan	1	Active	Pending	NCT01811992	
GBM/AA/AMG	AdV-CMV-p53	Intratumoral, resection cavity	Tumor suppressor expression	Multicenter	1	Completed	Safe	n/a	Lang et al., 2003[50]

GBM	Ad5-CMV-p53	Intratumoral/ resection cavity	Tumor suppressor expression	North American Brain Tumor Consortium	1	Completed	Safe	NCT00004041	Papanastassiou et al., 2002[51]
GBM	HSV-1 (1716)	Intratumoral	Tumor-selective virus-mediated cell death	University of Glasgow	1	Completed	Safe, possibly effective	n/a	Papanastassiou et al., 2002[51]
GBM	E1B-attenuated adenovirus (ONYX-015)	Resection cavity	Tumor-selective virus-mediated cell death	NABTT CNS Consortium	1	Completed	Safe, not effective	n/a	Chiocca et al., 2004[52]
GBM	Reovirus	Intratumoral	Tumor-selective virus-mediated cell death	University of Calgary	1	Completed	Safe, not effective	n/a	Forsyth et al., 2008[53]
GBM	HSV-1 (G207)	Intratumoral	Tumor-selective virus-mediated cell death	University of Alabama at Birmingham/Georgetown University Medical Center	1	Completed	Safe, possibly effective	n/a	Markert et al., 2000[54]
GBM	HSV-1 (1716)	Intratumoral	Tumor-selective virus-mediated cell death	University of Glasgow	1	Completed	Safe, possibly effective	n/a	Rampling et al., 2000[55]
GBM	Newcastle disease virus (HUJ)	Intravenous	Tumor-selective virus-mediated cell death	Hadassah University Hospital	1/2	Completed	Safe, possibly effective	n/a	Freeman et al., 2006[56]

GBM, glioblastoma multiforme; n/a, not applicable; AA, anaplastic astrocytoma; GS, gliosarcoma; AO, anaplastic oligodendroglioma; AMO, anaplastic mixed oligoastrocytoma; AE, anaplastic ependymoma; AMG, anaplastic mixed glioma; n/a, not available.

commonly intracerebral injections at multiple sites throughout the brain to achieve widespread expression. One trial is under way for the treatment of Sanfilippo type A syndrome by peripheral IV injections of AAV to deliver the SGSH gene (Table 2). The advantage of this delivery method is that it should achieve a wide spread throughout the brain. However, peripheral injection can lead to transduction of cells in organs other than the brain, which could result in undesirable effects. Furthermore, the blood-brain barrier prevents efficient transduction. These problems are mitigated by the use of AAV9, a serotype that has been shown to pass the blood-brain barrier,[10] and a self-complementary virus. AAV is normally a single-strand DNA virus; however, if a construct is generated with a self-complementary 3′ end, a double-stranded virus can be created. This process allows for increased expression at the cost of reduced viral carrying capacity.[25] For small genes, this limitation is not problematic.

Spinal muscular atrophy is a neuromuscular disease that results from mutation of the survival of motor neuron 1 (SMN1) gene. The severity of the disease depends on expression levels of SMN, but in all cases, the disease results in muscle atrophy; in the most severe cases, it results in death. Because this is a single gene disease, it is amenable to gene replacement therapy like the inherited metabolic disorders. A phase 1 trial is under way in which peripheral IV injections of self-complementary virus 9 are used to deliver a normal copy of the SMN1 gene (Table 2).

Gene therapy has also been used to treat neurodegenerative diseases such as Alzheimer disease (AD), amyotrophic lateral sclerosis (ALS), and Parkinson disease (PD) (Table 2). These diseases, with the exception of rare forms of ALS, are generally believed to be polygenic/multifactorial. Gene therapy treatment strategies are not as intuitive as those for the monogenic diseases discussed earlier. Because these diseases involve neurodegeneration, most gene therapy trials have been aimed at delivering genes that encode growth/survival factors with the goal of curtailing neuron loss.

For AD, the most common cause of dementia,[26] 2 phase 1 trials have shown

that providing nerve growth factor (NGF) via injection of the nucleus basalis of Meynert with AAV carrying the NGF gene (CERE-110) or transduced fibroblasts producing NGF is safe and results in axonal sprouting and neuronal hypertrophy.[11] A randomized phase 2 study of CERE-110 is under way (Table 2). This therapy is based on the observation that the nucleus basalis, the center for cholinergic output, undergoes degeneration in AD and that acetylcholinesterase inhibitors provide therapeutic benefit for patients with AD.[27]

ALS, also known as Lou Gehrig disease, is a neurodegenerative disease that results in motor neuron loss with death from respiratory failure. Most cases are not caused by defects in single gene; however, in 2% of cases (familial ALS) mutations in the gene SOD1 are responsible for the disease.[28] In familial ALS, disease-causing SOD1 mutations are toxic gain-of-function mutations. Hence, a phase 1 trial has been initiated in which antisense oligonucleotides are injected intrathecally to knock down aberrant gene expression (Table 2). This trial has shown safety of this therapy. For nonfamilial ALS, 2 phase 1/2 trials have used IM injection of plasmids carrying the genes for growth factors (either vascular endothelial growth factor or hepatocyte growth factor) to enhance motor neuron survival (Table 2). Results are pending.

