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Adv Genet. Author manuscript; available in PMC 2015 July 29.

Published in final edited form as:

Adv Genet. 2014 ; 87: 71–124. doi:10.1016/B978-0-12-800149-3.00002-0.

Clinical Applications Involving CNS Gene Transfer

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Abstract

Diseases of the central nervous system (CNS) have traditionally been the most difficult to treat by traditional pharmacological methods, due mostly to the blood–brain barrier and the difficulties associated with repeated drug administration targeting the CNS. Viral vector gene transfer represents a way to permanently provide a therapeutic protein within the nervous system after a single administration, whether this be a gene replacement strategy for an inherited disorder or a disease-modifying protein for a disease such as Parkinson's. Gene therapy approaches for CNS disorders has evolved considerably over the last two decades. Although a breakthrough treatment has remained elusive, current strategies are now considerably safer and potentially much more effective. This chapter will explore the past, current, and future status of CNS gene therapy, focusing on clinical trials utilizing adeno-associated virus and lentiviral vectors.

1. INTRODUCTION

Gene therapy is a promising treatment option for a number of neurological disorders. The potential benefits of using viral platforms for correcting these and other diseases are enormous, and as a result considerable efforts have been made to develop and improve vector systems for gene transfer to the central nervous system (CNS). Various viral platforms are tailored to their specific applications but generally should share a few key properties including low immunogenicity, lack of oncogenicity and pathogenicity, efficient gene transfer, long-term gene-of-interest expression, and scalable manufacture for clinical applications. It should be noted that no one gene transfer platform is perfectly suited for every disease application.

Traditional pharmacological approaches often run into considerable challenges when treating CNS disorders. It is difficult to get many compounds across the blood–brain barrier (BBB). Even for compounds that cross the BBB, very large doses must be administered into

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the blood to get enough of the drug into the brain to be effective. This can often lead to side effects in peripheral organs that must be considered. Methods to concentrate the drug within the nervous system, such as intrathecal administration, are possible but chronic administration of the drug has significant risk of complications. The benefit of gene transfer is that the therapeutic agent (protein, siRNA, etc.) can be produced within the CNS and provided on a permanent steady-state basis after a single administration.

1.1 Scope of the Book Chapter

The primary focus of this chapter is to provide the current state of clinical gene transfer research. The primary vectors for CNS gene transfer are adeno-associated virus (AAV) and lentiviral vectors, and these are the focus of this chapter. The biology and derivation of these vectors is described in greater detail in the accompanying chapter “Methods for Gene Transfer to the CNS,” and will expand on that chapter to describe clinical applications of the vector technology. A considerable amount of work has been done using a variety of viral vectors to treat brain tumors, but these are not discussed in this chapter.

Vectors derived from herpes simplex virus (HSV) have also been developed as gene transfer reagents to treat chronic pain. In the chapter “Methods for Gene Transfer to the CNS” the biology of HSV vectors and their utility for nervous system gene transfer were discussed. Clinical application of HSV vector for chronic pain involves injection of the vector in the skin, where it retrogradely transports along sensory axons to the dorsal root ganglia where the transgene is expressed. This topic was covered in the other chapter and will not be discussed here.

Completed and ongoing CNS-directed gene transfer trials are organized based on vector type (retrovirus vs AAV). While the focus of the chapter is on CNS clinical trials, some attention is given to other gene transfer trials as examples of key points. Attention is also given to advancements in vector technology that have not progressed to clinical trial but are poised to facilitate meaningful clinical advances in the near future. Where appropriate, challenges and obstacles to gene transfer trials are discussed, such as the possibility of vector-related oncogenesis and deleterious immune responses.

1.2 Statistics and Numbers on Gene Transfer Trials

The use of viral vectors to deliver genes to patients affected with neurological disorders is an attractive concept to researchers and clinicians. Due to the natural ability of viruses to infect cells with nucleic acid, they have gained much attention as a vector for delivery of genetic material since the 1980s. A total of 1843 gene therapy clinical trials have been initiated (current as of 2012) (Ginn, Alexander, Edelstein, Abedi, & Wixon, 2013). Of gene therapy clinical trials (including plasmid DNA) registered from 2000 to 2012 that focus on neurological disorders, 48% use an AAV vector delivery system, notably including Parkinson's disease (PD) and late infantile neuronal ceriod lipofuscinosis (Batten disease) (http://www.wiley.com//legacy/wileychi/genmed/clinical/) (Gray, Woodard, & Samulski, 2010).

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1.3 Distinction between In Vivo and Ex Vivo Gene Transfer

Although some overlap in approach exists (such as for PD), in general retroviral vector trials have been used for ex vivo gene transfer while AAV vectors have been used for in vivo gene transfer. *Ex vivo* refers to the transduction of cells outside of the body, then introduction of those cells into the patient's body; in this case the vector itself is not administered to the patient directly (Figure 2.1). In past gene transfer trials, this approach is analogous to a bone marrow transplant and for CNS diseases the nervous system is treated by one of three nonexclusive paths: (1) Bone marrow stem cells differentiate into microglia that migrate throughout the brain, serving as “factories” to secrete the missing factor to neural cells. (2) The immune system is corrected, which results in benefits to the nervous system, or (3) Bone marrow-derived cells secrete the missing factor into the blood, some of which translocates across the BBB into the nervous system. In vivo gene transfer involves injection of the gene transfer vector directly into the patient. Cells are transduced (corrected) within the patient's body, hopefully exerting a therapeutic effect.

2. CNS CLINICAL TRIALS UTILIZING RETROVIRAL VECTORS

2.1 Introduction

The concept of gene delivery using virus-derived vectors was introduced in the mid-twentieth century. This approach was widely perceived as ground-breaking for treating a wide spectrum of genetic diseases (Friedmann, 1976). Development of efficient viral vector platforms rapidly propelled human gene therapy to the forefront as a means to correct otherwise fatal disorders. Simple retroviral vectors (γ-retroviruses) were among the first that were utilized in preclinical and clinical studies due to low immunogenicity, long-term transgene expression, and a relatively simple manufacturing protocol. The first proof-of-principle study using γ-retroviruses to correct a genetic disease in humans was a trial attempting to correct a severe combined immunodeficiency disorder (SCID) carried out in Kenneth Culver's laboratory (Blaese et al., 1995). In this clinical trial, CD34-positive cells were isolated from two patients with inherited adenosine deaminase deficiency, transduced *ex vivo* with a γ-retrovirus, which carried the normal version of the gene, and readministered to the patients. One patient exhibited a temporary response, although she continued on enzyme replacement therapy (ERT). The response was far more limited in the second patient. Similar clinical trials were later conducted by Alain Fischer's group in France (Cavazzana-Calvo et al., 2000), and by Adrian Thrasher's group in London (Gaspar et al., 2004). Tragically, in both clinical trials, several children developed T-cell leukemia within 2–5 years after gene therapy, and one of these children died. Analysis of the patients with leukemia revealed insertional mutagenesis in the leukemic T-cell clone, which was correlated with the onset of leukemia. Integration of the provirus resulted in upregulation of adjacent proto-oncogenes due to the strong promoter elements in the γ-retrovirus long terminal repeats (LTR). Until this incidence, the risk of insertional mutagenesis of retroviruses was estimated to be only 10^{-6}–10^{-8} per integration event (Stocking et al., 1993). Currently, the frequency for a transforming insertion in a region of 10 kb around a proto-oncogene is calculated as 10^{-2}–10^{-3} (Baum & Fehse, 2003), which highlights the limitations of the γ-retroviral vector approach.

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The likelihood of insertional mutagenesis might be lower when utilizing lentiviral vectors. For example, in a model with tumor-susceptible mice, transplantation of γ-retroviral vector-transduced hematopoietic cells resulted in an accelerated tumorigenic process, whereas no additional adverse events were detected with lentiviral vectors (Montini et al., 2006). Furthermore, it has been demonstrated that a higher quantity of lentiviral vector is necessary to cause an oncogenic risk similar to that of γ-retroviral vectors (Montini et al., 2009). Thus, the use of lentiviral vectors should provide significant advantages in reducing the potential for adverse mutagenic events. Interestingly, this problem had already been taken into consideration from a theoretical point of view before the clinical trials mentioned above (Cline, 1985; Hacein-Bey-Abina, von Kalle, Schmidt, Le Deist, et al., 2003; Hacein-Bey-Abina, von Kalle, Schmidt, McCormack, et al., 2003b).

Another major disadvantage of using γ-retroviral vectors is the fact that they only transduce dividing cells. Infection of nondividing cells is possible, but the nuclear membrane must be disassembled for integration of the viral cDNA into the host-cell genome (Lewis & Emerman, 1994; Miller, Adam, & Miller, 1990). Thus, in order to target nondividing or terminally differentiated cells (e.g., postmitotic neurons), lentiviral vectors should be employed. Nuclear import of the lentiviral genome is maintained by the host proteins (Lewis & Emerman, 1994). Efficient transduction of neuronal cells in vivo was shown in the very first publication that utilized a lentiviral platform for the gene delivery (Naldini, Blomer, Gage, Trono, & Verma, 1996). Since then, hundreds of publications have reconnoitered the use of HIV-based vectors for therapeutic gene delivery in the CNS (Azzouz et al., 2002; Baekelandt et al., 2002; Bayer et al., 2008; Consiglio et al., 2001; Kantor et al., 2011; Perrin et al., 2007; Sergijenko et al., 2013; Wong et al., 2004; de Almeida, Zala, Aebischer, & Deglon, 2001).

Lentiviral vectors also have been shown to transduce most cell types within the CNS in vitro and in vivo, including premitotic and postmitotic neurons, adult neuronal stem cells, astrocytes, and oligodendrocytes (Blomer et al., 1997; Consiglio et al., 2004; Jakobsson, Ericson, Jansson, Bjork, & Lundberg, 2003; Miletic et al., 2004; Naldini, Blomer, Gage, et al., 1996). Lentiviral vectors can engender long-term, stable transgene expression in the CNS, persisting more than 6 months in the rat brain and as long as 12 months in other tissues (Bayer et al., 2008; Kantor et al., 2011; Yanez-Munoz et al., 2006). As mentioned above, no significant side effects have been associated with the delivery of lentiviral vectors. In addition, scalable systems for clinically graded vectors production have been developed and are currently in use (Kafri, van Praag, Ouyang, Gage, & Verma, 1999; Klages, Zufferey, & Trono, 2000). Furthermore, lentiviral vectors have been shown to achieve therapeutic levels of transgene expression in multiple animal models including Huntington's disease (HD) (Bensadoun, de Almeida, Dreano, Aebischer, & Deglon, 2001; Regulier, Pereira de Almeida, Sommer, Aebischer, & Deglon, 2002; Zala et al., 2004); PD (Azzouz, Ralph, Wong, et al., 2004; Lo Bianco et al., 2004); lysosomal storage diseases (LSDs) (Biffi et al., 2004; Bosch, Perret, Desmaris, Trono, & Heard, 2000; Consiglio et al., 2001); Alzheimer disease (Dodart et al., 2005; Pickford et al., 2008); ALS (Azzouz, Ralph, Storkebaum, et al., 2004a; Ralph et al., 2005; Raoul et al., 2005), and others. These results pave the road for the clinical trials employing lentiviral vectors.

