

Adeno-Associated Virus Vectors and Neurological Gene Therapy

David S. Ojala, Dominic P. Amara and David V. Schaffer

Neuroscientist published online 20 February 2014

DOI: 10.1177/1073858414521870

The online version of this article can be found at:

<http://nro.sagepub.com/content/early/2014/02/19/1073858414521870>

Published by:



<http://www.sagepublications.com>

Additional services and information for *The Neuroscientist* can be found at:

Email Alerts: <http://nro.sagepub.com/cgi/alerts>

Subscriptions: <http://nro.sagepub.com/subscriptions>

Reprints: <http://www.sagepub.com/journalsReprints.nav>

Permissions: <http://www.sagepub.com/journalsPermissions.nav>

>> [OnlineFirst Version of Record](#) - Feb 20, 2014

[What is This?](#)

Adeno-Associated Virus Vectors and Neurological Gene Therapy

The Neuroscientist
1–15
© The Author(s) 2014
Reprints and permissions:
sagepub.com/journalsPermissions.nav
DOI: 10.1177/1073858414521870
nro.sagepub.com



David S. Ojala¹, Dominic P. Amara², and David V. Schaffer^{1,3,4}

Abstract

Gene therapy has strong potential for treating a variety of genetic disorders, as demonstrated in recent clinical trials. There is unfortunately no scarcity of disease targets, and the grand challenge in this field has instead been the development of safe and efficient gene delivery platforms. To date, approximately two thirds of the 1800 gene therapy clinical trials completed worldwide have used viral vectors. Among these, adeno-associated virus (AAV) has emerged as particularly promising because of its impressive safety profile and efficiency in transducing a wide range of cell types. Gene delivery to the CNS involves both considerable promise and unique challenges, and better AAV vectors are thus needed to translate CNS gene therapy approaches to the clinic. This review discusses strategies for vector design, potential routes of administration, immune responses, and clinical applications of AAV in the CNS.

Keywords

gene therapy, directed evolution, adeno-associated virus (AAV), central nervous system, viral vectors

Introduction

Adeno-Associated Virus Biology

Adeno-associated viruses (AAVs) are non-enveloped, single-stranded DNA viruses that replicate only in the presence of a helper virus, primarily adenovirus (Schaffer and others 2008). The AAV viral genome is 4.7 kilobases and contains two inverted terminal repeats that flank the viral genes *rep* and *cap* (Schaffer and others 2008). Multiple proteins are produced from each viral gene through the use of alternative splicing and start codons (Fig. 1). The *rep* open reading frame (ORF) encodes four proteins necessary for AAV replication, site-specific integration, transcriptional regulation of viral promoters, and viral assembly. The *cap* ORF serves as a template for the production of three structural proteins, VP1-3, that assemble to form a 60-mer viral capsid approximately 25 nm in diameter (Fig. 2) (Schaffer and others 2008). Finally, an alternative ORF nested in *cap* encodes assembly-activating protein (AAP), which interacts with the capsid proteins VP1-3 and is necessary for capsid assembly (Sonntag and others 2010).

Vectors based on AAV are particularly promising gene delivery vehicles in large part because they exhibit low immunogenicity, can mediate long-term gene expression in both dividing and non-dividing cells, and have a low risk of insertional mutagenesis (Kaeppl and others 2013). To generate a recombinant AAV for gene delivery, the viral genome is removed and replaced with the

therapeutic transgene and a promoter. The viral inverted terminal repeats are the only *cis* elements needed to ensure packaging of the genetic payload into particles, and *rep* and *cap* can be supplied separately during virus production (Kwon and Schaffer 2008). Infection with recombinant AAV vectors results in primarily episomal transgene expression that is persistent in non-dividing cells for at least 10 years (Mingozzi and High 2013). AAV has been used in an increasing number of clinical trials, including recent successes for hemophilia B and the retinal degenerative disorder Leber's congenital amaurosis type 2 (Bainbridge and others 2008; Cideciyan and others 2009; Nathwani and others 2011; Testa and others 2013), as well as efforts that led to the first approval of a gene therapy product (for lipoprotein lipase deficiency) in Western nations in late 2012 (Miller 2012).

¹Department of Chemical and Biomolecular Engineering, University of California, Berkeley, CA, USA

²Department of Molecular and Cell Biology, University of California, Berkeley, CA, USA

³Department of Bioengineering, University of California, Berkeley, CA, USA

⁴The Helen Wills Neuroscience Institute, University of California, Berkeley, CA, USA

Corresponding Author:

David V. Schaffer, Department of Chemical and Biomolecular Engineering, University of California, 274 Stanley Hall, Berkeley, CA, 94720-1462, USA.
Email: schaffer@berkeley.edu

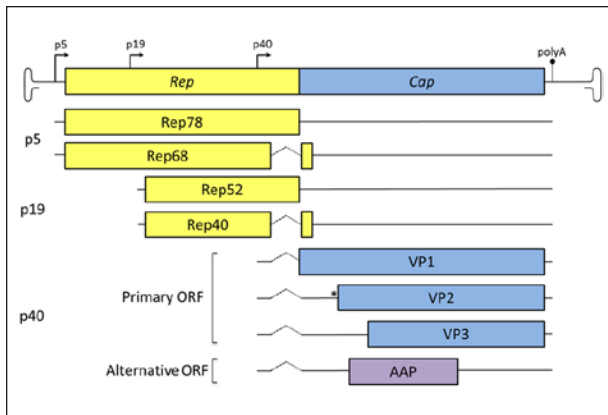


Figure 1. Key features of the AAV genome. Through alternative splicing, *rep* encodes four proteins—Rep78, Rep68, Rep52, and Rep40—that are involved in viral genome replication. The *cap* open reading frame (ORF) encodes three capsid proteins, VP1-3, that assemble to form a 60-mer viral capsid. There are two splice forms of the *cap* mRNA, where the longer form encodes VP1. In the shorter splice form the start codon for VP1 is removed, and translation initiates either from the VP3 start codon or an alternative ACG* start codon for VP2. The protein AAP, which assists in viral capsid assembly, is encoded by an alternative open reading frame with a nonconventional CUG start codon. Gene expression is driven by the p5, p19, or p40 promoter as indicated. AAV = adeno-associated virus; VP1 = viral capsid protein 1; VP2 = viral capsid protein 2; VP3 = viral capsid protein 3; AAP = assembly-activating protein.

Targeting the CNS

Because recombinant AAV vectors are well suited for gene therapy in the central nervous system, where most cells are post-mitotic and many chronic neurological diseases necessitate long-term transgene expression, AAV has entered into numerous CNS clinical studies (Table 1) (Asokan and others 2012). These trials have highlighted clinical challenges to successful gene therapy in the CNS. Biological transport barriers limit viral access to the brain parenchyma. For example, the blood-brain barrier (BBB) prevents global transduction of the CNS following intravascular vector administration, and clinical trials to date have thus employed intracranial injections that result primarily in localized transduction due to limited vector dispersion in the brain parenchyma. In addition, the ability to target specific cell types and regions in the CNS that are implicated in disease is important for safe and effective therapies for several indications; however, it is difficult to control AAV tropism and transgene expression. Finally, AAV vectors have an excellent safety record, though the implementation of new routes of administration and serotypes could increase the risk of an immune response to the vector and/or transgene. These challenges motivate the careful selection of

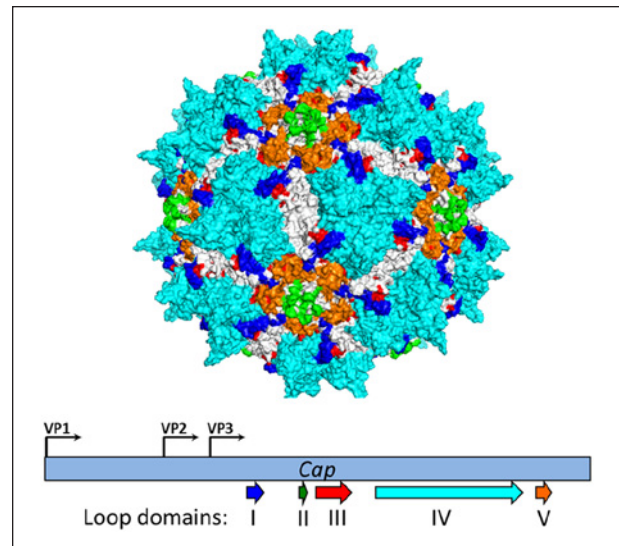


Figure 2. Adeno-associated virus (AAV) capsid structure. The locations of interstrand loop domains are indicated by the colored arrows on the *cap* gene and mapped to the three-dimensional AAV capsid structure, and these loop domains are highly diversified regions of *cap* that play a role in many virus–host interactions. The right-angle arrows indicate the translation start sites for viral proteins 1-3. This image was generated in PyMOL (The PyMOL Molecular Graphics System, Version 1.3r1, 2010, Schrödinger, LLC, Cambridge, MA) based on the crystal structure coordinates of AAV2 (Protein data bank accession number 1LP3).

natural AAV serotypes—and engineering of novel AAV variants—to yield transduction profiles that are optimally suited to specific therapeutic needs.

Adeno-Associated Virus Vector Design

Natural Serotypes in the CNS

There are 11 naturally occurring AAV serotypes and more than 100 variants of AAV (Wu and others 2006), which were isolated from human and non-human primate tissue, and which mediate a range of different cellular transduction and vector spread profiles in the central nervous system following direct injection into brain parenchyma. Most serotypes studied to date preferentially transduce neurons after intraparenchymal injection, and AAV2, for example, has particularly strong neuronal tropism (Bartlett and others 1998). However, while this vector has been favored in clinical trials because of its established safety record and historical use in the early AAV clinical studies (Ginn and others 2013), research has shown AAV1 and AAV5 to be more efficient in transducing neurons—and to a lesser extent some glia—in a number of rat and non-human primate brain regions (Mandel and

Table 1. Summary of AAV Clinical Trials in the CNS.