PD is a neurodegenerative disorder characterized by tremor, rigidity, bradykinesia, and postural instability. The hallmark of this disease is the degeneration of dopaminergic neurons in the substantia nigra pars compacta.[29] This disease has received the most attention in the gene therapy field. As for the neurodegenerative diseases discussed earlier, growth factor therapy has also been pursued as a treatment for PD (Table 2). One phase 1 trial[20] showed that injections of AAV carrying the Neurturin gene (a growth factor) into the putamen bilaterally were safe. A subsequent phase 2 trial did not show the treatment to be effective.[21] This group has an active phase 1/2 trial with injection of the same construct in the substantia nigra in addition to the putamen (Table 2). The growth factor therapy may be more effective if it is directly delivered to the area of cell loss. Another group is running a phase 1 trial to explore the safety of putaminal injections of AAV carrying the glial cell line–derived neurotrophic factor gene (Table 2). An alternative strategy to growth factor gene delivery that is being explored for the treatment of PD is the delivery of genes to enhance dopamine synthesis.[19,22] Two phase 1 studies have shown the safety of striatal injections of AAV carrying the AADC gene.[19,22] AADC converts levodopa to dopamine.[30] Hence, by expressing this enzyme in the striatum, the efficacy of levodopa treatment may be enhanced along with minimization of the often debilitating side effects that occur with high levodopa doses. Another phase 1 trial and a phase 1/2 trial are under way to investigate the safety and efficacy of this treatment (Table 2). A creative genetic-based neuromodulatory therapy for PD has been investigated in phase 1 and phase 2 trials.[17,18] This therapy involved the injection of AAV carrying the glutamate decarboxylase (GAD) gene into the subthalamic nucleus (STN). GAD converts glutamate, the major excitatory neurotransmitter of the brain, to γ-aminobutyric acid, the major inhibitory neurotransmitter of the brain.[31] The goal of this therapy was to increase inhibitory tone in the STN. This strategy was guided by the belief that the STN is hyperactive in PD and that STN inhibition is therapeutic for these patients.[32] The phase 1 trial showed safety and the phase 2 trial showed efficacy.[17,18] However, the treatment was abandoned presumably because of the increased efficacy of deep brain stimulation (DBS) for PD.[33]

GENE THERAPY APPROACHES TO TREAT ONCOLOGIC DISEASE

For the diseases discussed earlier, gene therapy is used to introduce genetic material to compensate for defective endogenous genes or introduce new genes to modify neural circuitry or enhance cell survival. For the treatment of tumors, gene therapy has been used to render tumor cells susceptible to a cell-killing drug (Table 3). The most common approach is to introduce the HSV thymidine kinase (TK) gene to tumor cells. This gene phosphorylates thymidine and is essential for DNA synthesis, and consequently, cell division. Drugs such as acyclovir, ganciclovir, and valacyclovir are nucleoside analogues that target viral forms of these kinases.[57] Hence, introducing the HSV form of this gene renders dividing cells susceptible to drugs such as ganciclovir.

Many phase 1 and phase 1/2 trials have shown the safety of TK gene therapy and ganciclovir treatment.[36-40,42-44,47-49] These trials used injection of fibroblasts producing retrovirus carrying the HSV-TK gene[36,38-40,48,49] or adenoviruses carrying the HSV-TK gene into tumors or more commonly into the resection cavity after a tumor resection.[37,42-44,47] One study did note a high number of adverse events (53%).[40] However, this study used not only resection cavity injections but also implantation of an Ommaya reservoir and subsequent injections into the reservoir. Many of the adverse events were related to the implantation and use of the Ommaya.[40]

Despite phase 2 trials showing efficacy of HSV-TK plus ganciclovir treatment,[41,45,46] a phase 3 trial failed to show survival benefit in the treatment group.[35] The phase 3 trial used injection of fibroblasts producing retroviruses carrying the HSV-TK gene. Hence, the failure of this study to show survival benefit was attributed to low efficiency of tumor cell transduction. This situation may be because the fibroblasts are unable to migrate and spread the retrovirus. The investigators posited that improved surgical delivery methods and improved vectors with greater spread could overcome this problem. After the failure of this phase 3 trial, subsequent trials have focused on use of adenoviral vectors.[41,46]

In addition to the HSV-TK plus nucleoside analogue strategy, a phase 2/3 trial is under way that uses injections of retrovirus carrying the yeast cytosine deaminase gene (Table 3). This enzyme converts 5-fluorocytosine, an antifungal drug, to fluorouracil, an antitumor drug. Hence, like the HSV-TK gene therapy, the cytosine deaminase gene therapy strategy is aimed at rendering tumor cells susceptible to tumor-killing drugs.