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The first clinical trials that employed lentiviral vectors to treat inherited disorders for adrenoleukodystrophy (ALD) (Phase I/II) (Cartier et al., 2009) and β-thalassemia (currently Phase III) were conducted in Europe. These clinical trials provided evidence of therapeutic efficacy in several patients for at least 6 years. Another clinical trial utilized lentiviral vectors for delivery of multiple genes involved in dopamine biosynthesis. This vector (ProSavin) is currently being tested in Phase I/II trials for PD (Grosset, 2010; Stewart et al., 2011). Finally, two more recent trials utilized lentiviral vectors for gene therapy of inherited diseases; metachromatic leukodystrophy (MLD) (Biffi et al., 2013) (Phase I/II), and Wiskott–Aldrich syndrome (WAS) (Aiuti et al., 2013) (Phase I/II) are discussed in this chapter. A list of retroviral gene transfer clinical trials focused on CNS-related disorders is provided in Table2.1.

2.1.1 Life Cycle of Lentiviral Vectors—Lentiviral vectors have evolved from HIV-1, one of the most intensively studied viruses (Figure 2.2) (reviewed in Coffin, Hughes, and Varmus (1997)). The virus is 80–100 nm in diameter, and its genome contains two copies of single-stranded (ss) RNA. HIV-1 is an enveloped virus that targets hematogenous host cells expressing a CD4-receptor and a CCR5- or CXCR4-coreceptor (reviewed in Coffin et al. (1997)). Following cell entry, HIV reverse transcribes the RNA molecule resulting in synthesis of linear, double-stranded DNA, which is translocated into the nucleus with the help of cellular proteins (Lewis & Emerman, 1994). Viral DNA comprises two identical repetitive sequences: the 5′-LTR and 3′-LTR harboring the promoter and polyadenylation signal (Poly-A) of the virus, respectively. Other essential elements within the HIV-genome include the primer-binding site (PBS) and polypurine tract (PPT). A PBS is a region located just downstream from the 5′-LTR, provided a primer-binding site for initiation of minus-strand synthesis. This primer is carried by a $tRNA_3^{Lys}$, although other tRNAs can be utilized (Hansen, Schulze, Mellert, & Moelling, 1988; Panganiban & Fiore, 1988). The plus-strand primer is provided by a 15-nucleotide PPT, a purine-rich sequence. The PPT is highly conserved in most retroviruses and has been shown to be selectively used as the site of plus-strand initiation. Soon after completion of DNA synthesis, the viral integrase protein (*Int*) recognizes and cleaves within the *att* sites located on the both ends of the viral DNA, eliminating the terminal two bases from each 3′ end. The resulting recessed 3′-OH group defines the provirus attachment sites utilized by the viral cDNA for integrating into host chromosomes (Colicelli & Goff, 1985, 1988; Craigie, Fujiwara, & Bushman, 1990; Leavitt, Rose, & Varmus, 1992). Following integration, the DNA of the HIV replicates along with the host genome and is passed on to the original cell's offspring; all descendants of the infected cell also will bear proviruses in their genomes (Buchow, Tschachler, Gallo, & Reitz, 1989). The proviral DNA expresses three core genes: *gag* (encodes a viral matrix, capsid, and nucleocapsid proteins); *pol* (encodes a protease, reverse transcriptase, and integrase); and *env* (encodes a viral envelope). In addition, the HIV-1 genome carries two regulatory genes (*rev* and *tat*) and four accessory genes (*nef*, *vif*, *vpr*, and *vpu*), involved in the viral entry, replication, and particle release (reviewed in Coffin et al. (1997)). In contrast to the regulatory proteins, the accessory proteins are dispensable for the vector's production, and can be deleted to create space for a transgene. Oppositely, the regulatory protein *Rev* (product of the *rev* gene) is essential for exporting full-length and partially spliced RNAs harboring a Rev response element from the nucleus to the cytoplasm (reviewed in Cockrell

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and Kafri (2007)). When transcription initiates, the host RNA-splicing machinery in the nucleus quickly splices the RNA, so that only the regulatory proteins *Rev* and *Tat* and the accessory protein *Nef* are produced. In the presence of the *Rev* protein, RNA is exported from the nucleus before it can be spliced, so that the structural proteins and RNA genome can be produced. This creates a positive feedback loop allowing the virus to overwhelm the host's defenses, and providing time-dependent regulation of replication (Dayton, Powell, & Dayton, 1989; Emerman, Vazeux, & Peden, 1989; Hadzopoulou-Cladaras et al., 1989). HIV-1 completes the life cycle by budding out after the virus reassembles in the cytoplasm membrane (reviewed in Coffin et al. (1997)).

2.1.2 Development of Lentiviral Vectors for CNS Gene Delivery Applications—

To reduce a risk of replication-competent retroviruses (RCRs), essential viral genome components are expressed from two plasmids, delivering *in trans* structural and enzymatic proteins (Naldini, Blomer, Gallay, et al., 1996). The plasmids were designed with very limited overlap in their sequence in order to minimize the likelihood of homologous recombination between them. Furthermore, if replication-competent virus is inadvertently generated throughout recombination between the plasmids, it will lack all of the accessory proteins and the pathogenic characteristics of wild-type HIV. Importantly, the transgene cassette carries all the *cis*-acting elements allowing the viral RNA to be packaged into the viral particles; thus, only transgenic DNA can be delivered into targeted cells. The packaging and envelope cassettes do not carry any of the *cis*-acting packaging elements, thus they cannot be transferred into the produced virions.

A further step toward improving the vector's safety was achieved when heterologous envelope proteins were incorporated into the viral particles. Importantly, the use of these envelopes dramatically expanded the host range (Canivet, Hoffman, Hardy, Sernatinger, & Levy, 1990; Chesebro, Wehrly, & Maury, 1990). Among the most popular envelopes used to pseudotype lentiviral vectors is a vesicular stomatitis glycoprotein G (VSV-G that has been demonstrated by Friedmann's group being capable of efficiently incorporated into Moloney murine leukemia virus (MoMLV)-based retroviral vectors encoding the gene for neomycin phosphotransferase (Neo) (Emi, Friedmann, & Yee, 1991). Although the VSV-G is one of the commonly employed proteins, lentiviral vectors can be pseudotyped with a wide spectrum of other heterologous envelopes (see later in this section).

Replacement of the parental promoter of HIV located in the viral LTRs by heterologous promoters, such as a cytomegalovirus (CMV) promoter or a Rous sarcoma virus promoter, further contributes to improved vector safety. In addition, deletions introduced in the viral LTRs eliminated viral enhancer/promoter sequences and the TATA box, creating a self-inactivating (SIN) lentiviral vector (Dull et al., 1998; Iwakuma, Cui, & Chang, 1999; Miyoshi, Blomer, Takahashi, Gage, & Verma, 1998). Incorporated initially into the 3′-LTR of the expression cassette, these deletions extended to the 5′-LTR during reverse transcription, thus preventing the full-length mRNA synthesis (Iwakuma et al., 1999; Miyoshi et al., 1998; Zufferey et al., 1998). This also implies that SIN-vectors are defective for mobilization by replication-competent HIV. Furthermore, the lack of enhancer/promoter elements in the SIN-vector reduces the risk of inadvertently activating silent host-cell promoters reducing any possible influence on transgene expression. Finally, the

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incorporation of the woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) (Zufferey, Donello, Trono, & Hope, 1999) and the central polypurine tract (Zennou et al., 2000) into the vector cassette increased the transduction efficiency and expression strength of lentiviral vectors (Figure 2.3). It should be noted that WPRE has been successfully incorporated into the expression cassette of other vectors including AAV and γ-retroviruses. Importantly, all these modifications did not reduce vector yield or the ability of the vectors to transduce nondividing cells, including terminally differentiated neurons (Dull et al., 1998; Kafri et al., 1999; Naldini, Blomer, Gage, et al., 1996; Naldini, Blomer, Gallay, et al., 1996; Zufferey, Nagy, Mandel, Naldini, & Trono, 1997).

To better control the transgene expression in specific cell populations including those of the brain, different tissue-specific promoters have been exploited. This is discussed in detail on the chapter “Methods for Gene Transfer to the Central Nervous System.”

As it has been mentioned above, pseudotyping the lentiviral vectors by heterologous envelope proteins contributed immensely to broadening the viral tropism. Besides VSV-G, other proteins have been employed to target the CNS. Most prominent are a lymphocytic choriomeningitis virus (LCMV), Mokola virus (MV), Moloney murine leukemia virus (MoMLV), Ross River virus, and rabies virus (RV), which have all demonstrated value for transduction into the different cell types of the CNS (for a detailed review, see Cronin, Zhang, and Reiser (2005)). Envelopes of the RV and the MV viruses are closely related proteins and were the first to be incorporated into lentiviral vectors. Both proteins have been demonstrated to be efficient in transducing neurons of the brain (Conzelmann, Cox, Schneider, & Thiel, 1990; Mochizuki, Schwartz, Tanaka, Brady, & Reiser, 1998). A major difference between MV and RV envelopes is that the glycoprotein of RV supports both retrograde and anterograde transport, while the glycoprotein of the MV is capable of utilizing predominantly the retrograde axonal transport path (Desmaris et al., 2001; Sacramento, Badrane, Bourhy, & Tordo, 1992). In contrast to the predominate neuronal affinity of the RV and MV vectors, LCMV- and MoMLV-pseudotyped lentiviral vectors selectively transduce astrocytes (Cannon, Sew, Montero, Burton, & Greenamyre, 2011). A more comprehensive overview discussing different envelope proteins can be found in the chapter “Methods for Gene Transfer to the Central Nervous System.”