Disease	Clinical Trial	Serotype	Promoter	Transgene Product	ClinicalTrials.gov identifier	Refs
Alzheimer's	Phase 2	AAV2	CAG	Nerve growth factor (NGF)	NCT00876863	Mandel and others, 2010
Canavan	Phase I	AAV2	NSE	Aspartoacylase (ASPA)	NA	McPhee and others, 2006
Late infantile neuronal ceroidlipofuscinosis	Phase I	AAV2	CAG	Human CLN2	NCT00151216	Worgall and others, 2008
Late infantile neuronal ceroidlipofuscinosis	Phase I/2 recruiting	AAVrh.10	CAG	Human CLN2	NCT01414985	
Parkinson's	Phase I/2	AAV2	CAG	Neurturin (NTN)	NCT00400634 NCT00985517	Bartus and others, 2013
Parkinson's	Phase I recruiting	AAV2	NA	Glial cell-derived neurotrophic factor (GDNF)	NCT01621581	
Parkinson's	Phase 2	AAV2	CAG	Glutamate decarboxylase I (GAD)	NCT00643890	LeWitt and others, 2011
Sanfilippo syndrome (MPSIIIB)	Phase I/2 recruiting	AAV5	NA	Human α -N-acetylglucosaminidase	ISRCTN ^a 19853672	Ellinwood and others, 2011

Abbreviations: AAV = adeno-associated virus; CAG = chicken β -actin promoter with CMV enhancer; CLN2 = also known as tripeptidyl peptidase I; CMV = cytomegalovirus; NA = not available; NSE = neuron-specific enolase.

^aInternational Standard Randomized Controlled Trial Number.

Burger 2004). By comparison, AAV4, one of the more phylogenetically distant AAV serotypes, preferentially transduces ependymal cells, even after intrastriatal injection (Davidson and others 2000; Markakis and others 2010). Finally, among more recently isolated AAVs, intracerebral injection of AAV7, 8, 9, and rh.10 results in primarily neuronal transduction, and vector spread is greatest with AAV9 and rh.10 (Cearley and Wolfe 2006).

In addition to neurons, glia represent potential targets. These cells play important supporting roles for neurons, including functions that could be enhanced via gene delivery. They are also involved in disorders such as amyotrophic lateral sclerosis and are thus potential direct disease targets (Ilieva and others 2009). Recently discovered serotypes hu.32, hu.11, pi.2, hu.48R3, and rh.8 are able to transduce both astrocytes and oligodendrocytes with varying efficiencies in the adult mouse brain (Cearley and others 2008).

In addition to cellular transduction, vector spread through tissue is a significant challenge, particularly for disorders that afflict large regions of the CNS such as lysosomal storage diseases. Vector can undergo transport either extracellularly or intracellularly. Intracellularly, axonal transport of AAV can occur in the retrograde (Kaspar and others 2002) or the anterograde direction, and AAV serotypes differ in their potential for such transport (Kaspar and others 2003). AAV1, 9, and rh.10 can all be disseminated along axonal projections in both the retrograde and anterograde directions after injection into the ventral tegmental area, a region dense in efferent and

afferent projections (Cearley and Wolfe 2007). Axonal transport has been proposed as a mechanism to enhance therapeutic efficacy of AAV by protecting potentially both presynaptic neurons and their projective fields (Kaspar and others 2002). Such axonal transport can also be used to trace and manipulate neural circuits in the CNS (Betley and Sternson 2011).

The discovery that AAV9 can cross the BBB in both neonatal and adult mice (Foust and others 2009) has raised the exciting possibility that intravascular (IV) AAV administration could mediate widespread CNS gene expression. IV delivery leads to both neuronal and astrocytic transduction in neonates, though expression was primarily restricted to astrocytes in adults. Since intracranial injection results in mixed astrocytic and neuronal transduction, this astrocytic tropism may be due to astrocytic endfeet interfacing with endothelial cells within the BBB. Follow-up studies have demonstrated that rAAVrh.10, rAAVrh.39, rAAVrh.43 are also capable of parenchymal cell transduction after intravascular infusion in neonatal mice (Zhang and others 2011). One challenge associated with IV administration, however, is that the majority of the human population harbors neutralizing antibodies against one or more AAV serotypes because of natural exposure, which can severely limit IV-mediated AAV delivery (Bartel and others 2011). Finally, intracerebroventricular (ICV) administration (see section Therapeutic Routes of Administration) may also enable AAV to gain access to large volumes of the CNS, though ICV administration of AAV2, 4, or 5 shifts tro-

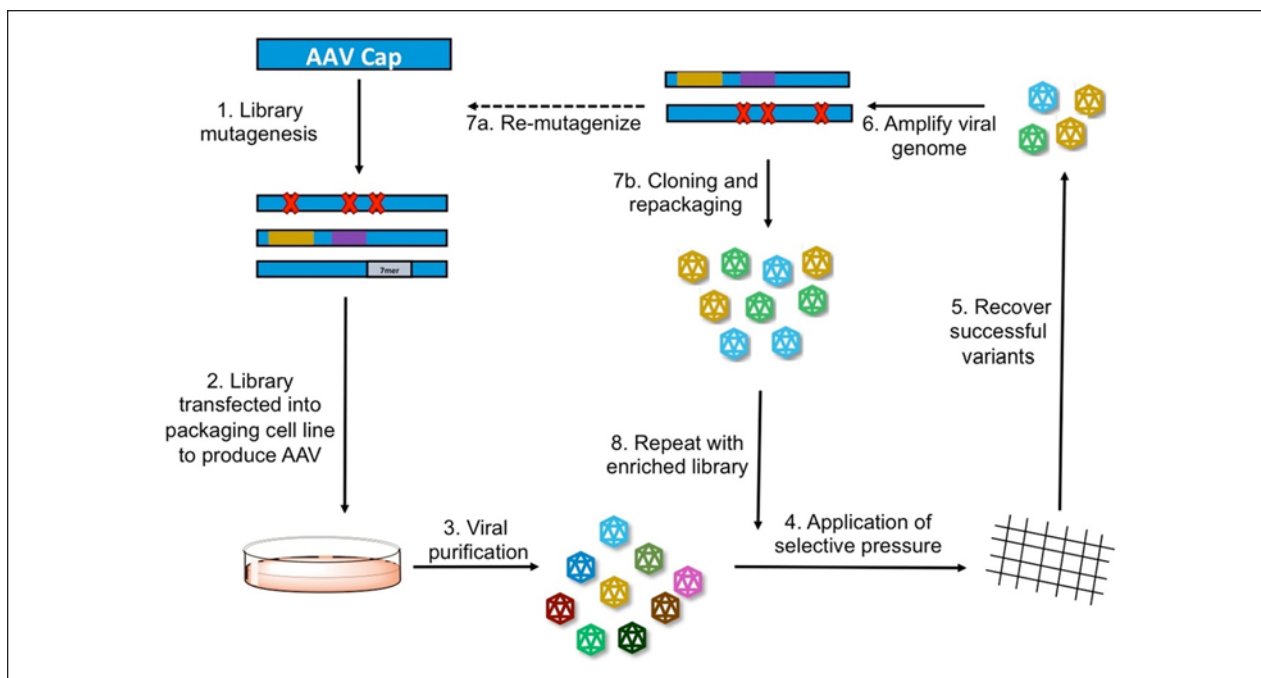


Figure 3. Adeno-associated virus (AAV)-directed evolution algorithm. (1) The AAV *cap* gene is mutagenized by techniques such as error-prone PCR, DNA shuffling, or the staggered extension process. (2) The mutagenized library is transfected into a packaging cell line (HEK293T) to produce viral particles. (3) Viral libraries are harvested and purified. (4) A selective pressure is applied. (5) Successful variants are recovered. (6) *Cap* genes are amplified by PCR. (7a) Additional mutagenesis can be conducted to increase library diversity. (7b) The enriched library is repackaged into viral particles. (8) The process is iterated to increase viral fitness.

pism primarily to ependymal cells with sparse transduction in the parenchyma (Davidson and others 2000).

Directed Evolution

Adeno-associated virus is thus a promising gene therapy vehicle; however, human therapeutic needs demand delivery properties that at best probably conferred AAVs with no selective advantages during natural evolution (i.e., CNS infection is not a prominent feature of AAV's life cycle) and at worst may be at odds with natural selection (e.g., a primary viral receptor that is promiscuously expressed at high levels can limit broad viral dispersal within tissue (Nguyen and others 2001)). As a result, there have been considerable efforts to engineer viral capsid proteins to meet biomedical needs (Schaffer and others 2008). However, the complexity of structure–function relationships in multimeric, 4 MDa viral particles renders rational design efforts difficult. An alternative approach, directed evolution, emulates how viruses naturally evolve—iterative rounds of genetic diversification and selection for improved function—but with selective pressures that can be designed to result in therapeutically useful viruses. Directed evolution has been applied to generate new viral variants with altered gene delivery

specificities and enhanced evasion of neutralizing antibodies (Asuri and others 2012; Dalkara and others 2013; Excoffon and others 2009; Jang and others 2011; Koerber and others 2006; Koerber and others 2008; Koerber and others 2009; Maheshri and others 2006).

Directed evolution of AAV (Fig. 3) first involves the generation of large libraries of mutated *cap* genes, using techniques such as error-prone PCR, DNA shuffling, or the staggered extension process (Schaffer and others 2008). These genetic libraries are then converted into viral particles, where each particle contains a viral genome that encodes its capsid shell, thereby linking the virus' genotype and phenotype. After applying a selective pressure for a specific improved function, *cap* genes can be recovered, amplified by PCR, and used for additional rounds of selection. Iteration drives library convergence toward top performers, which are then benchmarked to the best available natural serotypes. In addition to providing novel and useful vehicles, the resulting mutations can be reverse engineered to elucidate new basic structure–function relationship information. Moreover, beneficial mutations identified in different studies may also be combined on a single vector to address multiple therapeutic needs.