Different considerations must be made regarding vector selection and design for antitumor gene therapy versus gene therapy for the treatment of nononcologic diseases. Gene therapy for tumor treatment requires the intended genes to be delivered to

dividing cells, whereas gene therapy for other diseases often involves delivery of genes to neurons, which are nondividing cells. Hence, adenoviral vectors, which infect diving cells, are well suited for tumor applications. Furthermore, adenoviral vectors are immunogenic. Stimulation of the immune system is likely an added benefit of adenoviral vector use for tumor treatment, because immune system activation has been shown to have an antitumor effect.[58,59] In other applications, immune stimulation is not generally desired, so AAV, which is not immunogenic, is generally preferred. In addition to the possible immune stimulation of the viral vector itself, immune modulatory genes may also be delivered to enhance oncolytic therapy. A phase 1 trial has shown the safety of the interleukin 2 gene delivery alongside delivery of the TK gene using a bicistronic vector.[49] Another phase 1 trial combining TK gene delivery with delivery of the Flt3L gene, a gene that stimulates dendritic cells,[60] is under way (Table 3). A phase 1 trial of immune gene therapy without TK gene therapy has also been completed.[34] This study showed the safety of injections of adenovirus carrying the interferon β gene to gliomas. These investigators also observed increased tumor necrosis, an indication that immune modulation alone provides good antitumor treatment.[34]

Expression of the tumor suppressor gene p53 has been another strategy used to treat gliomas using gene therapy.[50] Two phase 1 trials have shown safety; however, efficacy remains to be tested (Table 3).

Direct virus-mediated tumor cell killing has been trialed using several vectors. In this strategy, oncolytic viruses are used to target and destroy tumor cells. Phase 1 trials have shown the safety of strains of HSV, adenovirus, reovirus, and Newcastle disease virus for this purpose.[51-56] Some of these trials have even shown possible efficacy.[51,54-56]

SURGICAL CONSIDERATIONS

Most of the trials discussed use intracerebral injections of gene delivery vectors, making gene therapy a neurosurgical treatment. Although some trials have used IV, IM, or intrathecal injections, the applications for these routes of delivery are limited. Systemic side effects and off-target gene expression make these delivery methods less favorable. Furthermore, many neurologic diseases require targeting of gene therapy to specific areas of the brain to modulate only the particular areas affected by a disease. Convection-enhanced delivery methods have been used for intracerebral administration of gene therapy vectors.[61] These techniques use positive pressure to infuse the vector through an intracerebral catheter. These methods of delivery allow for further spread of the therapeutic vector. Many early gene therapy trials were hampered by lack of vector spread. To guarantee that the vector is delivered to the entire extent of the intended target, a contrast agent such as gadoteridol may be mixed with the vector and injections can be given with real-time magnetic resonance imaging (MRI) visualization of vector spread.[62] With the advent of intraoperative MRI and frameless stereotactic delivery methods, real-time visualization of vector spread will become clinically feasible. Optimization of vector delivery is an important neurosurgical consideration.

Accurate vector delivery relies on stereotactic targeting. Stereotactic brain surgery was developed in the mid—twentieth century by Spiegel, Wycis, and Leksell.[63,64] The system developed by Leksell is still in use and remains a reliable and accurate means of stereotaxy. Other systems in use include the Brown-Roberts-Wells, Cosman-Roberts-Wells, and Kelly-Goerss COMPASS systems.[65] All these systems are subject to targeting errors because of mechanical inaccuracies, target calculation errors, and imaging inaccuracies, which may be significant.[65] These sources of error should be kept in mind to mitigate their effects.

Technological advancements such as optical tracking have enabled targeting without the use of a conventional stereotactic frame. These systems have been used for many applications including targeting of DBS electrodes. Several groups have found similar clinical outcomes with frame-based and frameless systems; however, accuracy typically remains higher with frame-based systems.[66,67] To improve the accuracy of frameless systems, some have used bone fiducial markers and have shown no significant difference in accuracy between frameless and frame-based systems.[68]

The ClearPoint (MRI Interventions, Irvine, California, USA) system has been developed as a tool for accurate stereotactic targeting using intraoperative MRI and an aiming platform. Clinical outcomes and lead placement accuracy for DBS surgery are similar with ClearPoint and frame-based systems.[69] The ClearPoint system has also been effectively used for laser ablation and drug/vector delivery.[70,71] A cannula for vector delivery is available for use with this system to enable accurate vector infusion.[71] Future advances may include optimization of delivery cannulas and/or delivery systems that could allow enhanced vector spread and reduce surgical time.

The development of genetic therapies to treat neurologic disease and the initiation of clinical trials for these therapies involves a multidisciplinary approach. Basic scientists with expertise in viral vectors and other gene therapy vectors are critical to the design of these treatments. Neurologists are essential for diagnosis, referral, and long-term patient management. The neurosurgeon plays a critical role in the delivery of the vector and should strive to enhance the safety and efficiency of vector delivery in the operating room. Clinical trials should be formulated with a multidisciplinary team so that all aspects of the trial from recruitment to vector delivery to follow-up are carefully planned and executed.

RISKS AND LIMITATIONS

Although many recent trials have shown safety of gene therapy (Table 1), there have been severe adverse immunologic reactions and even death after administration of viral vectors.[72,73] One of the first clinical gene therapy trials aimed to treat ornithine transcarbamylase deficiency by the administration of an adenoviral vector carrying the OTC gene in the late 1990s.[74] Mr. Jesse Gelsinger (the second patient to receive the vector at the highest dose in the dose escalation phase 1 study) died as a result of an exaggerated innate immune response and resultant multisystem organ failure.[72] The trial was promptly terminated

after Mr. Gelsinger's death. This event led to a public outcry and internal/external investigations regarding trial conduct. Mr. Gelsinger's death prompted institutional review board and clinical trial reform to protect future patients. Scientifically, more research was carried out on the immune response to vector administration and novel vectors such as AAV were discovered. Gene therapy has matured a great deal since this early trial; however, the risks of gene therapy, including severe adverse immune reactions, must not be overlooked or forgotten.