2.2 Lentiviral Vectors in Clinical Trials for Neurodegenerative Diseases

2.2.1 X-linked Adrenoleukodystrophy—X-linked adrenoleukodystrophy (X-ALD) is a genetic disorder with an incidence estimated between 1:20,000 and 1:50,000. ALD is caused by mutations in ABCD1, a member of the superfamily of ATP-binding cassette transporters that are involved in peroxisomal import of fatty acids and/or fatty acyl-CoAs in the organelle (reviewed in Kemp, Berger, and Aubourg (2012)). Mutations in the protein are associated with accumulation of very-long chain fatty acids, particularly cerotic acid in tissues throughout the body. The most severely affected tissues are the myelin in the CNS, the adrenal cortex, and the Leydig cells in the testes. As an X-linked disorder, ALD presents most commonly in males, however, approximately 50% of heterozygote females show some symptoms later in life. More than one-third of ALD patients suffer from the cerebral form of the disease, which is the most severe form. In boys affected with the childhood cerebral

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form of ALD, initial symptoms show up around 6–8 years old and include emotional instability, hyperactivity, and disruptive behavior. If untreated, cerebral ALD is characterized by progressive demyelination, rapid regression to a vegetative state, and death before adolescence.

Hematopoietic cell transplantation (HCT) is a treatment approach with some efficacy for ALD, but it is limited by donor-related constraints and carries a considerable risk of mortality. Hematopoietic stem cell (HSC) gene therapy provides an alternative approach. In vitro data demonstrated that lentiviral vectors expressing wild-type ABCD1 protein are capable of attaining biochemical correction in ABCD1-deficient monocytes/macrophages originated from the $CD34^+$ cells isolated from the patients (Benhamida et al., 2003). In vivo, reinfusion of ALD Sca-1^+ cells (a functional equivalent of $CD34^+$ cells in humans) transduced with lentiviral vector that expresses wild-type ABCD1 protein into ALD mice resulted in robust and long-term expression of the protein in brain cells (Cartier et al., 2009). More importantly, human CD34-positive cells have been shown to reach and populate the brain of recipient mice, where they successfully differentiated into microglial cells expressing human ALD protein (Asheuer et al., 2004).

This preclinical data paved the way for a clinical trial employing lentiviral vectors to deliver the *abcd1* gene to two patients diagnosed with X-ALD. These patients suffered from moderate cerebral demyelination that had developed prior to treatment, and had no human leukocyte antigen-matched donor, or cord blood for allogeneic HCT (Cartier et al., 2009). CD34-positive cells were isolated from the patients and transduced *ex vivo* with a replication-incompetent (SIN) version of the lentiviral vector expressing the wild-typed *abcd1* gene from the myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev PBS-substituted (MND) promoter (Cartier et al., 2009). Transduced cells were evaluated for RCRs before reinfusion to the patients. Data of this clinical study demonstrated that about 50% of the cells expressed ALD protein after infusion in both patients expressing similar levels of the functional protein (Cartier et al., 2009). Typically for lentiviral vector's integration, the proviral sequences were mostly found in coding regions without preferences for proto-oncogene or tumor suppressor gene integration. More importantly, no clonal expansion was detected over the time. Importantly, the demyelinating lesions declined dramatically in both patients 12 months after reinfusion, indicating reversibility of the demyelination process. Also, neurological symptoms associated with the disease were greatly alleviated by the treatment. Indeed, both patients have demonstrated normal cognitive and motor functions for a period of almost 6 years after treatment. It has to be noted that ALD patients, which do not receive treatment, all develop devastating progression of cerebral demyelination that is associated with motor and cognitive degeneration.

2.2.2 Lysosomal Storage Diseases—LSDs are a group of approximately 50 rare genetic metabolic disorders that result from defects in lysosomal function. Although the pathological mechanisms vary depending on the mutated gene, the enzyme malfunction generally leads to abnormal accumulation of substances inside the lysosome, which gradually interfere with cell function and lead to the cell death. LSDs are usually a consequence of the deficiency of a single enzyme required for the metabolism of lipids or

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glycoproteins. Individually, LSDs occur with incidences of less than 1:100,000; however, as a group the incidence is about 1:5000–1:10,000. Most of these disorders are inherited in an autosomal recessive manner such as Niemann–Pick disease, Krabbe disease, MLD, and WAS. However, a few are X-linked recessively inherited, such as Fabry disease and Hunter syndrome (Mucopolysaccharidosis (MPS) II) (reviewed in Boustany (2013)). LSDs are neurodegenerative syndromes characterized by progressive and severe developmental delay, movement disorders, seizures, dementia, deafness, and/or blindness. However, symptoms vary considerably depending on the particular disorder and other variables like the age of onset. These diseases affect mostly children, and in some severe diseases they can be fatal by 2–3 years of age. In general, there are no cures for LSDs and treatment is mostly symptomatic, although some recent approaches have shown some success for specific diseases. Those include hematopoietic stem cell transplantation (HSCT), umbilical cord blood transfusion, and ERT (Bruni, Loschi, Incerti, Gabrielli, & Coppa, 2007; Clarke & Iwanochko, 2005; Escolar et al., 2005).

In many cases for LSDs, relatively low amounts of enzyme (5–10% of normal levels) are required to halt disease progression, and there is a bystander effect by which an enzyme-producing cell can treat neighboring cells by cross-correction. The missing enzyme is secreted from the expressing cell and can be taken up by neighboring cells via the mannose-6-phosphate pathway (Sands & Davidson, 2006). For LSDs, hypothetically, the ideal approach would be to broadly transduce cells throughout the CNS in order to secrete enough therapeutic enzyme in a spatially appropriate manner to reduce the toxic substrate in the entire CNS. Lentiviral vectors employed for treatment have been shown to be effective in at least two LSDs, MLD, and WAS.

MLD is commonly listed in the family of the sphingolipidoses, because it affects metabolism of sphingolipids. Since sphingolipids play a key role in myelin synthesis, the disease affects both branches of the nervous systems: the peripheral and the central. MLD is caused by a deficiency in the enzyme, arylsulfatase A (ARSA) (Poeppel et al., 2005). It has been demonstrated that the enzyme activity in the majority of cases declines to the level of 10% or lower compared to that of unaffected controls. The absence of functional ARSA protein causes the sulfatides to accumulate in many tissues of the body, eventually damaging and destroying the myelin sheath of the nerve fibers of the PNS and the CNS that is ultimately manifested as severe progressive motor and cognitive impairments. The disease primarily affects children and invariably leads to premature death. MLD has been classified into several clinical forms depending on the onset of symptoms. The most severe symptoms are exhibited by the late-infantile form (LI), where symptoms appear by the second year of life with ensuing death a few years thereafter.

Disease progression is reversible in mice by overexpressing the *arca* gene in transduced hematopoietic stem cell gene therapy (HSC-GT) using lentiviral vectors. A similar degree of rescue has not been achieved by HSCT (Biffi et al., 2004, 2006). These results support other data suggesting that HSCT is insufficient to provide consistent benefits in MLD patients (Biffi, Aubourg, & Cartier, 2011; Boelens, Prasad, Tolar, Wynn, & Peters, 2010; Rovelli & Steward, 2005). Successful preclinical data paved the way for a clinical trial involving three presymptomatic children with MLD (all patients were tested for ARSA deficiency and had

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siblings with the LI form of the disease) (Biffi et al., 2013). After the lentiviral vector was generated following an enhanced clinical protocol, autologous HSPCs collected from the patients were transduced ex vivo by the vector expressing functional ARSA enzyme (Biffi et al., 2013). Because the treated patients carried nonfunctional and truncated ARSA protein, it was predicted that the immune response toward the vector-derived ARSA is likely to be insignificant. This consideration allowed exclusion of an immunosuppressive regimen.

All three patients treated with HSC-GT have been monitored from 18 to 24 months manifesting no sign of the disease (7–21 months after predicted disease onset) (Biffi et al., 2013). Patients demonstrated high-level stable engraftment of the lentiviral vector-transduced cells in bone marrow and peripheral blood. Remarkably, high levels of functional ARSA protein's expression have been achieved in different populations of myeloid cells and in some patients those levels were even higher than in unaffected controls. Importantly, no replication-competent virus has been detected, by means of anti-p^{24} ELISA (Biffi et al., 2013).

WAS is an X-linked inherited immunodeficiency disorder characterized by infections, thrombocytopenia, eczema, immune deficiency, and a high susceptibility to develop malignancies (Notarangelo, Miao, & Ochs, 2008). WAS is caused by mutations in *was* gene, which expresses the WASP enzyme and plays a key role in cytoskeletal functions. Patients deficient in WASP demonstrate incompetence in cell proliferation responses, cell migration, immunological synapsis formation, and more (Bosticardo, Marangoni, Aiuti, Villa, & Grazia Roncarolo, 2009).

γ-retroviruses were among the first employed for delivering the wild-type *was* gene to patients and expressing the WASP enzyme (Seymour & Thrasher, 2012). The very promising initial results were overshadowed by several leukemia cases developed by the patients that were ascribed to retroviral vector integration within the proto-oncogene, LMO2. This integration has been directly linked to pathological activation of the gene (Boztug et al., 2010). It should be noted that the risk of leukemogenesis is a particular concern for the cancer-prone WAS patients. Lentiviral vectors appear to be significantly safer, likely due to the different integration pattern discussed above.

The lentiviral vector platform has been employed by Aiuti and colleagues in a clinical trial (Phase I/II) in three children with WAS disease (Aiuti et al., 2013). Similar to the approach of the MLD clinical trial, autologous bone marrow-derived CD34-positive cells were isolated from the patients and transduced *ex vivo* with a self-inactivated lentiviral vector carrying the wild-type *was* gene. All three patients were symptomatic at the time of the enrollment and suffered from recurrent infections, eczema, bleeding, and thrombocytopenia. Patients were confirmed to carry mutations in the X-linked WAS gene that severely interfered with the functional activity of the WASP enzyme. In all cases, no compatible allogeneic donors for the HSCP were identified (Aiuti et al., 2013). Transduced cells were reinfused back to the patients 3 days after transduction. Remarkably, the vector transduction was shown to be very efficient as more than two copies of the viral genome per transduced cell were found by the real-time PCR. Furthermore, all three patients showed robust and multilineage engraftment of gene-corrected cells in bone marrow and peripheral blood.

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Importantly, transduction with the lentiviral vectors associated with neither expansion of the cells in bone marrow nor cellular proliferation in peripheral blood. In addition, no p^{24} protein has been detected in samples isolated from the bone marrow and peripheral blood, indicating that the reinfused vector remained to be replication-defective (Aiuti et al., 2013). Importantly, patients showed significant improvement in symptoms of the disease. In fact, the frequency and severity of infections progressively decreased in all three patients. The immune system gained full control of preexisting CMV replication, allowing withdrawal of anti-infection prophylaxis in two patients. In addition, pretreatment eczema resolved between 6 and 12 months after gene therapy and has not reoccurred (Aiuti et al., 2013).

Although a definitive conclusion on the results must await the long-term observations, the data presented in both the MLD and WAS clinical trials support the idea that lentiviral vector-based gene therapy is a safer alternative, compared with the gene transfer approach employing γ-retroviruses (Aiuti et al., 2013; Biffi et al., 2013).