Numerous in vitro selections to target cell types of the central nervous system have been conducted.

As discussed above, most AAV vectors predominantly transduce neurons in the central nervous system after intraparenchymal injection. Koerber and others (2009) evolved AAV variants to infect human and rat astrocytes *in vitro* up to 15-fold more efficiently than their corresponding parent serotypes. In addition, several evolved variants transduced astrocytes up to 5.5-fold more efficiently than parental serotypes in the rat striatum, despite the *in vitro* nature of the original selection. Directed evolution has also been applied to target neural stem cells (NSCs), which natural AAV serotypes do not efficiently infect. After seven rounds of selection on rat hippocampal NSCs, the variant AAV r3.45 was recovered (Jang and others 2011). This AAV, which included a peptide insertion on the capsid surface, was 15- to 50-fold more infectious on rat NSCs relative to AAV2 and AAV5. In addition, though it was selected on rat NSCs, AAV r3.45 also supported efficient gene delivery to both murine and human NSCs. Furthermore, the vector was harnessed to mediate gene correction by homologous recombination, an advantageous feature of AAV gene delivery (Khan and others 2011). Finally, this variant is selective for NSCs within the adult rodent brain (data not shown).

Glioblastoma multiforme is the most common brain cancer and has a very poor prognosis. Maguire and others (2010) conducted seven rounds of selection using a shuffled AAV library selected on glioblastoma U87 cells. A resulting, chimeric capsid with elements from AAV1, 2, rh.8, rh.10, and several point mutations transduced 97% of glioblastoma U87 cells at a dose of 10^4 genome copies/cell and also outperformed the AAV2 parental serotype on multiple glioma cell lines. Future *in vivo* analysis may explore the promise of such variants for gene therapy in solid tumors.

While *in vitro* selections have been the primary focus of work to date, numerous cellular and tissue complexities of the CNS cannot be emulated in culture. Consequently, several studies have shifted toward animal models that better represent the transport barriers that gene therapy vectors must overcome. For example, Gray and others (2010) employed directed evolution to select variants that crossed a seizure-compromised BBB. A shuffled library of AAV serotypes 1-6, 8, and 9 was injected via tail vein into rats 24 hours after kainic acid-induced limbic seizure, and AAV variants were recovered from seizure-sensitive brain sites. After three rounds of selection, two clones primarily composed of AAV1, 8, and 9 were found to selectively transduce regions in the ventral hippocampus and piriform cortex where seizures had compromised the BBB, but they did not cross the intact BBB. The evolved vectors displayed a transduction profile similar to AAV8, infecting mostly neurons and oligodendrocytes with few astrocytes or microglia transduced. Finally, the biodistribution of evolved clones was

detargeted from peripheral organs when compared with the parent serotypes AAV 1, 8, and 9.

The retina is also part of the CNS, and the retinal degenerative disorder Leber's congenital amaurosis type 2 has been successfully treated in gene therapy clinical trials (Bainbridge and others 2008; Cideciyan and others 2009). The majority of monogenic retinal diseases involve mutations in genes expressed in photoreceptors and the retinal pigment epithelium, which lie several hundred microns deep within the retina, and transducing these targets with existing AAV vehicles requires an injection into subretinal space between photoreceptors and retinal pigment epithelium. The resulting transient retinal detachment can damage retinas already undergoing degeneration, and subretinal injections only transduce cells that come into contact with the "bleb" of injected liquid. Dalkara and others (2013) applied directed evolution to engineer an AAV variant that can reach the outer retina after injection into the readily accessible vitreous humor. Six rounds of selection in adult mice led to a dominant variant containing a seven-amino acid sequence inserted into loop 4 of the capsid. The evolved variant (7m8) mediated widespread transduction of the outer retina and was able to rescue disease phenotypes in two mouse models of eye diseases, X-linked retinoschisis and Leber's congenital amaurosis. Finally, the vector also showed promising clinical potential in its ability to transduce photoreceptors from the vitreous in non-human primate.

Rational Design

In some cases where specific capsid structure–function relationships are known, rational design can be effective. For example, tyrosine residues on the capsid surface are subject to phosphorylation by tyrosine kinases, leading to capsid ubiquitination and proteasomal degradation (Zhong and others 2008). Work by Srivastava and colleagues showing that mutation of tyrosines to phenylalanines can overcome this problem and enable more efficient gene delivery (Qing and others 1997), and Dalkara and others (2012) built on this work by introducing Y to F substitutions at two highly conserved, surface-exposed residues on the AAV9 capsid. The resulting tyrosine mutant AAV9-scCAG-GFP vector was administered by tail vein injection in neonatal mice, and transduced CNS cells included both neurons and astrocytes in the hippocampus, hypothalamus, cortex, and cerebellum—a pattern similar to but more efficient than wild type AAV9. Tyrosine mutant AAV9 vectors have also been demonstrated to significantly enhance gene delivery to the CNS after intracardiac injections in adult mice (Iida and others 2013). Tyrosine mutations may not substantially shift the natural tropism of parent serotypes;

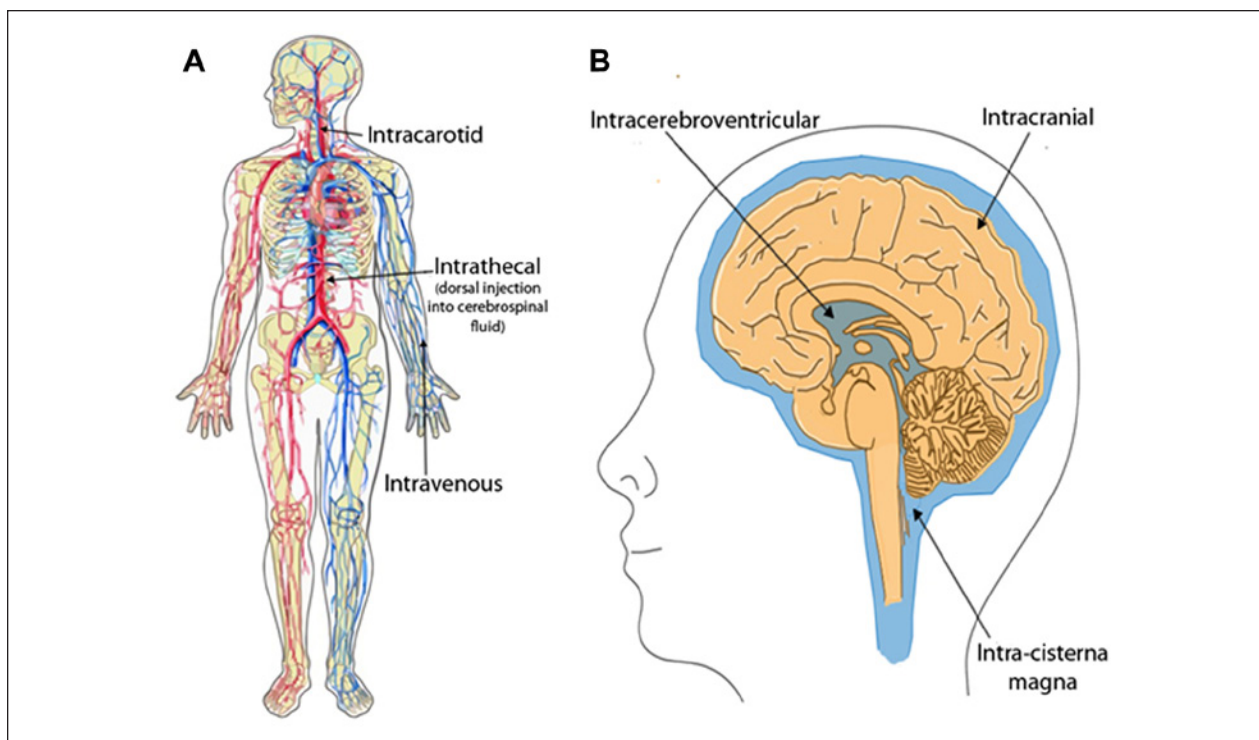


Figure 4. Routes of administration. (A) Illustration of intracarotid and intravenous injections into the bloodstream. The intrathecal route is a dorsal injection into the cerebrospinal fluid. (B) The more invasive intracranial, intracisterna magna, and intracerebroventricular injections into the CSF (blue) or brain tissue. Illustrations adapted from ChemBioDraw (Version 13.0.2.3021, Cambridge Software, Waltham, MA).

however, they may enable a reduction in vector dose and thereby lower the risk of an immune response.

Therapeutic Routes of Administration

Intracranial Administration

To date, intracranial administration of AAV—which involves insertion of flexible fused-silica infusion catheters through burr holes into the brain parenchyma, followed by slow infusion of vector (Lowery and Majewska 2010)—has been the most commonly employed route for gene delivery to the brain parenchyma (Bartus and others 2013; Ellinwood and others 2011; LeWitt and others 2011; Mandel 2010; McPhee and others 2006; Worgall and others 2008). This approach circumvents the biological transport barriers that render other administration routes challenging (Fig. 4). In addition, this route of administration does reduce the risk of vector neutralization by circulating antibodies, though anti-AAV neutralizing antibody titers in the brain parenchyma can reach 1% of levels found in systemic circulation (Treleaven and others 2012).