FUTURE DIRECTIONS

The applications of gene therapy discussed earlier include only those that have been trialed in humans. Nearly any gene can be expressed or knocked down using current molecular tools. Hence, the potential diseases that could be treated using gene therapy are numerous. Further refinements of gene therapy will be multifaceted, including improved vector design, improved vector delivery, and the use of novel neuromodulatory/neuroprotective genes.

There are many avenues for vector design improvements. These avenues may include further development of cell-type specific vectors. For example, most clinically used vectors have used nonspecific gene promoters such as the CMV (cytomegalovirus) or CAG (chicken β-actin) promoters (Table 2). Future studies could use more specific promotors to target gene expression to, for example, solely excitatory neurons. In addition to promoter development, discovery and use of novel vector serotypes may allow for more targeted gene delivery. No currently available vector is perfect. For example, although AAV is less immunogenic, it has a small carrying capacity. Discovery of novel vectors could eliminate these limitations.

Vector delivery is also an important area for improvement. There should be a move toward monitoring vector delivery spread with real-time imaging so that trial failures are not the result of incomplete gene delivery. In addition, there are some reports that certain additives may improve vector spread.[75] Refinements in the physical delivery of vectors such as the use of convection-enhanced delivery or novel catheter designs to prevent backflow may improve spread and reduce tissue damage.[61] Vector delivery is particularly a concern for lesions that require a large area of vector spread such as large tumors or diseases that require brainwide expression of a gene. AAV is a very small virus that can spread through the parenchyma relatively easily; however, high volumes and/or multiple injection sites are often required to transduce large areas. An alternative solution is to use intraventricular injections with the goal of transducing the ependyma. This strategy would enable a protein to be produced and then distributed throughout the cerebrospinal fluid to treat areas of the brain distant from the transduction. A recent trial[76] has shown that intraventricular infusion of cerliponase alfa is an effective treatment for CLN2 disease. This treatment required placement of an intraventricular reservoir and intraventricular cerliponase alfa delivery every 2 weeks. If gene therapy were used, the ependyma could produce cerliponase alfa with only a single delivery of vector. A gene therapy approach would also importantly obviate an intraventricular reservoir. For nononcologic gene therapy, most trials have focused on replacement of mutated genes or delivery of growth factor/cell survival genes (Table 2). In the basic science realm, many molecular tools have been developed for neuromodulation.[77] Optogenetics uses light-gated channels such as Channelrhodopsin (excitation) or Halorhodopsin (inhibition) to modulate neural activity. Although implementing this treatment in humans would require implantation of a light generator, many therapies including DBS require implantation of devices. The advantages of these neuromodulatory techniques include cell-type specific targeting and modulation of neurons without modulation of fibers of passage. Electric stimulation may excite not only neurons near the electrode but also axons in that area. By expressing light-gated channels in a population of neurons, light provided via a fiber-optic catheter only stimulates neurons expressing the channel. Hence, off-target stimulation can be avoided. A limitation of this technique is limited light spread through brain parenchyma. Novel probes are being developed to overcome this limitation.

NOVEL GENOME MODIFICATION TECHNIQUES

We have described numerous strategies used to treat neurologic disease with gene therapy. The treatments typically involve transduction of a gene that produces a therapeutic enzyme or growth factor. For diseases in which an aberrant gene product must be eliminated, knockdown of gene expression can be achieved at the RNA level with antisense oligonucleotides, as is being performed in an ALS trial (Table 1).

When complete knockout of a gene is desired, genome editing must be performed. An exciting novel and extremely versatile tool, the CRISPR (clustered, regularly interspaced, short palindromic repeat)/Cas9 system, has been developed to target specific DNA sequences for knockout, modification, or activation.[78] Before the discovery of the CRISPR/Cas9 system, zinc finger nucleases and transcription activator–like effector nucleases had to be generated to target specific DNA sequences. The disadvantage of the previous systems was the difficulty in designing them to target particular DNA sequences because of reliance on protein–DNA interactions. The CRISPR/Cas9 system, on the other hand, uses complementary guide RNAs to target specific sequences. Hence, rational design of sequence targeting is more intuitive with the CRISPR/Cas9 system.

The CRISPR/Cas9 system has been coopted from bacterial immune systems. In bacteria, CRISPR sequences target foreign genetic material for degradation by recruitment of the Cas9 endonuclease. When engineered for mammalian use, targeting Cas9 to a gene results in a double-stranded break, which allows for either DNA sequence modification or knockout. This system can therefore be used to correct endogenous mutations or knock out deleterious genes. However, correction of a mutation is difficult because editing DNA after an endonuclease cut is a low probability event. In other studies, Cas9 has also been modified to remove its endonuclease capability and instead carry transcriptional

activators or repressors to modify gene expression levels.