2.2.3 Parkinson's Disease—PD is the second most common age-related progressive neurodegenerative disorder. It affects about 4 million people worldwide and this number is expected to grow as the average age of the population increases. PD characterized by degenerative processes in the CNS resulted in progressive loss of dopamine-producing cells in the substantia nigra (Feng & Maguire-Zeiss, 2010). Early symptoms of the disease include shaking, slowness, and difficulty with walking. As disease progresses, cognition-related symptoms arise; these include difficulty in learning, patterns recognition, perception, and thinking. In the advanced stages of the disease dementia commonly occurred. PD also characterized by behavioral and emotional complications. The disease is more common in older people; most cases occur after the 50 years of age. PD is idiopathic, although some cases have been shown to have a genetic basis (Feng & Maguire-Zeiss, 2010).

In most instances PD patients receive levodopa treatment, the precursor to the dopamine. However, as the disease progresses and dopaminergic neurons continue to decline, these drugs eventually become ineffective at treating the symptoms. Moreover, long-term use of the drugs has been found to be associated with dyskinesia, marked by involuntary writhing movements. Clearly, new treatment strategies are needed for the treatment of PD.

The most straightforward strategy is to attain therapeutic level of dopa-mine by overexpressing enzymes that contribute to dopamine synthesis. Vector-mediated gene delivery of these enzymes is advantageous because (1) The therapeutic delivery remains restricted to the transduced area of brain; (2) Vectors are capable of supporting long-term gene expression; thus, repeated treatments are unnecessary; (3) Vectors can efficiently transduce postmitotic neurons and express those enzymes involved in the dopamine biosynthesis. Unlike AAV vectors, lentiviral vectors can accommodate multiple transgenes.

As mentioned above, lentiviral vectors provide an excellent choice for PD gene therapy, because these vectors can carry complex polycistronic cDNAs, which allows the expression of the three enzymes involved in dopamine biosynthesis. Lentiviral vectors have been employed by Oxford BioMedica, for a Phase I/II trial for PD. This clinical trial utilizes a multicistronic self-inactivated lentiviral vector, EIAV, to incorporate three transgenic

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sequences required for the synthesis of dopamine from tyro-sine: tyrosine hydroxylase, GTP cyclohydrolase I (required for the synthesis of tetrahydrobiopterin, an essential aromatic L-amino acid decarboxylase's (AADC) cofactor), and AADC (ProSavin; Lenti-TH-AADC-CH1) targeting striatal neurons (clinical trial identifier, NCT00627588). Furthermore, the tyrosine hydroxylase enzyme has been mutated to prevent the normal dopamine feedback inhibition (Kumer & Vrana, 1996; Wachtel, Bencsics, & Kang, 1997). Also, like AAV clinical trials with AADC, the lentiviral treatment includes the administration of levodopa to stimulate dopamine synthesis (Azzouz et al., 2002). The initial Phase of the clinical trial involved a small cohort of patients aimed to evaluate different doses of the lentiviral vector. Both doses have been found to be well tolerated in the patients, with no evidence of immunotoxicity or other adverse effects. In comparison to the low-dose group, the higher dose group showed higher level of motor improvement after 6 months. However, even the lower dose was sufficient to induce dopamine expression in a statistically significant manner, as seen in an improved UPDRS III “off” score. The Phase II part of the clinical trial is set to enroll more patients diagnosed with bilateral idiopathic PD for a period that is greater than 5 years, positively responding to dopaminergic therapy.

Alternative approach evolved for the treatment of PD based on overexpression of trophic factors playing role in protecting dopaminergic neurons. Overexpression of lentiviral-derived glial cell line-derived neurotrophic factor (GDNF) has proven sufficient to protect neurons in an animal model of HD (Schapira & Olanow, 2003). A potential caveat of this approach is that high levels of GDNF may trigger serious side effects arising from the overexpression of dopamine neurotransmitter in inappropriate cells, tyrosine hydroxylase silencing, and alteration of the dopamine levels in the striatum, all demonstrated in rodents (Ferreira et al., 2007; Shoulson, 1998; Simola, Morelli, & Pinna, 2008). Conversely, low levels of expression of the GDNF could be insufficient for achieving therapeutic effects, as evidenced in clinical trials-delivered GDNF by means of intraputaminal injections (Barker, 2006; Evans & Barker, 2008; Nutt et al., 2003). In addition, more work should be done to ensure safety of the therapy, if the GDNF is pursued as a target (Yu & Neimat, 2008).

As pointed out earlier in this chapter, the formation of cytoplasmic inclusions in the form of Lewy bodies that contain α-synuclein is a hallmark of the PD. Overexpression of α-synuclein and associated accumulation in neurons are thought to be neurotoxic. Therefore, α-synuclein downregulation could be beneficial for alleviation of the associated neurotoxicity. This approach has been implemented by Sapru and colleagues demonstrated that efficient silencing of human α-synuclein could be achieved by lentiviral vector delivered anti-α-synuclein RNA*i* to rat's striatum following over-expression of human α-synuclein gene (Sapru et al., 2006). Furthermore, Fountaine and colleagues demonstrated 80% reduction in the protein level associated with improvement in cellular dopamine homeostasis using similar approach (Fountaine et al., 2008; Fountaine & Wade-Martins, 2007).

2.3 Conclusions for Retroviral Vector Gene Transfer Trials

The most common form of gene therapy involves using cDNAs that express a therapeutic gene delivered by a vector. γ-Retroviral vectors and lentiviral vectors are among the most

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commonly employed viral platforms for gene delivery. Both vector platforms are capable of delivering large genetic payloads, maintaining stable and long-term transgene expression, and integrating genetic material into the host chromosomes. Transduced cells can then be transplanted back into the patient and proliferate with the correct gene, producing healthy cells. More than 50 patients affected by genetic diseases have been treated so far, predominantly with γ-retroviral vectors. Most patients have benefited from the treatment, however the incidence of leukemia and myelodysplasia in some patients with SCID-X1, chronic granulomatous disease, and WAS has raised questions about the biosafety of γ-retroviral vectors. The adverse events have generally been ascribed to vector integration in the vicinity of specific proto-oncogenes or tumor-specific genes result in the aberrant expression.

Lentiviral vectors brought striking improvements reflected in the current clinical trials, primarily because their integration profile is different from the γ-retroviral counterparts. Integration of lentiviral vectors has been shown to be associated with significantly lower frequency of insertional mutagenesis (Hematti et al., 2004; Modlich et al., 2009). Furthermore, lentiviral vectors are superior to the γ-retroviral vectors in many applications given their ability to transduce nondividing, quiescent cells. In addition, the vector's production protocol has been significantly improved for the latest clinical trials, resulted in higher purity and yields of the vector (Aiuti et al., 2013; Biffi et al., 2013).

In this chapter we discussed current clinical trials employing retroviral and lentiviral vectors, in the treatments of fatal neurodegenerative diseases including X-ALD, PDs, WAS, and leukodystrophies. Collectively, these trials are very encouraging. Patients treated for MLD, X-ALD, and WAS have demonstrated robust and multilineage engraftment of transgenes into bone marrow and peripheral blood cells. Lentiviral vectors employed in the clinical trials have been shown to remain replication-incompetent, assayed by a p^{24} ELISA. More importantly, no clonal expansion was detected over time, supporting the hypothesis that lentiviral vectors have a “safer” integration profile compared to other retroviral vectors. In fact, in the clinical trials of X-ALD, MLD, and WAS diseases investigators showed that the vectors demonstrated a heterogeneous pattern of integration into chromosomal sites, as observed after pan-genomic monitoring of blood cells. This is a presumptive indicator that no particular genomic areas carrying the vector may result in premalignant outgrowth of an HSC or another blood cell clone. Importantly, the findings were associated with a clear therapeutic benefit, because the diseases did not progress in any of the treated patients, as would be projected from untreated cases (Aiuti et al., 2013; Biffi et al., 2013). These observations correlated to the above-normal values of protein activity achieved by the lentiviral vector gene transfer.

Promising results from these clinical trials must await the long-term observations before a more definitive conclusion can be drawn on safety of the lentiviral vectors. Although the overall data thus far from clinical trials strongly support the improved safety of lentiviral vectors, it should be noted that lentiviral vectors are not completely devoid of potential detriments from insertional oncogenesis. For instance, EIAV vectors have been shown to be associated with the formation of tumors in the livers of mice following in utero and neonatal vector administration (Themis et al., 2005). Nevertheless, in the same study HIV-1-based

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vectors were not found to induce tumorigenesis. In addition, a partially dominant cell clone bearing an integration site within the HMGA2 gene in a β-thalassaemia's clinical trial raised anxiety among observers about the possibility of cancer development (Cavazzana-Calvo et al., 2010). Nevertheless, 6 years after the treatment this clone was no longer found to be dominant, although vector copies and the levels of expression of the β-globin gene remained sufficiently high to obviate the need blood transfusions. Altogether, the prospects are promising for continued growth of lentiviral vector use in certain clinical applications.

3. CNS CLINICAL TRIALS UTILIZING AAV VECTORS

3.1 Introduction

Wild-type AAV is a nonenveloped virus with a 4.7 kb ssDNA genome and it belongs to the parvovirus family (Goncalves, 2005; McCarty, Young, & Samulski, 2004). Although AAV infects humans, it is a nonpathogenic and it is classified as a dependovirus because it is unable to execute a lytic infection without coinfection with a helper virus such as adenovirus or herpesvirus (Goncalves, 2005; McCarty et al., 2004). Important for CNS gene therapy applications, AAV can transduce nondividing cells and has the ability to confer long-term stable gene expression without associated inflammation or toxicity (Bessis, GarciaCozar, & Boissier, 2004; Goncalves, 2005; Haberman, Samulski, & McCown, 2003). AAV-based gene transfer vector genomes do not integrate into the chromosome in nondividing cells, instead generally persisting as nonintegrated episomal concatamers (Carter, 2005; Schnepp, Clark, Klemanski, Pacak, & Johnson, 2003). Importantly, recombinant adeno-associated virus (rAAV) vectors have been designated as nonintegrating by the EMEA Expert Committee on Medicinal Products Gene Therapy Expert Committee (EMEA/CPMP/1879/04) and FDA (Draft Guidance for Industry: Gene Therapy Clinical Trials—Observing Participants for Delayed Adverse Events August 2005). rAAV vectors contain no AAV coding sequences, and they package any DNA cassette within its size constraints (approximately 4.7 kb) as long as the DNA is flanked by ~145 bp AAV inverted terminal repeats (ITR). Over 100 different naturally occurring AAV capsid variants have been identified with potentially different cell tropisms, providing a broad toolkit of vectors for delivery to target cells (Gao, Vandenberghe, & Wilson, 2005; Wu, Asokan, & Samulski, 2006). Clinical grade AAV can be manufactured to high titers appropriate for human use. A detailed description of how AAV has been developed into a CNS gene transfer reagent can be found in the chapter “Methods for Gene Transfer to the Central Nervous System.”