Intracranial injections do have significant drawbacks. As described above (see section Adeno-Associated Virus Vector Design), poor vector spread limits transgene expression to the vicinity of the injection site, a major shortcoming for diseases that affect large CNS regions such as Parkinson's or Alzheimer's diseases, or the entire CNS such as lysosomal storage disorders. It is estimated that complete transduction of the entire human infant brain for treatment of lysosomal storage disorders would require 50 to 350 injection tracts based on the limited diffusion distance (~1-3 mm) of AAV vectors (Cunningham and others 2008; Vite and others 2003). Each injection presents a risk of hemorrhaging, edema, and bacterial contamination. The spread of AAV vector throughout the brain parenchyma can be improved by convection-enhanced delivery (Cunningham and others 2008; Kells and others 2009), which increases vector transport through the interstitial fluid by inducing convective flow in addition to diffusion. Since bulk flow depends only on the pressure gradient, the injection pressure is maintained at a sufficient level to overcome the hydrostatic pressure of the interstitial fluid and thereby distribute the vector throughout the brain, though flow rates should be conservative to avoid uncontrolled vector spread along paths of

least resistance such as white matter tracts (Linninger and others 2008). Convection-enhanced delivery has been used safely in a clinical trial for Parkinson's disease (Eberling and others 2008), and the development of new cannula designs to prevent reflux (Krauze and others 2005) and sophisticated MRI guidance systems to ensure accurate cannula placement (San Sebastian and others 2012; van der Bom and others 2013) will further improve safety and efficacy.

Intravascular Administration

Intravascular administration in principle offers the potential for noninvasive transduction of the entire brain with a single vector infusion, given the high density of CNS capillaries (Pardridge 2005). However, AAV transcytosis to the brain parenchyma is obviously limited by the BBB, whose tight junctions between endothelial cells preclude paracellular transport of AAV. Serotypes such as AAV9 that do cross the BBB are thought to undergo receptor-mediated transcytosis in endothelial cells (Shen and others 2011).

There are, however, several disadvantages of IV delivery: The vector circulates throughout the entire body where it is exposed to circulating antibodies and can transduce peripheral organs in addition to the CNS. The latter two represent a loss of vector, can lead to off-target side effects, and increases the risk of an immune response. In addition, due to the inefficiency in crossing the BBB, only a small fraction of injected virus reaches the brain and spinal cord, necessitating in principle high vector doses on the order of 10^{15} viral genomes to treat an adult human (Samaranch and others 2012). Moreover, transduction is primarily limited to astrocytes in adult organisms as previously discussed (see section Adeno-Associated Virus Vector Design), though vector engineering may broaden tropism.

Intra-CSF

Delivery to the CSF places vector near the CNS parenchyma, has potential to reduce peripheral off-target transduction, and limits exposure to serum neutralizing antibodies. That said, analogous to the BBB, tight junctions between ependymal cells limit the efficiency of vector penetration into the brain parenchyma.

Samaranch and others (2012) demonstrated that AAV9 infusion into the cisterna magna of non-human primates promotes significantly stronger transgene expression throughout the cortex and cerebellum compared to intravascular delivery. Transgene expression was observed primarily in astrocytes, scattered pyramidal neurons, and almost no microglia or oligodendrocytes. Moderate serum titers of preexisting anti-AAV antibodies (1:200) did

prevent brain transduction, indicating that delivery to the CSF did not offer complete immunological protection.

Gray and others (2013) compared the transduction profiles of AAV2.5 (Bowles and others 2012) and AAV9 after injection into the cisterna magna and lumbar intrathecal space in non-human primates. Both vectors achieved widespread transduction of neurons and astrocytes in the brain and spinal cord, and intrathecal injections were more effective than intracisternal injections in promoting gene delivery to the dorsal root ganglia. Cross-reactivity of neutralizing antibodies between AAV2.5 and AAV9 was not observed in two of four monkeys tested, supporting the possibility of serotype switching for a second administration of vector. Furthermore, circulating neutralizing antibody titers up to 1:128, which prevent gene delivery after IV administration (Gray and others 2011), had no inhibitory effect on CNS gene transfer.

Retrograde Transport

Non-invasive gene delivery to the CNS can also be accomplished via vector administration to peripheral muscle tissue and retrograde transport along motor neuron projections to cell bodies residing in the CNS (Kaspar and others 2003). This approach is particularly relevant for diseases such as amyotrophic lateral sclerosis and spinal muscular atrophy that primarily afflict motor neurons. Hollis and others (2008) investigated the efficiency of retrograde transport of AAV serotypes 1-6 after peripheral injection into either extensor carpi muscle or sciatic nerve. AAV1 performed best in retrograde infection of lower motor neurons (1% to 4.1% of all motor neurons were transduced) after both intramuscular and intranerve injection, and the latter was more efficient.

AAV8 is also capable of retrograde transport in both neonatal and adult mice (Foust and others 2008; Zheng and others 2010). In adult mice, AAV8 does not cross the blood-nerve barrier, limiting systemic dissemination to peripheral organs (Zheng and others 2010). In another study, AAV9 was shown to undergo retrograde transport after injection into the gastrocnemius muscle of adult mice, transducing both neurons and astrocytes equally well with up to 43% of total motor neurons transduced (Benkhalifa-Ziyyat and others 2013). Spread of vector from injected muscle to the CNS and peripheral organs was also observed, likely due to the ability of AAV9 to cross the blood-nerve barrier.

Immune Responses to AAV in the CNS

Recombinant AAV vectors do not encode viral genes, but immune responses can be mounted against the viral capsid and/or the transgene product. For example,

preexisting neutralizing antibodies generated from a prior exposure to AAV can opsonize and inactivate the vector. The extent of vector neutralization depends on the route of administration. IV routes that expose the vector to circulating neutralizing antibodies are sensitive to low antibody titers (Gray and others 2011), whereas relatively high titers of circulating anti-AAV neutralizing antibodies do not appear to significantly affect transduction after intracranial AAV administration in immune-primed mice (Treleaven and others 2012). Phase I clinical trials employing intracranial delivery of recombinant AAV have confirmed preclinical results, showing a minimal humoral response and limited adaptive immune response (Kaplitl and others 2007; McPhee and others 2006).

After viral transduction and the onset of transgene expression, immune responses to the transgene product can lead to clearing of transduced cells with subsequent loss of gene expression and inflammation in the CNS. Most CNS gene therapy trials have employed AAV2, a vector with neuronal tropism, but newer vectors with broader tropism such as AAV9 may increase the likelihood of an immune response by infecting antigen-presenting cells. Ciesielska and others (2013) administered AAV9 encoding human aromatic L-amino acid decarboxylase (hAADC) or green fluorescent protein into rat striatum or thalamus. Both vectors provoked a full immune response, with upregulation of MHC II in glia, lymphocytic infiltration, and inflammation leading to significant loss of neurons and generation of antibodies against the transgenes. A significant fraction of the MHC II positive cells were identified as microglia 1 week and 3 weeks after injection, and a smaller population of MHC II positive astrocytes was also observed at the 3-week time point. In contrast to AAV9, AAV2-mediated delivery of hAADC has elicited no safety concerns in human or animal studies (Christine and others 2009; Eberling and others 2008). The authors speculated that transduction of antigen-presenting cells and subsequent presentation of transgene antigen led to a full adaptive immune response. Cell-mediated responses have also been observed after intracerebral infusion of an AAV1-GFP vector in non-human primate (Hadaczek and others 2009) and intracranial injection of AAV5- α -L-iduronidase in a dog model of Hurler syndrome (Ciron and others 2006). Therefore, immune responses to non-self proteins expressed from AAV vectors may limit therapeutic options for patients that lack immune tolerance due to null mutations in the endogenous gene. In light of these findings, engineered vectors that both overcome CNS transport barriers and do not infect antigen-presenting cells are needed.

Strategies for Limiting Off-Target Transduction

Some natural serotypes are selective for specific cell types, and directed evolution may be used to fundamentally

reengineer cell tropism (Pulicherla and others 2011). In addition, a cell type specific promoter can also be employed to restrict expression to specific transduced cells. One challenge is that such a promoter must be sufficiently small for it and the transgene to fit within the limited carrying capacity of the virus, roughly 5 kb ssDNA or 2.5 kb self-complementary dsDNA (Wang and others 2003). Neuron selective promoters that have been explored include synapsin-1, neuron-specific enolase, and human platelet-derived growth factor (Shevtsova and others 2005). In addition, the 2.5 kb rat tyrosine hydroxylase promoter has been used to drive transgene expression in mid-brain dopamine neurons (Oh and others 2009). Likewise, expression can be restricted to astrocytes and oligodendrocytes using glial fibrillary acidic protein or myelin basic protein promoters, respectively (von Jonquieres and others 2013). Ideally, a matching pair of cell type specific promoter and viral capsid would be developed for each disease target.

An alternate approach to reduce transgene expression in off-target tissues is the use of microRNA (miRNA) elements. MicroRNAs are non-coding regulatory RNAs that contribute to post-transcriptional gene silencing. miRNA target sequences matching miRNAs expressed only in peripheral organs can be introduced into AAV expression cassettes to reduce transgene mRNA levels in off-target tissues. For example, Qiao and others (2011) demonstrated that five copies of liver-specific miR-122 in the 3' untranslated region of AAV9 vectors reduced expression of the reporter genes luciferase and β -galactosidase by 50- and 70-fold, respectively, in liver tissue. Expression was not reduced in cardiac and muscle tissues, demonstrating the specificity of silencing. An analogous study (Geisler and others 2011) incorporated three copies of the same miR-122 element in AAV9 and provided additional evidence of reduced transgene expression in both hepatic cell lines and mouse liver. The discovery and cataloging of new miRNA elements (Kozomara and Griffiths-Jones 2011) will further expand applications of this technology.

Gene Therapy for Neurological Disorders

Lysosomal Storage Disorders

Lysosomal storage disorders are a family of inherited diseases involving deficiencies in enzymes that metabolize lipids, glycoproteins, or mucopolysaccharides. These deficiencies lead to the accumulation of undigested macromolecules in lysosomes, resulting in disruption of cellular function and clinical manifestations. More than 50 lysosomal storage disorders (LSDs) have been identified and collectively affect approximately 1 in 7700 births (Table 2) (Fuller and others 2006). Enzyme replacement therapies have been approved for the treatment of LSDs

Table 2. Several Lysosomal Storage Disorders Affecting the CNS.