Special considerations must be made for CRISPR/Cas9 use for gene therapy. If a gene editing approach is desired, the problem of low efficiency must be addressed. If gene knockout is pursued, transient expression (e.g., with plasmid transfection techniques) of the CRISPR/Cas9 system should be considered. Furthermore, CRISPR/Cas9 binding to off-target sites can result in serious deleterious consequences as a result of unintended gene editing. High-fidelity versions of Cas9 have been developed to mitigate this problem.[79] Transient expression would also help mitigate the problem of off-target effects. CRISPR/Cas9 technology has seen rapid adoption and development, and clinical trials in humans are being developed. In oncology, an ex vivo human cell editing strategy has been developed and is in a phase 1 trial for treatment of multiple myeloma (NCT03399448). Although further refinement is needed for neurologic applications, this technology may soon see application in human gene therapy for neurologic disease as well.

CONCLUSIONS

Gene therapy is a burgeoning field with many applications for neurosurgical and neurologic diseases. We have reviewed completed human gene therapy trials and others that are under way. The clinical trials reviewed are nearly all phase 1 or 2 trials. Future phase 3 trials have the promise to show efficacy of these therapies. Many disorders are amenable to gene therapy; further development and refinement of these techniques will allow for even more diseases to be treated. Neurosurgeons will play an essential role in designing, developing, and providing these therapies.

REFERENCES

1. Finkel RS, Mercuri E, Darras BT, Connolly AM, Kuntz NL, Kirschner J, et al. Nusinersen versus sham control in infantile-onset spinal muscular atrophy. *N Engl J Med*. 2017;377:1723-1732.

2. Maude SL, Laetsch TW, Buechner J, Rives S, Boyer M, Bittencourt H, et al. Tisagenlecleucel in children and young adults with B-cell lymphoblastic leukemia. *N Engl J Med*. 2018;378:439-448.

3. Mercuri E, Darras BT, Chiriboga CA, Day JW, Campbell C, Connolly AM, et al. Nusinersen versus sham control in later-onset spinal muscular atrophy. *N Engl J Med*. 2018;378:625-635.

4. Russell S, Bennett J, Wellman JA, Chung DC, Yu Z-F, Tillman A, et al. Efficacy and safety of voretigene neparvovec (AAV2-hRPE65v2) in patients with RPE65-mediated inherited retinal dystrophy: a randomised, controlled, open-label, phase 3 trial. *Lancet*. 2017;390:849-860.

5. Warrington KH, Herzog RW. Treatment of human disease by adeno-associated viral gene transfer. *Hum Genet*. 2006;119:571-603.

6. Srivastava A. In vivo tissue-tropism of adeno-associated viral vectors. *Curr Opin Virol*. 2016;21:75-80.

7. Hirsch ML, Wolf SJ, Samulski RJ. Delivering transgenic DNA exceeding the carrying capacity of AAV vectors. *Methods Mol Biol Clifton NJ*. 2016;1382:21-39.

8. Latchman DS. Gene delivery and gene therapy with herpes simplex virus-based vectors. *Gene*. 2001;264:1-9.

9. Sengupta R, Mukherjee C, Sarkar N, Sun Z, Lesnik J, Huang J, et al. An optimized protocol for packaging pseudotyped integrase defective lentivirus. *Biol Proced Online*. 2016;18:14.

10. Saraiva J, Nobre RJ, Pereira de Almeida L. Gene therapy for the CNS using AAVs: the impact of systemic delivery by AAV9. *J Control Release*. 2016;241:94-109.

11. Tuszynski MH, Yang JH, Barba D, U H-S, Bakay RAE, Pay MM, et al. Nerve growth factor gene therapy: activation of neuronal responses in Alzheimer disease. *JAMA Neurol*. 2015;72:1139-1147.

12. Leone P, Shera D, McPhee SWJ, Francis JS, Kolodny EH, Bilaniuk LT, et al. Long-term follow-up after gene therapy for Canavan disease. *Sci Transl Med*. 2012;4:165ra163.

13. Leone P, Janson CG, Bilianuk L, Wang Z, Sorgi F, Huang L, et al. Aspartoacylase gene transfer to the mammalian central nervous system with therapeutic implications for Canavan disease. *Ann Neurol*. 2000;48:27-38.

14. Fink DJ, Wechuck J, Mata M, Glorioso JC, Goss J, Krisky D, et al. Gene therapy for pain: results of a phase I clinical trial. *Ann Neurol*. 2011;70:207-212.

15. Worgall S, Sondhi D, Hackett NR, Kosofsky B, Kekatpure MV, Neyzi N, et al. Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. *Hum Gene Ther*. 2008;19:463-474.

16. Tardieu M, Zérah M, Husson B, de Bournonville S, Deiva K, Adamsbaum C, et al. Intracerebral administration of adeno-associated viral vector serotype rh.10 carrying human SGSH and SUMF1 cDNAs in children with mucopolysaccharidosis type IIIA disease: results of a phase I/II trial. *Hum Gene Ther*. 2014;25:506-516.

17. Kaplitt MG, Feigin A, Tang C, Fitzsimons HL, Mattis P, Lawlor PA, et al. Safety and tolerability of gene therapy with an adeno-associated virus (AAV) borne GAD gene for Parkinson's disease: an open label, phase I trial. *Lancet*. 2007;369:2097-2105.