3.2 Canavan Disease

3.2.1 Overview of Canavan (Van Bogaert-Bertrand) Disease—Canavan disease (CD) is a rare pediatric leukodystrophy caused by inactivating mutations of the aspartoacylase (ASPA) gene, located on human chromosome 17p13.2, and encoding for the ASPA protein. ASPA is normally expressed in oligodendrocytes, where it ordinarily catabolizes N-acetyl-aspartate (NAA) into free acetate and aspartate. The loss of function associated with CD mutations results in the gross accumulation of NAA in the brain. As a consequence, large amounts of NAA are present in plasma and urine (Matalon et al., 1988). NAA is the second most abundant amino acid in the human brain after glutamate and is mostly localized in neurons, which constitute about 50% of brain volume. Over 40

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mutations of the ASPA gene associated with a severe phenotype in CD have been identified. CD occurs in all ethnic groups (Zeng et al., 2002), but it is present with high frequency (carrier rate of 1:40) in Ashkenazi Jewish descendants; two mutations (E285A and Y231X) account for almost all cases of CD in this population (Matalon, Michals, & Kaul, 1995). CD, like the majority of rare neurometabolic diseases, is inherited in autosomal recessive fashion. The disease was originally described in a case report by Myrtelle Canavan in 1931 as a subacute demyelinating disease of the CNS, which she presumed to be a variant of Schilder's “encephalitis periaxialis diffusa,” which instead was unrelated (Canavan, 1931). Several decades later, Van Bogaert and Bertrand described the distinctive spongiform pathology (Van Bogaert & Bertrand, 1949), which was further characterized by others on the ultrastructural level (Adachi, Schneck, Cara, & Volk, 1973). Interestingly, there have been no modern ultrastructual studies of CD since the discovery of the metabolic (Hagenfeldt, Bollgren, & Venizelos, 1987) and genetic (Kaul, Gao, Balamurugan, & Matalon, 1993) deficits, and details of the pathology are still emerging. The spongiform changes are quite distinctive and involve diffuse hypomyelination and dysmyelination throughout large white matter tracts of the brain, including the corpus callosum, region semiovale, and U-fibers. Spongiform degeneration is usually seen in the cerebrum, cerebellum, and brainstem (Sacks, Brown, & Aguilar, 1965). Distinctive features include a loss of demarcation between the cortical white and gray matter, with multiple vacuoles up to 200 μm in diameter throughout deep cortex and subcortical white matter. The white matter pathology is characterized by an accumulation of extracellular fluid between the myelin lamellae of oligodendrocytes, and is also evident in astrocytes with grossly swollen processes and abnormally elongated mitochondria (Gambetti, Mellman, & Gonatas, 1969). Vacuolated oligodendroglia are present, but most oligodendroglia are reported to have normal morphology even in areas of expanded extracellular space and disintegrating myelin sheaths. Of note, the nuclei and cell bodies of neurons are generally intact without evidence of abnormality, consistent with a primary disease of white matter, but neurotransmission along the large myelinated fiber tracts is severely impaired.

Clinical signs of CD begin in early infancy with hypotonia and megaloencephaly, followed by severe motor and cognitive developmental delay. Similar to other global degenerative disorders, there is poor head control and floppiness, which progresses to loss of developmental milestones with rigidity, spasticity, myoclonus, and lethargy. Patients may develop cortical blindness with abnormal visual evoked potentials and hearing impairment due to abnormal cochlear development, thus contributing to an already profound mental retardation. Epilepsy is a nearly universal manifestation of CD in later stages and commonly includes a mixture of absence, myoclonic, and tonic–clonic seizures. Generally speaking, there does not appear to be a clear genotype–phenotype correlation; a wide variety of mutations have been described which result in a nearly total loss of *ASPA* residual activity, elevated whole-brain NAA, and a severe phenotype. Rare variants of CD have been reported with a mild phenotype (Janson, Kolodny, et al., 2006) and only subtle signs of psychomotor delay, but this presentation is highly atypical and appears limited to a very small number of ASPA mutations. In general, most Canavan patients are severely affected and the majority do not survive past adolescence. However, with the advent of more intense supportive care and nursing assistance, some patients may survive in a debilitated state through adulthood.

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There is presently no cure for CD and current drug therapies are limited to symptomatic treatment of seizures and oral acetate supplementation (Leone, Janson, McPhee, & During, 1999), although a few unblinded trials including lithium citrate and other therapeutics such as glyceryl triacetate have demonstrated some benefit (Assadi et al., 2010; Janson et al., 2005; Madhavarao et al., 2009).

An interesting feature of CD is the inherent compartmentalization of the substrate molecule for the ASPA enzyme, NAA, which is produced and sequestered in neurons with a high concentration gradient with respect to the extracellular space. In CD, NAA builds up in the vicinity of axons and is thought to cause injury to myelin by a combination of metabolic and osmotic effects. In normal brain homeostasis, NAA is extruded from neurons to the extracellular space where it is taken up by glia as a source of acetyl groups during the process of myelination. The ASPA enzyme is associated with glia where it is physically separated from the main site of NAA synthesis. It is thought that NAA and related compounds such as NAAG normally function as organic osmolytes (Taylor et al., 1995), as well as serving a biosynthetic and signaling function during development (Baslow & Guilfoyle, 2009). Data generated using the aspa-null Nur7 model (Traka et al., 2008) of CD indicated that in the absence of ASPA, disrupted oligodendrocyte development, metabolic deficits, and oxidative stress occur (Francis, Strande, Markov, & Leone, 2012). In turn, the extensive dysmyelination that occurs in CD may be caused by abnormal formation of myelin lipids (Burri, Steffen, & Herschkowitz, 1991; Wang et al., 2009), impaired signaling and migration of oligodendrocyte precursors during development via regulation of brain-derived neurotrophic factor (Francis, Olariu, McPhee, & Leone, 2006), metabolic deficits (Traka et al., 2008), as well as by direct osmotic injury to the CNS (Baslow, 1999).

3.2.2 Nonviral Gene Therapy for CD with LPD-ASPA—Gene therapy for CD is historically significant, because it represents the first clinical use of gene therapy for a neurodegenerative disease. By 1993, the gene for CD had been identified and expertise in gene therapy was developing to the point where a clinical trial was deemed possible. Although herpesvirus vectors were being used in very limited fashion as part of a “suicide gene” approach for brain cancer human gene therapy, and newer viral vectors such as AAV were also in development, viral gene therapy vectors had not yet advanced to the point where safe and effective options were available for human experimentation. At that time, nonviral gene therapy was seen as the safest and most efficient technique for clinical application, due to the perceived risks of viral reactivation and viral contamination, insertional mutagenesis, and potential neurotoxicity. Later work would show that viral vectors such as AAV were as safe and actually more effective for long-term transgene expression, but in the early 1990s the gene therapy field was still in its infancy.

Prior to gene therapy for CD, investigators at Yale University and the University of Auckland had been developing in vivo methodologies for gene therapy of PD, and were directly approached by patient-oriented research foundations to adapt those approaches in order to fast-track clinical gene therapy for CD. The process by which the first clinical trial took place was controversial at the time, since no animal model of the disease existed and the initial clinical trial was approved on the basis of a strong clinical rationale together with animal biodistribution and toxicity studies, rather than preliminary efficacy studies (Coney,

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1996; During, 1996). Preclinical studies demonstrated that the intracerebroventricular delivery of the nonviral “lipid-encapsulated condensed plasmid DNA” (LPD) containing a LacZ reporter gene resulted in conspicuous gene expression in periventricular cells that penetrated into the brain parenchyma. Moreover, studies performed in rodents demonstrated that LPD–plasmid complexes efficiently transduced neurons and gene expression could persist for over 10 months. ASPA expression was tested in vitro and high levels of enzyme activity were obtained. The clinical LPD was combined with an expression plasmid containing the early CMV promoter, the human ASPA full-length cDNA and an SV40 poly-A flanked by AAV 145 base pair ITR. The lipid component, 3b-[*N*-(*N*9,*N*9dimethyl-aminoethane)carbamoyl] cholesterol (DC-Chol)/dioleoylphosphatidyl-ethanolamine (DOPE), was developed at the University of Pittsburgh and consisted of a mixture of a neutral lipid DOPE and a cationic liposome DC-Chol (Gao & Huang, 1996). Poly-L-Lysine was used as a DNA-condensing cationic polymer. The first two patients with CD underwent nonviral human ASPA gene therapy via direct intracerebroventricular delivery in 1996 in New Zealand (Leone et al., 2000). A subsequent Phase I clinical protocol using LPD-CMV-hASPA with a protamine-condensing polymer formulation for CD was initiated in the United States (US) in 1998, following the review of the Recombinant DNA Advisory Committee and FDA approval under Investigational New Drug Application #7307.

The first US-based Phase I Clinical Gene Therapy Protocol for CD, unlike the one conducted in New Zealand, provided the placement of Ommaya reservoirs, which were permanently implanted into the lateral ventricle of the brain, as part of an initial neurosurgical pregene therapy procedure. After the patients had completely recovered from that surgery, LPD-CMV-hASPA with condensed protamine polymer was injected into the reservoir under the scalp and administered together with systemic mannitol to improve penetration, and the vector was infused directly into the cerebrospinal fluid (CSF). In this manner, any side effect associated with either the surgical procedure or the vector delivery could be clearly distinguished and identified, in addition the reservoir enabled CSF collection without the risks, complications, and discomfort typically associated with lumbar puncture. Hence after the first two patients were treated in New Zealand in 1996, a larger cohort of 14 patients was enrolled and treated in the United States in 1998 as part of a larger Phase I pilot clinical trial. Outcome measures included noninvasive brain imaging with proton magnetic resonance spectroscopy to precisely quantify NAA levels, serial clinical and neurodevelopmental assessments, and EEG with visual and auditory evoked potentials. Although ASPA-LPD was well tolerated and the data suggested a partial stabilization of cerebral NAA levels and mild improvement in periventricular myelination, the overall improvements were of such a small magnitude that viral-based approaches were ultimately adopted.

3.2.3 Viral-Based Gene Therapy for CD with an Adeno-Associated Viral Vector (AAV2)—As with the original clinical trial using ASPA-LPD, the second CD gene therapy study, which began enrolling patients in 2001, was unique in many important respects. Particularly novel was the fact that rather than contracting with an outside pharmaceutical company, clinical-grade AAV vector was produced in an FDA-approved academic laboratory at the University of North Carolina. This was the first AAV-based gene therapy

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produced by a U.S. academic institution to be approved for neurological use by the FDA, under an Investigational New Drug Protocol #9119. However, the most groundbreaking aspect was obviously related to the fact that this was the first clinical use of AAV vectors in the human brain, and thus CD was seen as a potential gateway for gene therapy in other diseases such as PD and LSDs.