Disease	Mutated Gene	Size of Coding Region (bp) ^a	Disease Prevalence ^b
MPSI-Hurler	Iduronidase, α -L (IDUA)	1962	1:100,000 (severe); 1:500,000 (attenuated)
MPSII-Hunter	Iduronate 2-sulfatase (IDS)	1032	1:100,000 males
MPSIIIB-Sanfilippo type B	N-acetylglucosaminidase, α (NAGLU)	2231	1:200,000
MPSVII-Sly	Glucuronidase, β (GUSB)	1956	1:250,000
Neuronal ceroid lipofuscinosis (Batten) ^a	Ceroid lipofuscinosis neuronal 2 gene (CLN2)	1669	1:25,000
Tay-Sachs	Hexosaminidase A, α polypeptide (HEXA)	1590	1:3500 (Ashkenazi Jewish population); 1:320,000 (general population)

^aPubMed nucleotide.

^bEstimates from the U.S. National Library of Medicine genetics home reference.

(Ohashi 2012) but are ineffective in the CNS since LSD enzymes do not cross the BBB. Given the short half-life of LSD enzymes, repeat intracranial infusions of enzyme would be necessary to achieve a therapeutic effect. In contrast, AAV vectors can provide sustained expression of LSD enzymes with a single vector dose. Moreover, cross-correction of non-transduced cells is possible since many LSD enzymes can be secreted and internalized by neighboring cells.

Late infantile neuronal ceroid lipofuscinosis (LINCL, also known as Batten disease) is caused by mutations in the ceroid lipofuscinosis neuronal 2 gene (CLN2). A phase 1 clinical trial for LINCL, involving AAV2 vector delivery of the CLN2 gene to 10 children via intracranial injection, has been completed (Worgall and others 2008). Gene delivery yielded a statistically significant slowing of disease progression as measured by a clinical rating scale. A secondary variable, neuroimaging results based on quantitative MRI parameters, was suggestive of improvement but did not yield a statistically significant change relative to the control group. The most common serious adverse events included seizures and the loss of one subject with severe LINCL 49 days after administration following development of status epilepticus. However, none of these adverse events were unequivocally attributed to the vector.

Alternative administration routes and vectors with increased spread (AAVrh.10; Sondhi and others 2012) are being explored to achieve the robust CNS transduction needed for correction of LSDs. Haurigot and others (2013) studied the impact of intra-CSF delivery of AAV9 vectors encoding sulfamidase in a mouse model of MPS IIIA (Sanfilippo syndrome type A). A high dose (5×10^{10} μ g per adult mouse) mediated sulfamidase activity in all brain regions with up to 11% to 36% of normal expression levels in males and 7% to 39% of expression levels in females. Gene delivery resulted in reduced glycosaminoglycan accumulation in most brain regions, correction

of behavioral responses, and extended survival. Intracisternal administration of the same vector in dogs resulted in primarily neuronal with some scattered astrocytic transduction, though approximately 3% to 5% of hepatocytes were also transduced, indicating that the vector exited the CSF. Expression of canine sulfamidase was sustained for weeks, but delivery of human sulfamidase caused expression to peak after 3 weeks and then decline, implicating an immune response to non-self protein in the dog. As intracisternal injections are not favored in pediatric patients, authors also evaluated intracerebroventricular administration, which resulted in widespread AAV9 vector distribution and transgene expression comparable to the intracisternal route. Serum antibody titers rose rapidly after vector exposure but remained low in the CSF (<1:10) in the absence of severe CNS inflammation. Treatment of dogs with preexisting immunity against AAV resulted in moderate gene expression in the CNS and severely reduced transduction of peripheral tissues, likely because CSF antibody titers (1:1 to 1:3.1) were much lower than in the periphery (1:1000).

Amyotrophic Lateral Sclerosis

Amyotrophic lateral sclerosis (ALS) is a progressive neurodegenerative disease characterized by the death of motor neurons. Of familial ALS cases (which collectively account for 10% of ALS), the most common inheritance pattern is autosomal dominant, and several genes have been implicated. Approximately 20% of these familial cases are traced to mutations in superoxide dismutase 1 (SOD1) (Nizzardo and others 2012). The mechanism of SOD1 toxicity is controversial, but it is thought to involve misfolded SOD1 aggregates.

Foust and others (2013) intravenously injected AAV9 encoding SOD1 short hairpin RNA (shRNA) at P1 and P21 in a mouse model of ALS. Transgene expression was robust in astrocytes (P1, 34% \pm 2%; P21, 54%

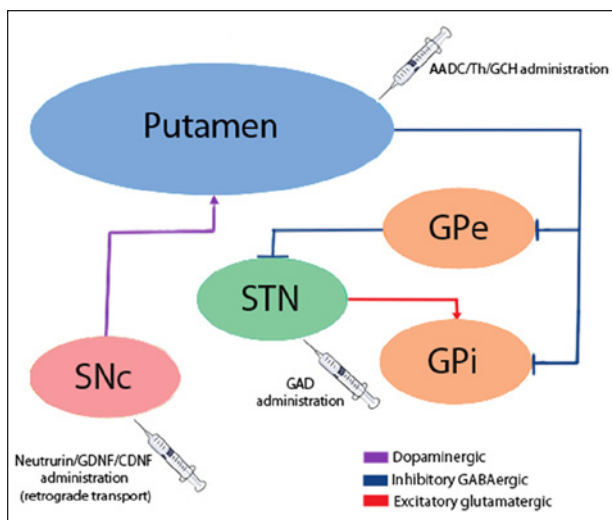


Figure 5. Therapeutic strategies for Parkinson's disease. Delivery of neurotrophic factors to the substantia nigra compacta (SNc) for retrograde delivery to the putamen. Gene therapy using glutamic acid decarboxylase (GAD) to quiet neurons in the subthalamic nucleus (STN). Delivery of neurotransmitter synthetic enzymes involved in dopamine production to the putamen. AADC = aromatic L-amino acid decarboxylase; Th = tyrosine hydroxylase; GCH = guanosine triphosphate cyclohydrolase; GDNF = glial-derived neurotrophic factor; CDNF = cerebral-derived neurotrophic factor; GPe = globus pallidus external; GPi = globus pallidus internal. Illustrations adapted from ChemBioDraw (Version 13.0.2.3021, Cambridge Software, Waltham, MA).

$\pm 3\%$) and motor neurons (P1, $62\% \pm 1\%$; P21, $8\% \pm 1\%$) and persisted throughout the life span of the mice. One shRNA mediated 60% and 45% reductions in mutant SOD1 protein in P1 and P21 injected mice, and it extended survival times by 39% when treatment was initiated at birth, one of the longest extensions of survival reported in this mouse model. In addition, vector administration was impressively able to slow disease progression even after disease onset (injection at P85). The same vector administered via intrathecal injection in cynomolgus macaques led to widespread gene expression in both neurons and astrocytes in the grey and white matter of the spinal cord. The percentage of ChAT⁺ motor neurons expressing the transgene was 50% in the cervical region, 65% in the thoracic region, and 80% in the lumbar region. SOD1 knockdown matched this pattern, with a 60% decrease in SOD1 mRNA in the cervical region, 70% decrease in thoracic region, and 88% decrease in lumbar region. These promising results provide support for human clinical trials. In another approach, Kaspar and others (2003) treated a mouse model of ALS with an intramuscular injection of an AAV vector encoding insulin-like growth factor 1. Retrograde gene delivery improved motor neuron survival, delayed deterioration

of motor function, and extended overall survival (Kaspar and others 2003).

Neuropathic Pain

AAV-mediated delivery of shRNAs has also recently been applied to treat injury-induced neuropathic pain. Na_v1.3 channels are up-regulated in DRG sensory neurons after injuries to the nervous system (Hains and others 2003), and are therefore potential targets for treatment of chronic pain. Samad and others (2013) injected an AAV2/5 vector encoding shRNA against Na_v1.3 into the lumbar dorsal root ganglion of adult rats with spared nerve injury. Two independent AAV-shRNA vectors were able to transduce $\sim 45\%$ of neurons and achieve $\sim 50\%$ knockdown of Na_v1.3 compared with a scrambled shRNA control. Importantly, off-target effects against other sodium channels were not observed. Moreover, Na_v1.3 down-regulation resulted in a significant, partial attenuation of mechanical allodynia, with up to a sixfold recovery of pain threshold; a promising proof of principle for gene therapy approaches to treat chronic neuropathic pain.

Parkinson's Disease

Parkinson's disease is a neurodegenerative disorder characterized by loss of motor function accompanying the death of dopaminergic neurons in the substantia nigra. In contrast to monogenic disorders, the mechanisms of pathogenesis in Parkinson's disease involve multiple genes and environmental factors (Shadrina and others 2010), and consequently gene therapy is not as straightforward as supplying a functional copy of a defective gene. Instead, clinical trials have utilized neurotrophic factors that prevent neuronal cell death, or alternatively neurotransmitter synthetic enzymes to modulate neuronal activity (Fig. 5). Translation to the clinic has demonstrated a strong safety profile and is progressing toward therapy efficacy.

In a clinical trial conducted by Ceregene, delivery of CER-120—an AAV2 vector encoding the neurotrophic factor neurturin under a constitutive CAG promoter—to the putamen resulted in an excellent safety profile in a phase 1 clinical trial (Bartus and others 2013). A multicenter, double-blind, sham surgery controlled phase 2 trial strengthened claims of vector safety but failed to yield a statistically significant benefit on the primary endpoint, the motor-off component of the Unified Parkinson's Disease Rating Scale (UPDRS) evaluated after 12 months. Some clinical benefits were evident: 19 of 25 efficacy endpoints were favorable compared with the sham control. Autopsy analysis of two subjects (who died due to unrelated causes) confirmed neurturin

expression in the putamen with co-localized tyrosine hydroxylase. Interestingly, neurturin staining was sparse in the substantia nigra, indicating that retrograde transport of the AAV vector and neurturin was inefficient. While unexpected based on preclinical studies in animal models, this result may indicate that Parkinson's disease in humans is characterized by accelerated degeneration of axonal transport capabilities well before the death of the neuron (Burke and O'Malley 2013).