18. LeWitt PA, Rezai AR, Leehey MA, Ojemann SG, Flaherty AW, Eskandar EN, et al. AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial. *Lancet Neurol*. 2011;10:309-319.

19. Christine CW, Starr PA, Larson PS, Eberling JL, Jagust WJ, Hawkins RA, et al. Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. *Neurology*. 2009;73:1662-1669.

20. Marks WJ Jr, Ostrem JL, Verhagen L, Starr PA, Larson PS, Bakay RA, et al. Safety and tolerability of intraputaminal delivery of CERE-120 (adeno-associated virus serotype 2—neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. *Lancet Neurol*. 2008;7:400-408.

21. Marks WJ Jr, Bartus RT, Siffert J, Davis CS, Lozano A, Boulis N, et al. Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol*. 2010;9:1164-1172.

22. Muramatsu S, Fujimoto K, Kato S, Mizukami H, Asari S, Ikeguchi K, et al. A phase I study of aromatic L-amino acid decarboxylase gene therapy for Parkinson's disease. *Mol Ther*. 2010;18:1731-1735.

23. Katz ML, Tecedor L, Chen Y, Williamson BG, Lysenko E, Wininger FA, et al. AAV gene transfer delays disease onset in a TPP1-deficient canine model of the late infantile form of Batten disease. *Sci Transl Med*. 2015;7:313ra180.

24. Jackson RJ. The current status of vertebrate cellular mRNA IRESs. *Cold Spring Harb Perspect Biol*. 2013;5.

25. McCarty DM. Self-complementary AAV vectors; advances and applications. *Mol Ther J Am Soc Gene Ther*. 2008;16:1648-1656.

26. Canter RG, Penney J, Tsai L-H. The road to restoring neural circuits for the treatment of Alzheimer's disease. *Nature*. 2016;539:187-196.

27. Mesulam M-M. Cholinergic circuitry of the human nucleus basalis and its fate in Alzheimer's disease. *J Comp Neurol*. 2013;521:4124-4144.

28. Al-Chalabi A, van den Berg LH, Veldink J. Gene discovery in amyotrophic lateral sclerosis: implications for clinical management. *Nat Rev Neurol*. 2017;13:96-104.

29. Olanow CW. The scientific basis for the current treatment of Parkinson's disease. *Annu Rev Med*. 2004;55:41-60.

30. Nagatsua T, Sawadab M. L-dopa therapy for Parkinson's disease: past, present, and future. *Parkinsonism Relat Disord*. 2009;15(suppl 1):S3-S8.

31. Erlander MG, Tillakaratne NJK, Feldblum S, Patel N, Tobin AJ. Two genes encode distinct glutamate decarboxylases. *Neuron*. 1991;7:91-100.

32. Obeso JA, Rodriguez-Oroz MC, Rodriguez M, Macias R, Alvarez L, Guridi J, et al.

Pathophysiologic basis of surgery for Parkinson's disease. *Neurology*. 2000;55(12 suppl 6):S7-S12.

33. Deuschl G, Schade-Brittinger C, Krack P, Volkmann J, Schäfer H, Bötzel K, et al. A randomized trial of deep-brain stimulation for Parkinson's disease. *N Engl J Med*. 2006;355:896-908.

34. Chiocca EA, Smith KM, McKinney B, Palmer CA, Rosenfeld S, Lillehei K, et al. A phase I trial of Ad.hIFN-beta gene therapy for glioma. *Mol Ther*. 2008;16:618-626.

35. Rainov NG. A phase III clinical evaluation of herpes simplex virus type 1 thymidine kinase and ganciclovir gene therapy as an adjuvant to surgical resection and radiation in adults with previously untreated glioblastoma multiforme. *Hum Gene Ther*. 2000;11:2389-2401.

36. Izquierdo M, Martín V, de Felipe P, Izquierdo JM, Pérez-Higueras A, Cortés ML, et al. Human malignant brain tumor response to herpes simplex thymidine kinase (HSVtk)/ganciclovir gene therapy. *Gene Ther*. 1996;3:491-495.

37. Chiocca EA, Aguilar LK, Bell SD, Kaur B, Hardcastle J, Cavaliere R, et al. Phase IB study of gene-mediated cytotoxic immunotherapy adjuvant to up-front surgery and intensive timing radiation for malignant glioma. *J Clin Oncol*. 2011;29:3611-3619.

38. Shand N, Weber F, Mariani L, Bernstein M, Gianella-Borradori A, Long Z, et al. A phase 1-2 clinical trial of gene therapy for recurrent glioblastoma multiforme by tumor transduction with the herpes simplex thymidine kinase gene followed by ganciclovir. GLI328 European-Canadian Study Group. *Hum Gene Ther*. 1999;10:2325-2335.

39. Klatzmann D, Valéry CA, Bensimon G, Marro B, Boyer O, Mokhtari K, et al. A phase I/II study of herpes simplex virus type 1 thymidine kinase "suicide" gene therapy for recurrent glioblastoma. Study Group on Gene Therapy for Glioblastoma. *Hum Gene Ther*. 1998;9:2595-2604.