AAV-ASPA gene therapy for CD was designed as a Phase I/II safety study, with selected efficacy measures as secondary outcomes. In terms of obtaining required regulatory approvals, the main advantage of this second study, compared to the initial clinical trial with LPD-ASPA, was the strong rationale based on prior human data, as well as additional proof-of-principle and safety data from newly characterized transgenic mouse and rat models (McPhee et al., 2005). Although information on acute or long-term immunological effects in humans was lacking, accumulated experience in different animal species strongly suggested that the proposed procedure was safe. Extensive preclinical dosing and toxicology testing had been performed, including having tested the neurosurgical procedure and vector delivery protocol on young primates in 2000 to fullfil FDA requirements. Because clinical safety was of paramount concern, the protocol included numerous outcome measures with a focus on safety monitoring (Janson et al., 2002). These included a team-based assessment protocol for the acute postoperative period, as well as numerous scheduled follow-up studies during the post-hospitalization period after patients were discharged from the hospital. The clinical effects of treatment were assessed primarily with standardized clinical examinations and serial cerebral NAA measurements using noninvasive proton magnetic resonance spectroscopy. The study was powered for 21 patients based on changes in NAA levels that were expected to be clinically significant, on the order of a >2 mM decrease in whole-brain concentrations. A total of 28 patients were ultimately enrolled, which included a parallel natural history study in which serial measurements of brain NAA and morphology were measured in order to model the expected course of disease (Janson, McPhee, et al., 2006). In the end, the magnitude of effect of AAV2-ASPA on cerebral NAA was so large that the final protocol treated only 13 subjects, one less than the original nonviral study, but demonstrated a highly significant drop in pathologically elevated NAA in multiple brain regions in treated patients compared to untreated and pretreatment controls, as well as preliminary evidence of slowing brain atrophy, seizures, and neurological deterioration (Leone et al., 2012). Some important limitations of the study included the fact that most patients (13:1) were treated well into the progression of their disease, and moreover the dosing regimen was much lower than initially proposed, on the basis of FDA concerns about possible toxicity. There were no clinical adverse events specifically related to the viral vector. Immunological data showed that a minority of patients (less than 30%) had a neutralizing antibody response to the AAV capsid, and there was no correlation with clinical outcomes. In fact, one of the younger subjects with a more favorable clinical response to gene therapy had the highest transient peak in her AAV neutralizing antibody response, which was up to 10-fold over baseline levels at 3 months posttreatment, that subsequently resolved to baseline. Further investigations conducted on the serum samples demonstrated that this patient was possibly exposed to wild-type AAV within 10 months preceding treatment with recombinant AAV2-ASPA (McPhee et al., 2006). In summary, this gene

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therapy study showed favorable changes in predefined markers of disease progression, with no long-term adverse events and no deaths at over 10 years from time of treatment.

3.2.4 Future Directions for CD Gene Therapy—Gene therapy for CD has been through several iterations, which reflect important improvements in vector design. The next step in therapeutic development for CD is likely to be a larger scale efficacy study with dose escalation, using a next-generation vector together with improved vector delivery methods. The neurosurgical delivery protocol implemented was the best option available at the time. Viral vector targeting for neurogenetic disorders affecting glia still need to be optimized and less invasive delivery techniques through the CSF or blood should be considered. One major issue in CD is that NAA levels are globally elevated, but the specific location of the ASPA enzyme in association with myelin sheaths may be critical to preventing osmotic injury from excessive NAA. The majority of the currently available viral vectors do not target glia selectively and this technical limitation should be overcome. Our rationale for targeting neurons, which are the source of brain NAA, was that even a small relative decrease in NAA production and export from neurons might forestall the effects of periaxonal spongiform changes, as well as other deleterious effects related to cellular membrane properties and cellular energetics, including seizures and abnormal swelling of astrocytes. The study did in fact show a global decrease in NAA as well as a relative decrease in seizure frequencies, which supports the original rationale. However, since ASPA is temporally and spatially correlated with early migration and differentiation of glia, and NAA is a known acetyl source for lipid biosynthesis as well as a key osmolyte, alternative and complementary treatment approaches should also be explored (Baslow & Guilfoyle, 2013). These may include targeted drug approaches to modulating NAA synthesis or catabolism or anapleurotic support to the CNS as well as transplantation of normal oligodendrocyte precursors (Francis, Markov, & Leone, 2014). Moreover, gene-corrected HSCs via *ex vivo* gene therapy in the future could serve as an alternative approach to in vivo gene therapy for neurogenetic disorders (Biffi et al., 2013). While decreasing the abnormal extracellular milieu of pathologically elevated NAA showed benefits in both clinical nonviral and viral gene therapy protocols, the entire mechanistic model of CD remains elusive. Although a considerable amount of work lies ahead, targeted complementary therapeutic strategies should be explored.

3.3 Batten Disease and Mucopolysaccharidosis-Type IIIA

The clinical approaches to treat late-infantile Batten disease (late-infantile neuronal ceroid lipofuscinosis, LINCL) and MPS-type IIIA were very similar and were essentially modeled after the Canavan gene transfer trial. LINCL and MPS IIIA are both LSDs, which can benefit from cross-correction such that one transduced cell could potentially express and provide the missing enzyme to neighboring cells. LINCL (OMIM # 204500) is caused by loss-of-function mutations in the CLN2 gene, which encodes the protein tripeptidyl peptidase 1 (TPP1). In humans, onset of LINCL is around 1–6 years of age leading to mental deterioration and death around 10–12 years of age. MPS IIIA (OMIM # 252900) is caused by loss-of-function mutations in the N-sulfoglucosamine sulfohydrolase (SGSH) gene, which encodes for the protein SGSH. Onset is around 2–6 years old followed by mental degeneration and death in the second or third decade. Because of their clear genetic

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cause, lack of available treatments, and potential for cross-correction, these have been recognized as promising candidates for gene therapy.

Early results showing efficacy in rodents using intracranial administration of an AAV2/CLN2 vector (Passini et al., 2006; Sondhi et al., 2005) coincided with the dissemination of a clinical approach for CD gene therapy (Janson et al., 2002). This, along with supporting safety data (Hackett et al., 2005), allowed rapid translation to a Phase I clinical trial to treat symptomatic LINCL children. The treatment approach was to inject 2.5×10^{12} vg of AAV2/CLN2 vector to 12 locations throughout the brain, via six burr holes in the skull (Souweidane et al., 2010; Worgall et al., 2008). The procedure was found to be safe in the 10 treated subjects, and humoral responses to the vector were mild and mostly transient. Any potential treatment efficacy was minimal and not statistically significant. It was not clear whether this was due to minimal spread of the AAV2 vector or selection of subjects at the late stages of the disease. Data from CLN2 knockout mice clearly demonstrated an age-related effect to the treatment outcome using AAV/CLN2 vectors, with diminishing efficacy as mice where treated later in the disease course (Cabrera-Salazar et al., 2007; Sondhi et al., 2008). To address the very limited spread of AAV2 vectors, the rh10 AAV capsid was identified as having considerably better distribution and expression in mice and rats after intracranial injection, which correlated with better therapeutic efficacy in CLN2 knockout mice (Sondhi et al., 2007). In fact, in African green monkeys using AAVrh10 vectors by intracranial injection, expression of TPP1 was more than two standard deviations above wild type (WT) levels in approximately 32% of the entire brain (Sondhi et al., 2012). These nonhuman primate studies demonstrated safety of this new vector, prompting a second gene transfer clinical trial for LINCL (clinicaltrials.gov registration NCT01161576 and NCT01414985). Thus far, no results of this trial have been reported.

Modeling the approach taken for LINCL using AAVrh10, Tardieu et al. conducted a Phase I/II clinical trial for MPS IIIA in four patients, by intracranial administration of an AAVrh10/SGSH-SUMF1 vector (Tardieu et al., 2014). The vector expressed not only the missing enzyme, SGSH, but also the SGSH activation factor SUMF1 via an internal ribosome entry site. The vector and surgical procedure was well tolerated and deemed safe, but no clear benefit could be concluded on the four patients after follow-up for 1 year. One of these patients was 8 months old, and this early intervention would be expected to yield the strongest therapeutic response. While there is still potential for significant long-term benefits, the lack of greater efficacy even at an early age of intervention suggests that the approach is suboptimal.

Another AAV capsid, AAV9, was shown to cross the BBB after intravenous (IV) administration and transduce both neurons and glia in postnatal animals including mice, rats, cats, and nonhuman primates (Duque et al., 2009; Foust et al., 2009; Gray, Blake, et al., 2010; Gray et al., 2011). Using this vector to carry the SGSH gene, a near total rescue could be achieved in SGSH knockout mice (Ruzo et al., 2012). Similar results could be achieved at a lower dose if the vector was injected into the CSF of the cisterna magna, and the approach translated to canines (Haurigot et al., 2013). This alternative approach utilizing an AAV9 capsid with an IV or intra-CSF route of administration could represent a safer and more comprehensive gene transfer strategy to treat MPS IIIA.

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3.4 Parkinson Disease

PD presents an excellent clinical target for viral vector gene therapy for several reasons. First, the primary pathological insult involves the slow degeneration of dopamine-containing neurons in the substantia nigra, where symptoms initially can be alleviated by the administration of L-DOPA, a precursor for dopamine synthesis. Second, the anatomical sites are well defined for the dopamine-containing cell bodies, as well as the projection sites. Thus, unlike many global neurological disorders, PD exhibits defined locations of pathology and a specific neurotransmitter deficit, two properties that appear ideal for the restricted sphere of viral vector influence. Given these characteristics, the basic in vivo properties of AAV vectors provide an attractive gene therapy vehicle for the treatment of PD. As previously mentioned AAV vectors support long-term, nontoxic transduction of post-mitotic neurons in the CNS, and even with the original AAV2 serotype, sufficient areas of transduction can be achieved. For example, Hadaczek et al. found that in MPTP-lesioned nonhuman primates, clinically relevant gene expression remained 8 years after AAV transduction (Hadaczek et al., 2010). Given these beneficial properties, successful basic research findings have provided extensive support for three distinct therapeutic gene therapies that have entered clinical trials. One approach involves altering the balance of neuronal activity. Another strategy focuses upon increasing dopamine production, while delivery of dopamine-related growth factors comprises the third approach.