Based on these results Ceregene initiated a phase 1/2b study incorporating direct injection of the substantia nigra and a fourfold increased dosage to the putamen. Given the strong placebo effect observed in the first trial, the time point for evaluation of the primary endpoint was increased to 15 months. Phase 1/2b results indicated no significant safety issues with gene delivery to the substantia nigra. Despite these modifications, initial phase 2b efficacy results did not demonstrate a statistically significant benefit on the primary endpoint (Ceregene 2013a). Similar to the first trial, a number of secondary endpoints produced statistically significant benefits, and a strong placebo effect was observed in the sham surgery control group. Additional analysis identified a better response on the primary endpoint in patients diagnosed 5 years prior to treatment compared with those diagnosed 10 years prior (Ceregene 2013b). Although the efficacy results were disappointing, the field should be encouraged by the exceptional safety profile and progress in vector delivery.

Other neurotrophic factors are being considered to treat Parkinson's disease. While a phase 1/2 double-blind trial with direct daily infusion of glial derived neurotrophic factor (GDNF) protein into the putamen did not produce a statistically significant benefit (Lang and others 2006), an AAV-GDNF vector may perform better than repeated GDNF infusions. A phase 1 clinical trial employing an AAV2-GDNF vector is currently recruiting (National Institute of Neurological Disorders and Stroke [NINDS]. AAV2-GDNF for Advanced Parkinson's Disease). In addition, multiple groups have shown that administration of cerebral dopamine neurotrophic factor (CDNF) to the striatum can prevent deterioration of mid-brain dopamine neurons in a rat model of Parkinson's disease (Back and others 2013; Ren and others 2013), indicating the promise of this molecule.

A different therapeutic strategy is to directly target the enzymatic pathway for dopamine production. L-dopa administration is currently the most effective treatment for relieving symptoms of Parkinson's disease; however, most patients experience a decrease in therapeutic efficacy after taking the medication for several years. One possible reason for such diminishing returns is reduced levels of aromatic L-amino acid decarboxylase (AADC), the enzyme that converts L-dopa to dopamine, and gene delivery of AADC could thus improve the response to

L-dopa. Furthermore, the degree of therapy can be controlled by modifying the dosing regimen of L-dopa. Preclinical studies in parkinsonian monkeys demonstrated increased L-dopa conversion lasting for more than 7 years after convection-enhanced delivery of AAV2-AADC into the striatum (Hadaczek and others 2006). Furthermore, an open label phase 1 clinical trial using an AAV2 vector for AADC delivery to the putamen improved the mean UPDRS rating scale score by 30% in the on and off states (Christine and others 2009; Eberling and others 2008). Given the strong placebo effect observed in the CERE-120 trials, double-blinded sham surgery controls will likely be included in future trials.

Additional enzymes involved in dopamine production include tyrosine hydroxylase (TH), the rate-limiting enzyme for conversion of L-tyrosine to L-dopa, and guanosine triphosphate cyclohydrolase I (GCH), the rate-limiting enzyme for production of the TH co-factor tetrahydrobiopterine. These genes have not yet been delivered in a clinical setting with AAV, but preclinical results in rat models are promising (Bjorklund and others 2010; Carlsson and others 2005). Most studies have delivered each gene in a separate virus, but a new vector designed to co-express both TH and GCH1 from a single virus (Cederfjall and others 2013) provided a dose-dependent functional recovery based on enhanced dopamine production in 6-OHDA lesioned rats. Unexpectedly, the same vector administered to parkinsonian monkeys resulted in transgene expression of GCH but not TH for unknown reasons.

Another approach involves delivery of the gene glutamic acid decarboxylase (GAD)—the rate-limiting enzyme for GABA production—into the subthalamic nucleus (STN). Increases in GABA production reduce output from the STN, an overactive region in Parkinson's disease. In a completed double-blind sham surgery controlled clinical trial, administration of AAV2-GAD vector to the STN via intracranial injection was well tolerated and resulted in improvements in the UPDRS motor score of 23.1% in patients receiving the vector compared to 12.7% in the sham control group after 6 months, the first statistically significant difference in the primary endpoint of a double-blind phase 2 trial employing AAV to treat Parkinson's disease (LeWitt and others 2011). Despite benefit in the primary endpoint, most quality-of-life measures did not improve, and as a result the trial sponsor (Neurologix) was unable to proceed to a phase 3 trial.

Conclusion

Promising clinical trials and the first gene therapy product approval underscore the exciting potential of gene delivery using AAV. Recombinant AAV vectors can provide long-lasting gene expression to treat chronic

neurological disorders and have demonstrated a strong safety profile, making them promising for both monogenic and idiopathic CNS disease. That said, clinical translation in the CNS has been hindered by biological transport barriers, immune responses, and infection of off-target cells. However, these challenges have motivated the development of engineered vectors to further and fully realize the potential of gene therapy in the CNS.

Declaration of Conflicting Interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: DVS is an inventor on patents involving AAV directed evolution.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by a National Science Foundation (NSF) graduate research fellowship to DSO and was also supported by the National Institutes of Health (NIH Grant No. EY018241-09).

References

- Asokan A, Schaffer DV, Samulski RJ. 2012. The AAV vector toolkit: poised at the clinical crossroads. *Mol Ther* 20(4):699–708.
- Asuri P, Bartel MA, Vazin T, Jang JH, Wong TB, Schaffer DV. 2012. Directed evolution of adeno-associated virus for enhanced gene delivery and gene targeting in human pluripotent stem cells. *Mol Ther* 20(2):329–38.
- Back S, Peranen J, Galli E, Pulkkila P, Lonka-Nevalaita L, Tamminen T, and others. 2013. Gene therapy with AAV2-CDNF provides functional benefits in a rat model of Parkinson's disease. *Brain Behav* 3(2):75–88.
- Bainbridge JW, Smith AJ, Barker SS, Robbie S, Henderson R, Balaggan K, and others. 2008. Effect of gene therapy on visual function in Leber's congenital amaurosis. *N Engl J Med* 358(21):2231–9.
- Bartel M, Schaffer D, Buning H. 2011. Enhancing the clinical potential of AAV vectors by capsid engineering to evade pre-existing immunity. *Front Microbiol* 2:204.
- Bartlett JS, Samulski RJ, McCown TJ. 1998. Selective and rapid uptake of adeno-associated virus type 2 in brain. *Hum Gene Ther* 9(8):1181–6.
- Bartus RT, Baumann TL, Brown L, Kruegel BR, Ostrove JM, Herzog CD. 2013. Advancing neurotrophic factors as treatments for age-related neurodegenerative diseases: developing and demonstrating "clinical proof-of-concept" for AAV-neurturin (CERE-120) in Parkinson's disease. *Neurobiol Aging* 34(1):35–61.
- Benkhelifa-Ziyyat S, Besse A, Roda M, Duque S, Astord S, Carcenac R, and others. 2013. Intramuscular scAAV9-SMN injection mediates widespread gene delivery to the spinal cord and decreases disease severity in SMA mice. *Mol Ther* 21(2):282–90.
- Betley JN, Sternson SM. 2011. Adeno-associated viral vectors for mapping, monitoring, and manipulating neural circuits. *Hum Gene Ther* 22(6):669–77.
- Bjorklund T, Carlsson T, Cederfjall EA, Carta M, Kirik D. 2010. Optimized adeno-associated viral vector-mediated striatal DOPA delivery restores sensorimotor function and prevents dyskinesias in a model of advanced Parkinson's disease. *Brain* 133(Pt 2):496–511.
- Bowles DE, McPhee SW, Li C, Gray SJ, Samulski JJ, Camp AS, and others. 2012. Phase 1 gene therapy for Duchenne muscular dystrophy using a translational optimized AAV vector. *Mol Ther* 20(2):443–55.
- Burke RE, O'Malley K. 2013. Axon degeneration in Parkinson's disease. *Exp Neurol* 246:72–83.
- Carlsson T, Winkler C, Burger C, Muzyczka N, Mandel RJ, Cenci A, and others. 2005. Reversal of dyskinesias in an animal model of Parkinson's disease by continuous L-DOPA delivery using rAAV vectors. *Brain* 128(Pt 3):559–69.
- Cearley CN, Vandenberghe LH, Parente MK, Carnish ER, Wilson JM, Wolfe JH. 2008. Expanded repertoire of AAV vector serotypes mediate unique patterns of transduction in mouse brain. *Mol Ther* 16(10):1710–8.
- Cearley CN, Wolfe JH. 2006. Transduction characteristics of adeno-associated virus vectors expressing cap serotypes 7, 8, 9, and Rh10 in the mouse brain. *Mol Ther* 13(3):528–37.
- Cearley CN, Wolfe JH. 2007. A single injection of an adeno-associated virus vector into nuclei with divergent connections results in widespread vector distribution in the brain and global correction of a neurogenetic disease. *J Neurosci* 27(37):9928–40.
- Cederfjall E, Nilsson N, Sahin G, Chu Y, Nikitidou E, Bjorklund T, and others. 2013. Continuous DOPA synthesis from a single AAV: dosing and efficacy in models of Parkinson's disease. *Sci Rep* 3:2157.
- Ceregene. 2013a. April 19. Ceregene reports data from Parkinson's disease phase 2b study.
- Ceregene. 2013b. May 21. Ceregene reports additional efficacy data from Parkinson's disease phase 2b study.
- Christine CW, Starr PA, Larson PS, Eberling JL, Jagust WJ, Hawkins RA, and others. 2009. Safety and tolerability of putaminal AADC gene therapy for Parkinson disease. *Neurology* 73(20):1662–9.
- Cideciyan AV, Hauswirth WW, Aleman TS, Kaushal S, Schwartz SB, Boye SL, and others. 2009. Human RPE65 gene therapy for Leber congenital amaurosis: persistence of early visual improvements and safety at 1 year. *Hum Gene Ther* 20(9):999–1004.
- Ciesielska A, Hadaczek P, Mittermeyer G, Zhou S, Wright JF, Bankiewicz KS, and others. 2013. Cerebral infusion of AAV9 vector-encoding non-self proteins can elicit cell-mediated immune responses. *Mol Ther* 21(1):158–66.
- Ciron C, Desmaris N, Colle MA, Raoul S, Joussemet B, Verot L, and others. 2006. Gene therapy of the brain in the dog model of Hurler's syndrome. *Ann Neurol* 60(2):204–13.
- Cunningham J, Pivrotto P, Bringas J, Suzuki B, Vijay S, Sanftner L, and others. 2008. Biodistribution of adeno-associated virus type-2 in nonhuman primates after convection-enhanced delivery to brain. *Mol Ther* 16(7):1267–75.