40. Prados MD, McDermott M, Chang SM, Wilson CB, Fick J, Culver KW, et al. Treatment of progressive or recurrent glioblastoma multiforme in adults with herpes simplex virus thymidine kinase gene vector-producer cells followed by intravenous ganciclovir administration: a phase I/II multi-institutional trial. *J Neurooncol*. 2003;65:269-278.

41. Immonen A, Vapalahti M, Tyynelä K, Hurskainen H, Sandmair A, Vanninen R, et al. AdvHSV-tk gene therapy with intravenous ganciclovir improves survival in human malignant glioma: a randomised, controlled study. *Mol Ther*. 2004;10:967-972.

42. Trask TW, Trask RP, Aguilar-Cordova E, Shine HD, Wyde PR, Goodman JC, et al. Phase I study of adenoviral delivery of the HSV-tk gene and ganciclovir administration in patients with current malignant brain tumors. *Mol Ther*. 2000;1:195-203.

43. Germano IM, Fable J, Gultekin SH, Silvers A. Adenovirus/herpes simplex-thymidine kinase/ganciclovir complex: preliminary results of a phase I trial in patients with recurrent malignant gliomas. *J Neurooncol*. 2003;65:279-289.

44. Smitt PS, Driesse M, Wolbers J, Kros M, Avezaat C. Treatment of relapsed malignant glioma with an adenoviral vector containing the herpes simplex thymidine kinase gene followed by ganciclovir. *Mol Ther J Am Soc Gene Ther*. 2003;7:851-858.

45. Sandmair AM, Loimas S, Puranen P, Immonen A, Kossila M, Puranen M, et al. Thymidine kinase gene therapy for human malignant glioma, using replication-deficient retroviruses or adenoviruses. *Hum Gene Ther*. 2000;11:2197-2205.

46. Wheeler LA, Manzanera AG, Bell SD, Cavaliere R, McGregor JM, Grecula JC, et al. Phase II multicenter study of gene-mediated cytotoxic immunotherapy as adjuvant to surgical resection for newly diagnosed malignant glioma. *Neuro-Oncol*. 2016;18:1137-1145.

47. Alavi JB, Eck SL. Gene therapy for high grade gliomas. *Expert Opin Biol Ther*. 2001;1:239-252.

48. Ram Z, Culver KW, Oshiro EM, Viola JJ, DeVroom HL, Otto E, et al. Therapy of malignant brain tumors by intratumoral implantation of retroviral vector-producing cells. *Nat Med*. 1997;3:1354-1361.

49. Palù G, Cavaggioni A, Calvi P, Franchin E, Pizzato M, Boschetto R, et al. Gene therapy of glioblastoma multiforme via combined expression of suicide and cytokine genes: a pilot study in humans. *Gene Ther*. 1999;6:330-337.

50. Lang FF, Bruner JM, Fuller GN, Aldape K, Prados MD, Chang S, et al. Phase I trial of adenovirus-mediated p53 gene therapy for recurrent glioma: biological and clinical results. *J Clin Oncol*. 2003;21:2508-2518.

51. Papanastassiou V, Rampling R, Fraser M, Petty R, Hadley D, Nicoll J, et al. The potential for efficacy of the modified (ICP 34.5(-)) herpes simplex virus HSV1716 following intratumoural injection into human malignant glioma: a proof of principle study. *Gene Ther*. 2002;9:398-406.

52. Chiocca EA, Abbed KM, Tatter S, Louis DN, Hochberg FH, Barker F, et al. A phase I open-label, dose-escalation, multi-institutional trial of injection with an E1B-Attenuated adenovirus, ONYX-015, into the peritumoral region of recurrent malignant gliomas, in the adjuvant setting. *Mol Ther*. 2004;10:958-966.

53. Forsyth P, Roldán G, George D, Wallace C, Palmer CA, Morris D, et al. A phase I trial of intratumoral administration of reovirus in patients with histologically confirmed recurrent malignant gliomas. *Mol Ther*. 2008;16:627-632.

54. Markert JM, Medlock MD, Rabkin SD, Gillespie GY, Todo T, Hunter WD, et al. Conditionally replicating herpes simplex virus mutant, G207 for the treatment of malignant glioma: results of a phase I trial. *Gene Ther*. 2000;7:867-874.

55. Rampling R, Cruickshank G, Papanastassiou V, Nicoll J, Hadley D, Brennan D, et al. Toxicity evaluation of replication-competent herpes simplex virus (ICP 34.5 null mutant 1716) in patients with recurrent malignant glioma. *Gene Ther*. 2000;7:859-866.

56. Freeman AI, Zakay-Rones Z, Gomori JM, Linetsky E, Rasooly L, Greenbaum E, et al. Phase I/II trial of intravenous NDV-HUJ oncolytic virus in recurrent glioblastoma multiforme. *Mol Ther*. 2006;13:221-228.

57. Black ME, Newcomb TG, Wilson HM, Loeb LA. Creation of drug-specific herpes simplex virus type 1 thymidine kinase mutants for gene therapy. *Proc Natl Acad Sci U S A*. 1996;93:3525-3529.