The first clinical trial emerged from the discovery by Luo et al. who demonstrated that AAV-mediated expression of glutamic acid decarboxylase (GAD) in the subthalamic nucleus shifted neuronal responses from excitatory to inhibitory, a change that attenuated behavioral pathology in a rat Parkinson's model (Luo et al., 2002). Based upon these findings a subsequent clinical trial was initiated where an AAV2-GAD vector was infused into the subthalamic nucleus of Parkinson's patients (Kaplitt et al., 2007). Given the positive safety and tolerability findings, a Phase II trial was initiated with 45 subjects that included both treatment and sham groups (LeWitt et al., 2011). Although the results proved significant on the primary measure (UPDRS motor-off scale), the effects were quite modest. Moreover, many other measures were not significant, even though five subjects were removed from the analysis due to injection targeting issues. The modest nature of these Phase II results led to a discontinuation of the program by the sponsor, Neurologix.

A second approach to Parkinson's gene therapy involves the expression of an enzyme directly involved in dopamine synthesis, AADC. Basic research studies in MPTP-treated primates demonstrated that AAV vector-derived AADC expression supported positive therapeutic effects, when L-DOPA was administered, even up to 8 years post-AAV transduction (Bankiewicz et al., 2000; Hadaczek et al., 2010). This series of positive preclinical findings supported subsequent Phase I clinical trials. As found in the AAV2-GAD, no toxicity occurred from the vector infusion into the putamen, but again the therapeutic outcomes were modest at best (Christine et al., 2009). A long-term follow-up to the Christine et al. (2009) Phase I study and found a significant elevation in the positron emission tomograghy (PET) value 4 years after the vector infusion (Mittermeyer et al., 2012). Although a slight improvement in the UPDRS scores were found for the first 12 months posttreatment, this modest improvement declined over the following years.

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Subsequently, another identical Phase I trial was initiated that used the same AAV-AADC vector (Muramatsu et al., 2010). Again there were no adverse events attributed to the vector infusion, and PET scanning verified the activity of the vector-derived AADC. However, as in the first study, outcome measures reflected only modest improvements. Upon reflection the original researchers hypothesized that the modest effects could be attributed to the modest level of transduction in the putamen. Thus, given the dramatic results in primate models (Hadaczek et al., 2010), another trial has been initiated with the goal of obtaining greater putamen transduction (ClinicalTrials.gov Identifier:NCT01973543). By increasing the dosage of vector these investigators expect to achieve a 60% transduction of the putamen. This ongoing Phase I study should provide more definitive evidence as to whether a significant level of efficacy can be achieved with this gene therapy approach. However, the one liability of greater transduction could be the appearance of dyskinesias, as was found after high vector doses in nonhuman primates (Bankiewicz et al., 2006).

As a side note, the approach taken to deliver the AADC gene for PD was recognized as a potential therapeutic strategy to address AADC deficiency, a genetic disease caused by loss-of-function mutations in the AADC gene. A Phase I clinical trial was conducted, using the same vector and injection approach for PD in pediatric subjects with inherited AADC deficiency. All four treated subjects (4–6 years of age) showed improved motor function, increased serotonin and dopamine in the CSF, and stable transgene expression following treatment (Hwu et al., 2012). These studies reinforced that the vector design and administration methodology results in successful expression of AADC in the putamen, in a simpler disease paradigm requiring straightforward gene replacement.

The final approach for Parkinson's gene therapy involves the expression of growth factors that in preclinical studies protect dopamine-containing neurons from cell death. For example, the naturally occurring analog of glial-derived neurotrophic factor, neurturin, prevented neurotoxic damage to dopamine-containing neurons (Gasmi, Brandon, et al., 2007a; Gasmi, Herzog, et al., 2007; Herzog et al., 2013). Given these convincing preclinical findings, Ceregene initiated clinical studies using AAV2-neurturin vectors (CERE-120). Both the Phase I (Marks et al., 2008) and initial Phase II trials (Marks et al., 2010) found no adverse effects attributed to the AAV vector, but also did not find significant improvement in the primary endpoint measures up to 12 months post AAV infusion. However, 15–18 months later, significant improvements were found. A subsequent Phase II study was initiated where the AAV2-neurturin vector was infused into both the putamen and the substantia nigra. However, this double-blind trial failed to find significant changes in the primary endpoint. Thus, this lack of positive results precluded moving to a Phase III trial.

In all of these clinical trials, the AAV vectors have performed as predicted from preclinical animal studies. Moreover, no adverse effects could be attributed to the vectors, even across the different gene products. Thus, at present these negative results suggest that like ALS models, preclinical Parkinson's models likely do not faithfully translate to the human condition. As such, effective gene therapy for PD may await newer animal models that better reflect the underlying neuropathology and progression of PD.

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3.5 Advances in Clinical Approaches Using AAV Vectors

AAV vector technology is advancing rapidly, facilitating new approaches for clinically relevant gene transfer. Changes to the capsid, genome design, and route of administration have made global CNS gene transfer possible in ways that are expected to translate to humans. This has led to a renaissance of sorts in gene therapy strategies to treat inherited neurological disorders.

3.5.1 Global Gene Transfer Strategies Using AAV Vectors—Emerging AAV vector technologies are allowing global delivery of a gene-based therapy to the entire CNS. An ideal global therapeutic approach would utilize an expressed factor that is expressed and secreted. In this scenario, the biodistribution of the expressed factor can be more pervasive than the vector biodistribution, possibly leading to disease correction even in the event of suboptimal vector delivery. Early and ongoing strategies based on this principle utilized one of two methods:

- Multiple intraparenchymal brain injections to provide pockets of gene expression throughout the brain, as described above for Canavan disease, Batten disease, and MPS IIIA.
- Transduction of the ependymal cells lining the ventricles as a means of expressing the factor into the CSF, where it would then be distributed throughout the brain.

Attempts have been made to scale up the results of intraparenchymal injections in mice and other animal models to humans. In contrast to results in mice, the relative lack of meaningful efficacy in clinical trials for CD, Batten disease, and MPS IIIA indicates the difficulty in scaling this approach from rodents to humans. This is likely attributable to limited spread of the vector from the injection site. While a large portion of the mouse brain can be transduced by a single injection, the approach simply does not scale linearly to a human brain approximately 1000 times larger. Because of this limitation, along with the highly invasive nature of stereotaxic neurosurgery, alternative approaches have been investigated to broadly and evenly transduce cells across the entire nervous system. As discussed below, newer AAV vector technology has made significant strides in this regard.

3.5.1.1 Self-complementary AAV: An advance in AAV vector technology has been the self-complementary (sc) vector, whose genome is comprised of complementary copies of the DNA insert linked in *cis* through a mutated AAV ITR. Sc AAV vectors have 10- to 100-fold higher transduction efficiency than traditional ss AAV vectors (McCarty et al., 2003; McCarty, Monahan, & Samulski, 2001), facilitating applications designed for global delivery of the virus particles, such as via IV or intra-CSF injection. For example, upon IV injection of AAV9 vectors, at equivalent doses at least 20 times more cells are transduced using sc AAV vectors compared to ss AAV vectors (Gray et al., 2011). Similarly, sc AAV vectors were found to be more efficient for intrathecal gene delivery compared to ss AAV vectors (Storek et al., 2006). However, a potential drawback of sc AAV vectors for some genes is that the packaging capacity is cut in half, to approximately 2.2 kb of foreign DNA.

3.5.1.2 IV Administration Using AAV Vectors: Multiple groups have now reported in detail the ability of AAV9 vectors to cross the BBB and transduce neurons and glia

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following IV injection in neonatal mice, adult mice, rats, cats, and nonhuman primates (Duque et al., 2009; Foust et al., 2009; Gray, Blake, et al., 2010; Gray et al., 2011). Using doses of sc AAV ranging from 5×10^{12} to 2×10^{14} vg/kg, strategies employing IV delivery of AAV9 vectors have successfully treated spinal muscular atrophy (SMA) (Foust et al., 2010), MPS IIIA (Ruzo et al., 2012), MPS IIIB (Fu, Dirosario, Killedar, Zaraspe, & McCarty, 2011), CD (Ahmed et al., 2013), and Rett syndrome (Gadalla et al., 2013; Garg et al., 2013) in mice. This is especially interesting for SMA, where the delivery efficiency is apparently high enough to achieve efficacy even though the transferred gene (*SMN1*) should exert only a cell-autonomous effect. A 10-fold lower dose can be efficacious for MPS IIIB compared with SMA, likely owing to the secretion of the expressed enzyme for MPS IIIB, and this lower dosing threshold may increase the translational feasibility of this approach. Although the intravascular approach utilizes a fluid volume that should be amenable for direct-dose scaling between rodents and humans, the translation of this approach can be challenging because of the reduced delivery efficiency in nonhuman primates compared to mice, the high amounts of vector required, the relatively high prevalence of AAV9-seropositive individuals, and the high biodistribution of the vector to peripheral tissues (Gray et al., 2011). In fact, with AAV9 about 100 copies of the vector will reach the liver for each copy delivered to the brain.

Another consideration for more global gene transfer approaches is the near-necessity for using sc AAV vectors, which limits the size of genes that can be delivered; with traditional ss AAV vectors the reduced efficiency creates a requirement for exceptionally high doses to achieve significant CNS transduction. While efficient CNS gene transfer with ss AAV9 vectors has been documented in mice, this is typically in mice injected as neonates. Astrocyte development is ongoing in newborn mice. With the important contributions of astrocytic processes to the BBB biology, there are significant alterations in the BBB composition between newborn and P7 mice. This coincides with a much higher CNS transduction efficiency with AAV9 in neonatal mice compared to juvenile or adult mice, along with an increased tropism for neurons relative to astrocytes. In adult mice the tropism shifts considerably toward astrocytes. Some groups report nearly exclusive astrocyte transduction in adult mice after IV administration (Foust et al., 2009), while others report approximately 2/3 of cells as neurons with mostly astrocytes (and some oligodendrocytes) making up the rest of transduced neural cells (Duque et al., 2009; Gray et al., 2011). This difference in the properties of AAV9 tropism and efficiency at different ages can create additional difficulties when assessing the potential for human translation. The developmental age of mice is different from humans at birth, such that a neonatal mouse is developmentally equivalent to a human that is still in utero. Thus, treatment effects in neonatal mice may overstate the potential efficacy that could be expected in humans.