- Dalkara D, Byrne LC, Klimczak RR, Visel M, Yin L, Merigan WH, and others. 2013. In vivo-directed evolution of a new adeno-associated virus for therapeutic outer retinal gene delivery from the vitreous. *Sci Transl Med* 5(189):189ra76.
- Dalkara D, Byrne LC, Lee T, Hoffmann NV, Schaffer DV, Flannery JG. 2012. Enhanced gene delivery to the neonatal retina through systemic administration of tyrosine-mutated AAV9. *Gene Ther* 19(2):176–81.
- Davidson BL, Stein CS, Heth JA, Martins I, Kotin RM, Derksen TA, and others. 2000. Recombinant adeno-associated virus type 2, 4, and 5 vectors: transduction of variant cell types and regions in the mammalian central nervous system. *Proc Natl Acad Sci U S A* 97(7):3428–32.
- Eberling JL, Jagust WJ, Christine CW, Starr P, Larson P, Bankiewicz KS, and others. 2008. Results from a phase I safety trial of hAADC gene therapy for Parkinson disease. *Neurology* 70(21):1980–3.
- Ellinwood NM, Ausseil J, Desmaris N, Bigou S, Liu S, Jens JK, and others. 2011. Safe, efficient, and reproducible gene therapy of the brain in the dog models of Sanfilippo and Hurler syndromes. *Mol Ther* 19(2):251–9.
- Excoffon KJ, Koerber JT, Dickey DD, Murtha M, Keshavjee S, Kaspar BK, and others. 2009. Directed evolution of adeno-associated virus to an infectious respiratory virus. *Proc Natl Acad Sci U S A* 106(10):3865–70.
- Foust KD, Nurre E, Montgomery CL, Hernandez A, Chan CM, Kaspar BK. 2009. Intravascular AAV9 preferentially targets neonatal neurons and adult astrocytes. *Nat Biotechnol* 27(1):59–65.
- Foust KD, Poirier A, Pacak CA, Mandel RJ, Flotte TR. 2008. Neonatal intraperitoneal or intravenous injections of recombinant adeno-associated virus type 8 transduce dorsal root ganglia and lower motor neurons. *Hum Gene Ther* 19(1):61–70.
- Foust KD, Salazar DL, Likhite S, Ferraiuolo L, Ditsworth D, Ilieva H, and others. 2013. Therapeutic AAV9-mediated suppression of mutant SOD1 slows disease progression and extends survival in models of inherited ALS. *Mol Ther* 21(12):2148–59.
- Fuller M, Meikle PJ, Hopwood JJ. 2006. Epidemiology of lysosomal storage diseases: an overview. In: Mehta A, Beck M, and Sunder-Plassmann G, editors. *Fabry disease: perspectives from 5 Years of FOS*. Oxford, England: Oxford PharmaGenesis. p 9–20.
- Geisler A, Jungmann A, Kurreck J, Poller W, Katus HA, Vetter R, and others. 2011. microRNA122-regulated transgene expression increases specificity of cardiac gene transfer upon intravenous delivery of AAV9 vectors. *Gene Ther* 18(2):199–209.
- Ginn SL, Alexander IE, Edelstein ML, Abedi MR, Wixon J. 2013. Gene therapy clinical trials worldwide to 2012—an update. *J Gene Med* 15(2):65–77.
- Gray SJ, Blake BL, Criswell HE, Nicolson SC, Samulski RJ, McCown TJ, and others. 2010. Directed evolution of a novel adeno-associated virus (AAV) vector that crosses the seizure-compromised blood-brain barrier (BBB). *Mol Ther* 18(3):570–8.
- Gray SJ, Matagne V, Bachaboina L, Yadav S, Ojeda SR, Samulski RJ. 2011. Preclinical differences of intravascular AAV9 delivery to neurons and glia: a comparative study of adult mice and nonhuman primates. *Mol Ther* 19(6):1058–69.
- Gray SJ, Nagabhushan Kalburgi S, McCown TJ, Jude Samulski R. 2013. Global CNS gene delivery and evasion of anti-AAV-neutralizing antibodies by intrathecal AAV administration in non-human primates. *Gene Ther* 20(4):450–9.
- Hadaczek P, Forsayeth J, Mirek H, Munson K, Bringas J, Pivrotto P, and others. 2009. Transduction of nonhuman primate brain with adeno-associated virus serotype 1: vector trafficking and immune response. *Hum Gene Ther* 20(3):225–37.
- Hadaczek P, Kohutnicka M, Krauze MT, Bringas J, Pivrotto P, Cunningham J, and others. 2006. Convection-enhanced delivery of adeno-associated virus type 2 (AAV2) into the striatum and transport of AAV2 within monkey brain. *Hum Gene Ther* 17(3):291–302.
- Hains BC, Klein JP, Saab CY, Craner MJ, Black JA, Waxman SG. 2003. Upregulation of sodium channel Nav1.3 and functional involvement in neuronal hyperexcitability associated with central neuropathic pain after spinal cord injury. *J Neurosci* 23(26):8881–92.
- Haurigot V, Marcó S, Ribera A, Garcia M, Ruzo A, Villacampa P, and others. 2013. Whole body correction of mucopolysaccharidosis IIIA by intracerebrospinal fluid gene therapy. *J Clin Invest*. Epub Jul 1.
- Hollis ER 2nd, Kadoya K, Hirsch M, Samulski RJ, Tuszyński MH. 2008. Efficient retrograde neuronal transduction utilizing self-complementary AAV1. *Mol Ther* 16(2):296–301.
- Iida A, Takino N, Miyauchi H, Shimazaki K, Muramatsu S. 2013. Systemic delivery of tyrosine-mutant AAV vectors results in robust transduction of neurons in adult mice. *Biomed Res Int* 2013:974819.
- Ilieva H, Polymenidou M, Cleveland DW. 2009. Non-cell autonomous toxicity in neurodegenerative disorders: ALS and beyond. *J Cell Biol* 187(6):761–72.
- Jang JH, Koerber JT, Kim JS, Asuri P, Vazin T, Bartel M, and others. 2011. An evolved adeno-associated viral variant enhances gene delivery and gene targeting in neural stem cells. *Mol Ther* 19(4):667–75.
- Kaeppl C, Beattie SG, Fronza R, van Logtenstein R, Salmon F, Schmidt S, and others. 2013. A largely random AAV integration profile after LPLD gene therapy. *Nat Med* 19(7):889–91.
- Kaplitt MG, Feigin A, Tang C, Fitzsimons HL, Mattis P, Lawlor PA, and others. 2007. 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* 369(9579):2097–105.
- Kaspar BK, Erickson D, Schaffer D, Hinh L, Gage FH, Peterson DA. 2002. Targeted retrograde gene delivery for neuronal protection. *Mol Ther* 5(1):50–6.
- Kaspar BK, Llado J, Sherkat N, Rothstein JD, Gage FH. 2003. Retrograde viral delivery of IGF-1 prolongs survival in a mouse ALS model. *Science* 301(5634):839–42.
- Kells AP, Hadaczek P, Yin D, Bringas J, Varenika V, Forsayeth J, and others. 2009. Efficient gene therapy-based method for the delivery of therapeutics to primate cortex. *Proc Natl Acad Sci U S A* 106(7):2407–11.