58. Freeman SM, Ramesh R, Shastri M, Munshi A, Jensen AK, Marrogi AJ. The role of cytokines in mediating the bystander effect using HSV-TK xenogeneic cells. *Cancer Lett*. 1995;92:167-174.

59. Gagandeep S, Brew R, Green B, Christmas SE, Klatzmann D, Poston GJ, et al. Prodrug-activated gene therapy: involvement of an immunological component in the "bystander effect". *Cancer Gene Ther*. 1996;3:83-88.

60. Watowich SS, Liu Y-J. Mechanisms regulating dendritic cell specification and development. *Immunol Rev*. 2010;238:76-92.

61. Lonser RR, Sarntinoranont M, Morrison PF, Oldfield EH. Convection-enhanced delivery to the central nervous system. *J Neurosurg*. 2015;122:697-706.

62. Salegio EA, Bringas J, Bankiewicz KS. MRI-guided delivery of viral vectors. *Methods Mol Biol*. 2016;1382:217-230.

63. Rahman M, Murad GJA, Mocco J. Early history of the stereotactic apparatus in neurosurgery. *Neurosurg Focus*. 2009;27:E12.

64. Spiegel EA, Wycis HT, Marks M, Lee AJ. Stereotaxic apparatus for operations on the human brain. *Science*. 1947;106:349-350.

65. Maciunas RJ, Galloway RL, Latimer JW. The application accuracy of stereotactic frames. *Neurosurgery*. 1994;35:682-694 [discussion 694-695].

66. Tai C-H, Wu R-M, Lin C-H, Pan M-K, Chen Y-F, Liu H-M, et al. Deep brain stimulation therapy for Parkinson's disease using frameless stereotaxy: comparison with frame-based surgery. *Eur J Neurol*. 2010;17:1377-1385.

67. Bjartmarz H, Rehncrona S. Comparison of accuracy and precision between frame-based and frameless stereotactic navigation for deep brain stimulation electrode implantation. *Stereotact Funct Neurosurg*. 2007;85:235-242.

68. Holloway KL, Gaede SE, Starr PA, Rosenow JM, Ramakrishnan V, Henderson JM. Frameless stereotaxy using bone fiducial markers for deep brain stimulation. *J Neurosurg*. 2005;103:404-413.

69. Ostrem JL, Ziman N, Galifianakis NB, Starr PA, Luciano MS, Katz M, et al. Clinical outcomes using ClearPoint interventional MRI for deep brain stimulation lead placement in Parkinson's disease. *J Neurosurg*. 2015;124:908-916.

70. Ho AL, Sussman ES, Pendharkar AV, Le S, Mantovani A, Keebaugh AC, et al. Improved

operative efficiency using a real-time MRI-guided stereotactic platform for laser amygdalohippocampotomy. *J Neurosurg*. 2018;128:1165-1172.

71. Chittiboina P, Heiss JD, Lonser RR. Accuracy of direct magnetic resonance imaging-guided placement of drug infusion cannulae. *J Neurosurg*. 2015; 122:1173-1179.

72. Wilson JM. Lessons learned from the gene therapy trial for ornithine transcarbamylase deficiency. *Mol Genet Metab*. 2009;96:151-157.

73. Wilson JM. Genetic diseases, immunology, viruses, and gene therapy. *Hum Gene Ther*. 2014;25: 257-261.

74. Raper SE, Yudkoff M, Chirmule N, Gao G-P, Nunes F, Haskal ZJ, et al. A pilot study of in vivo liver-directed gene transfer with an adenoviral vector in partial ornithine transcarbamylase deficiency. *Hum Gene Ther*. 2002;13:163-175.

75. Mastakov MY, Baer K, Xu R, Fitzsimons H, During MJ. Combined injection of rAAV with mannitol enhances gene expression in the rat brain. *Mol Ther*. 2001;3:225-232.

76. Schulz A, Ajayi T, Specchio N, de Los Reyes E, Gissen P, Ballon D, et al. Study of intraventricular cerliponase alfa for CLN2 disease. *N Engl J Med*. 2018;378:1898-1907.

77. Rajasethupathy P, Ferenczi E, Deisseroth K. Targeting neural circuits. *Cell*. 2016;165:524-534.

78. Sander JD, Joung JK. CRISPR-Cas systems for editing, regulating and targeting genomes. *Nat Biotechnol*. 2014;32:347-355.

79. Vakulskas CA, Dever DP, Rettig GR, Turk R, Jacobi AM, Collingwood MA, et al. A high-fidelity Cas9 mutant delivered as a ribonucleoprotein complex enables efficient gene editing in human hematopoietic stem and progenitor cells. *Nat Med*. 2018;24:1216-1224.

Conflict of interest statement: P.G.A. received licensing fees from Spark Therapeutics and consulting fees from Teva Pharmaceuticals and receives funding from the U.S. National Institutes of Health/National Institute of Neurological Disorders and Stroke (UH3-NS094355-01).

Received 28 June 2018; accepted 12 September 2018

Citation: World Neurosurg. (2019) 121:261-273.
https://doi.org/10.1016/j.wneu.2018.09.097

Journal homepage: ***www.journals.elsevier.com/world-neurosurgery***

Available online: ***www.sciencedirect.com***