- Khan IF, Hirata RK, Russell DW. 2011. AAV-mediated gene targeting methods for human cells. *Nat Protoc* 6(4):482–501.
- Koerber JT, Jang JH, Schaffer DV. 2008. DNA shuffling of adeno-associated virus yields functionally diverse viral progeny. *Mol Ther* 16(10):1703–9.
- Koerber JT, Klimczak R, Jang JH, Dalkara D, Flannery JG, Schaffer DV. 2009. Molecular evolution of adeno-associated virus for enhanced glial gene delivery. *Mol Ther* 17(12):2088–95.
- Koerber JT, Maheshri N, Kaspar BK, Schaffer DV. 2006. Construction of diverse adeno-associated viral libraries for directed evolution of enhanced gene delivery vehicles. *Nat Protoc* 1(2):701–6.
- Kozomara A, Griffiths-Jones S. 2011. miRBase: integrating microRNA annotation and deep-sequencing data. *Nucleic Acids Res* 39(Database issue):D152–7.
- Krauze MT, Saito R, Noble C, Tamas M, Bringas J, Park JW, and others. 2005. Reflux-free cannula for convection-enhanced high-speed delivery of therapeutic agents. *J Neurosurg* 103(5):923–9.
- Kwon I, Schaffer DV. 2008. Designer gene delivery vectors: molecular engineering and evolution of adeno-associated viral vectors for enhanced gene transfer. *Pharm Res* 25(3):489–99.
- Lang AE, Gill S, Patel NK, Lozano A, Nutt JG, Penn R, and others. 2006. Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. *Ann Neurol* 59(3):459–66.
- LeWitt PA, Rezai AR, Leehey MA, Ojemann SG, Flaherty AW, Eskandar EN, and others. 2011. AAV2-GAD gene therapy for advanced Parkinson's disease: a double-blind, sham-surgery controlled, randomised trial. *Lancet Neurol* 10(4):309–19.
- Linninger AA, Somayaji MR, Mekarski M, Zhang L. 2008. Prediction of convection-enhanced drug delivery to the human brain. *J Theor Biol* 250(1):125–38.
- Lowery RL, Majewska AK. 2010. Intracranial injection of adeno-associated viral vectors. *J Vis Exp* (45).
- Maguire CA, Gianni D, Meijer DH, Shaket LA, Wakimoto H, Rabkin SD, and others. 2010. Directed evolution of adeno-associated virus for glioma cell transduction. *J Neurooncol* 96(3):337–47.
- Maheshri N, Koerber JT, Kaspar BK, Schaffer DV. 2006. Directed evolution of adeno-associated virus yields enhanced gene delivery vectors. *Nat Biotechnol* 24(2):198–204.
- Mandel RJ. 2010. CERE-110, an adeno-associated virus-based gene delivery vector expressing human nerve growth factor for the treatment of Alzheimer's disease. *Curr Opin Mol Ther* 12(2):240–7.
- Mandel RJ, Burger C. 2004. Clinical trials in neurological disorders using AAV vectors: promises and challenges. *Curr Opin Mol Ther* 6(5):482–90.
- Markakis EA, Vives KP, Bober J, Leichter S, Leranthe C, Beecham J, and others. 2010. Comparative transduction efficiency of AAV vector serotypes 1–6 in the substantia nigra and striatum of the primate brain. *Mol Ther* 18(3):588–93.
- McPhee SW, Janson CG, Li C, Samulski RJ, Camp AS, Francis J, and others. 2006. Immune responses to AAV in a phase I study for Canavan disease. *J Gene Med* 8(5):577–88.
- Miller N. 2012. Glybera and the future of gene therapy in the European Union. *Nat Rev Drug Discov* 11(5):419.
- Mingozzi F, High KA. 2013. Immune responses to AAV vectors: overcoming barriers to successful gene therapy. *Blood* 122(1):23–36.
- Nathwani AC, Tuddenham EG, Rangarajan S, Rosales C, McIntosh J, Linch DC, and others. 2011. Adenovirus-associated virus vector-mediated gene transfer in hemophilia B. *N Engl J Med* 365(25):2357–65.
- Nguyen JB, Sanchez-Pernaute R, Cunningham J, Bankiewicz KS. 2001. Convection-enhanced delivery of AAV-2 combined with heparin increases TK gene transfer in the rat brain. *Neuroreport* 12(9):1961–4.
- Nizzardo M, Simone C, Falcone M, Riboldi G, Rizzo F, Magri F, and others. 2012. Research advances in gene therapy approaches for the treatment of amyotrophic lateral sclerosis. *Cell Mol Life Sci* 69(10):1641–50.
- Oh MS, Hong SJ, Huh Y, Kim KS. 2009. Expression of transgenes in midbrain dopamine neurons using the tyrosine hydroxylase promoter. *Gene Ther* 16(3):437–40.
- Ohashi T. 2012. Enzyme replacement therapy for lysosomal storage diseases. *Pediatr Endocrinol Rev* 10(Suppl 1):26–34.
- Pardridge WM. 2005. The blood-brain barrier: bottleneck in brain drug development. *NeuroRx* 2(1):3–14.
- Pulicherla N, Shen S, Yadav S, Debbink K, Govindasamy L, Agbandje-McKenna M, and others. 2011. Engineering liver-detargeted AAV9 vectors for cardiac and musculoskeletal gene transfer. *Mol Ther* 19(6):1070–8.
- Qiao C, Yuan Z, Li J, He B, Zheng H, Mayer C, and others. 2011. Liver-specific microRNA-122 target sequences incorporated in AAV vectors efficiently inhibits transgene expression in the liver. *Gene Ther* 18(4):403–10.
- Qing K, Wang XS, Kube DM, Ponnazhagan S, Bajpai A, Srivastava A. 1997. Role of tyrosine phosphorylation of a cellular protein in adeno-associated virus 2-mediated transgene expression. *Proc Natl Acad Sci U S A* 94(20):10879–84.
- Ren X, Zhang T, Gong X, Hu G, Ding W, Wang X. 2013. AAV2-mediated striatum delivery of human CDNF prevents the deterioration of midbrain dopamine neurons in a 6-hydroxydopamine induced parkinsonian rat model. *Exp Neurol* 248:148–56.
- Samad OA, Tan AM, Cheng X, Foster E, Dib-Hajj SD, Waxman SG. 2013. Virus-mediated shRNA knockdown of Na(v)1.3 in rat dorsal root ganglion attenuates nerve injury-induced neuropathic pain. *Mol Ther* 21(1):49–56.
- Samaranch L, Salegio EA, San Sebastian W, Kells AP, Foust KD, Bringas JR, and others. 2012. Adeno-associated virus serotype 9 transduction in the central nervous system of nonhuman primates. *Hum Gene Ther* 23(4):382–9.
- San Sebastian W, Richardson RM, Kells AP, Lamarre C, Bringas J, Pivrotto P, and others. 2012. Safety and tolerability of magnetic resonance imaging-guided convection-enhanced delivery of AAV2-hAADC with a novel delivery platform in nonhuman primate striatum. *Hum Gene Ther* 23(2):210–7.

- Schaffer DV, Koerber JT, Lim KI. 2008. Molecular engineering of viral gene delivery vehicles. *Annu Rev Biomed Eng* 10:169–94.
- Shadrina MI, Slominsky PA, Limborska SA. 2010. Molecular mechanisms of pathogenesis of Parkinson's disease. *Int Rev Cell Mol Biol* 281:229–66.
- Shen S, Bryant KD, Brown SM, Randell SH, Asokan A. 2011. Terminal N-linked galactose is the primary receptor for adeno-associated virus 9. *J Biol Chem* 286(15):13532–40.
- Shevtsova Z, Malik JM, Michel U, Bahr M, Kugler S. 2005. Promoters and serotypes: targeting of adeno-associated virus vectors for gene transfer in the rat central nervous system in vitro and in vivo. *Exp Physiol* 90(1):53–9.
- Sondhi D, Johnson L, Purpura K, Monette S, Souweidane MM, Kaplitt MG, and others. 2012. Long-term expression and safety of administration of AAVrh.10hCLN2 to the brain of rats and nonhuman primates for the treatment of late infantile neuronal ceroid lipofuscinosis. *Hum Gene Ther Methods* 23(5):324–35.
- Sonntag F, Schmidt K, Kleinschmidt JA. 2010. A viral assembly factor promotes AAV2 capsid formation in the nucleolus. *Proc Natl Acad Sci U S A* 107(22):10220–5.
- Testa F, Maguire AM, Rossi S, Pierce EA, Melillo P, Marshall K, and others. 2013. Three-year follow-up after unilateral subretinal delivery of adeno-associated virus in patients with Leber congenital Amaurosis type 2. *Ophthalmology* 120(6):1283–91.
- Treleaven CM, Tamsett TJ, Bu J, Fidler JA, Sardi SP, Hurlbut GD, and others. 2012. Gene transfer to the CNS is efficacious in immune-primed mice harboring physiologically relevant titers of anti-AAV antibodies. *Mol Ther* 20(9):1713–23.
- van der Bom IM, Moser RP, Gao G, Sena-Esteves M, Aronin N, Gounis MJ. 2013. Frameless multimodal image guidance of localized convection-enhanced delivery of therapeutics in the brain. *J Neurointerv Surg* 5(1):69–72.
- Vite CH, Passini MA, Haskins ME, Wolfe JH. 2003. Adeno-associated virus vector-mediated transduction in the cat brain. *Gene Ther* 10(22):1874–81.
- von Jonquieres G, Mersmann N, Klugmann CB, Harasta AE, Lutz B, Teahan O, and others. 2013. Glial promoter selectivity following AAV-delivery to the immature brain. *PLoS One* 8(6):e65646.
- Wang Z, Ma HI, Li J, Sun L, Zhang J, Xiao X. 2003. Rapid and highly efficient transduction by double-stranded adeno-associated virus vectors in vitro and in vivo. *Gene Ther* 10(26):2105–11.
- Worgall S, Sondhi D, Hackett NR, Kosofsky B, Kekatpure MV, Neyzi N, and others. 2008. Treatment of late infantile neuronal ceroid lipofuscinosis by CNS administration of a serotype 2 adeno-associated virus expressing CLN2 cDNA. *Hum Gene Ther* 19(5):463–74.
- Wu Z, Asokan A, Samulski RJ. 2006. Adeno-associated virus serotypes: vector toolkit for human gene therapy. *Mol Ther* 14(3):316–27.
- Zhang H, Yang B, Mu X, Ahmed SS, Su Q, He R, and others. 2011. Several rAAV vectors efficiently cross the blood-brain barrier and transduce neurons and astrocytes in the neonatal mouse central nervous system. *Mol Ther* 19(8):1440–8.
- Zheng H, Qiao C, Wang CH, Li J, Li J, Yuan Z, and others. 2010. Efficient retrograde transport of adeno-associated virus type 8 to spinal cord and dorsal root ganglion after vector delivery in muscle. *Hum Gene Ther* 21(1):87–97.
- Zhong L, Li B, Jayandharan G, Mah CS, Govindasamy L, Agbandje-McKenna M, and others. 2008. Tyrosine-phosphorylation of AAV2 vectors and its consequences on viral intracellular trafficking and transgene expression. *Virology* 381(2):194–202.