3.5.1.3 Intra-CSF Administration Using AAV Vectors: A potential strategy to overcome some of the translational barriers of intravascular AAV9 vector gene therapy is to physically concentrate the vector around the nervous system. This can be done by injecting the vectors into the CSF, either via the lumbar cistern or cisterna magna. This route of administration has been evaluated in mice, pigs, and nonhuman primates using reporter genes (Federici et al., 2012; Gray, Nagabhushan Kalburgi, McCown, & Jude Samulski, 2013; Samaranch et

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al., 2012, 2013; Snyder et al., 2011). These studies have shown that intrathecal administration of AAV9 vectors can very efficiently transduce motor neurons within the spinal cord, large sensory neurons in the dorsal root ganglia, Purkinje neurons in the cerebellum, as well as additional neuronal and glial subtypes throughout entire CNS. In the case of pigs where an intrathecal catheter was used to place vector injection at the cervical, thoracic, and lumbar regions of the spinal cord, motor neuron transduction efficiency was 50–100% along the entire spinal cord length (Federici et al., 2012). The advantage of an intra-CSF route of administration is the potential to avoid preexisting neutralizing antibodies in the blood, reduce the vector load delivered to peripheral organs, and reduce the overall dose required to transduce a sufficient number of cells in the CNS.

AAV1, 2, 3, 5, 6, and 8 have all been tested in rodent models via the intrathecal route (Kao et al., 2010; Storek et al., 2006; Towne, Pertin, Beggah, Aebischer, & Decosterd, 2009; Vulchanova et al., 2010). Of these serotypes, AAV1, AAV5, AAV6, and AAV8 were found to effectively transduce neurons within the dorsal root ganglia after intrathecal injection, while AAV2 and AAV3 are only minimally effective. A comparison of AAV serotypes (1, 6, 8, and 9) in mice found AAV6 and AAV9 to have the most efficient spread after a single intrathecal injection (Snyder et al., 2011). Consistent with this, Dirren et al. reported success in targeting spinal cord motor neurons with AAV6 in nonhuman primates after a cisterna magna injection (Dirren et al., 2014). AAV7 has also shown some propensity for global CNS gene transfer following cisterna magna administration in nonhuman primates (Samaranch et al., 2013).

Preclinical studies utilizing an intra-CSF route of administration for AAV9 vectors have been encouraging. Gigaxonin knockout mice modeling giant axonal neuropathy (GAN) normally display extensive pathological aggregates of peripherin in the brainstem and dorsal spinal cord, but 3 weeks following intracisternal injection of an AAV9/gigaxonin vector these aggregates were mostly cleared (Mussche et al., 2013). In a more comprehensive preclinical study for MPS IIIA, intracisternal injection of an AAV9/sulfamidase vector was able to restore nearly normal levels of sulfamidase throughout the entire nervous system and peripheral organs, as well as correct the behavioral and survival deficits of the knockout MPS IIIA mouse model (Haurigot et al., 2013). Importantly, the approach to treat MPS IIIA was successfully scaled to canines. An exploration of dose-responsive motor neuron transduction in nonhuman primates determined the appropriate dose to target ≥30% of spinal cord motor neurons after intrathecal AAV9 administration, which was the threshold estimated to achieve efficacy for SMA (Passini et al., 2014). The successful scaling of the intra-CSF approach to larger animals suggests greater potential for the encouraging rodent studies to translate to humans.

3.5.2 Pending Clinical Trials (SMA, GAN, MPS IIIB)—Three clinical trial proposals utilizing AAV9 vectors have been reviewed by the NIH Recombinant DNA Advisory Committee, with recommendations given to each to proceed to clinical trials. These represent the first in vivo gene therapy trials with the potential to globally deliver a gene across the entire nervous system in humans. The first of these is for SMA, using a sc AAV9/SMN1 vector administered intravenously in infants with SMA (http://osp.od.nih.gov/sites/default/files/RAC_Minutes_12-12.pdf). The second of these is for GAN, using a sc AAV9/

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gigaxonin vector administered into the CSF by a lumbar intrathecal injection into subjects with GAN (http://osp.od.nih.gov/sites/default/files/RAC_Minutes_06_13_0.pdf). The third proposed trial is for MPS IIIB, using a ss AAV9/NAGLU vector administered intravenously (http://osp.od.nih.gov/sites/default/files/RAC_Agenda_031214_0.pdf). These are Phase I or Phase I/II trials focused primarily on safety. If these approaches are found to be safe and even partially effective, this will establish a platform technology to potentially treat dozens of inherited neurological disorders.

3.6 Conclusions on AAV Clinical Trials

AAV was the first vector used to treat an inherited neurological disorder. Early trials for CD and Batten disease utilized the best vector technology at the time for "global" CNS gene transfer, namely AAV2 vectors injected to as many sites throughout the brain as practical. Despite these somewhat heroic attempts, these treatments were largely ineffective in terms of correcting disease symptoms. However, these early trials paved the way for the use of AAV2 vectors to be used more focally to target PD. The trials for PD have shown signs of efficacy, but they have been plagued by placebo effects that make the short- and long-term clinical outcomes difficult to interpret. Since the early Canavan and Batten trials, other AAV capsids have been characterized that are considerably more efficient than AAV2, namely AAV9 and AAVrh10. Newer technologies surrounding the use of AAV vectors for global nervous system distribution following IV or intra-CSF administration hold great promise for transformative therapeutic approaches for neurological diseases. With the favorable safety profile of AAV-derived vectors, an increasing amount of CNS-directed clinical trials can be expected in the coming years.

4. LESSONS LEARNED FROM RELATED STUDIES

4.1 Immune Responses

The potential for immune responses against the vector or expressed transgene are often overlooked, but these can have a profound effect on long-term treatment efficacy as well as the safety of treated subjects. As discussed previously, pre-existing antibodies against the vector can dramatically inhibit the efficacy of any IV gene transfer approach. In addition to the concern of neutralizing antibodies, a cytotoxic T-lymphocyte response against the AAV2 capsid was noted in a liver-directed gene transfer trial for Hemophilia, but the appearance of this immune response only occurred in the highest dose group (2×10^{12} vg/kg) (Manno et al., 2006; Mingozzi & High, 2007). In a subsequent clinical trial for hemophilia, a similar-appearing response has been seen in some but not all patients, but it was successfully resolved without loss of gene expression using a short course of prednisone (Nathwani et al., 2011). CNS-directed clinical trials for CD and Batten disease have shown sporadic generation of anticapsid-neutralizing antibodies, but there was no evidence of any deleterious immune reactions, capsid-specific or otherwise (Hackett et al., 2005; Leone et al., 2012; McPhee et al., 2006). The planned clinical trials aimed to globally target the CNS by IV administration (see Section 3.4.2) will use doses above 2×10^{12} vg/kg and the AAV9 capsid has a high tropism for the liver, so capsid-specific immune responses are a possibility to consider.

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Immune responses against the transgene are perhaps a greater concern. Most gene transfer trials would deliver wild-type human genes, which would not be expected to generate an immune response under normal circumstances. However, in standard gene replacement approaches in patients with genetic diseases, the patients may not produce any of the missing protein in any form. In this case, such as in patients with large homozygous deletions or nonsense mutations, the treated patients might view the wild-type protein expressed from vector-transduced cells as a foreign protein. This could cause a humoral response limiting the efficacy of a secreted protein, and/or a cytotoxic T-lympocyte response against transgene-expressing cells. The notion that the CNS is “immune-priveleged” and a safe harbor for foreign transgene expression was challenged by recent results in rats and nonhuman primates (Ciesielska et al., 2013; Samaranch et al., 2014). These studies indicated that transduction of antigen-presenting cells, such as astrocytes, within the CNS could lead to immune-mediated clearance of transduced cells. Not only could this cause a loss of transgene expression, but the destruction of transduced cells could lead to deleterious adverse effects. In nonhuman primates injected with AAV9/GFP vectors (modeling the expression of a foreign antigen), dosed animals developed severe ataxia requiring euthanasia within a few weeks of injection (Samaranch et al., 2014). Until this issue is resolved, it is advisable to place restrictions on trial subjects to include only those that would not be at high risk of developing an immune response against the wild-type expressed protein.

Ex vivo gene transfer approaches utilizing retroviral vectors may not face this challenge since HSC are the target cells. Bone marrow-derived stem cells repopulate the thymus, creating tolerance to any proteins expressed in those cells. Thus, the transduced bone marrow stem cells create tolerance for the replaced protein at the same time that they mediate a therapeutic effect by providing the missing protein. There is a possibility that bone marrow transplant of normal cells, or lentivirus-transduced autologous cells, could be used as a strategy to induce tolerance in combination with other gene transfer or enzyme replacement protocols.

4.2 Glybera

Although at least 75 clinical trials have been initiated using AAV vectors with no serious adverse effects related to the vector, few have advanced past Phase II. Similarly, trials utilizing lentiviral vectors have been mostly small Phase I or II trials. The gene therapy community achieved a milestone in October 2012 when a gene therapy product utilizing an AAV serotype 1 vector received full regulatory approval in Europe (Melchiorri et al., 2013). This vector, called Glybera, was used to deliver the lipoprotein lipase gene to treat lipoprotein lipase deficiency. Glybera is administered via a one-time series of up to 60 intramuscular injections. Lipoprotein lipase deficiency is an orphan disease, and approval was achieved based on the results from only 27 patients that participated in a series of three uncontrolled, open-label clinical trials. While Glybera is not used to treat nervous system disorders, it sets an important regulatory precedence for the development and approval of gene therapy products for rare diseases.

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5. CONCLUSION

Promising results have been obtained in small clinical trials for MLD and WAS using lentiviral vectors by ex vivo gene transfer approaches, but a clear understanding of the extent of rescue and confirmation of these preliminary results will require larger clinical trials. Early trials also identified the risk of insertional mutagenesis, ultimately leading to oncogenesis. Emerging technology for lentiviral vectors appears to overcome many of the early safety concerns for retroviral vectors.

For AAV vectors, early clinical trials demonstrated successful gene transfer, but large impacts on the disease symptoms were lacking except perhaps for the small AADC-deficiency trial. Clinical trials for PD have shown encouraging results, but are plagued by a placebo effect that has made assessing the potential of gene therapy difficult. LSDs represent a promising family of diseases that could benefit from gene therapy. The main obstacle in the translation of LSD gene therapies has been the availability of a global gene delivery system applicable to large animals; however, promising technological developments utilizing IV or intra-CSF AAV vector delivery are beginning to meet that need.

In summary, the clinical trials to date have laid important groundwork in the advancement of CNS-directed gene therapy. While an unequivocal clinical success for CNS gene therapy has remained elusive, upcoming clinical trials will be testing approaches that have much greater potential to successfully translate encouraging results from animal models into humans. In the upcoming years, clinical trials for SMA, GAN, MPS IIIB, and other diseases will test the promise of global AAV-mediated CNS gene transfer. Concurrently, the promising results from the MLD and WAS trials using lentiviral vectors are being translated to other related diseases. Once a gene therapy breakthrough is realized for one disease, no matter how rare, the vector platform and approach can serve as a template for the treatment of a wide range of neurological disorders.

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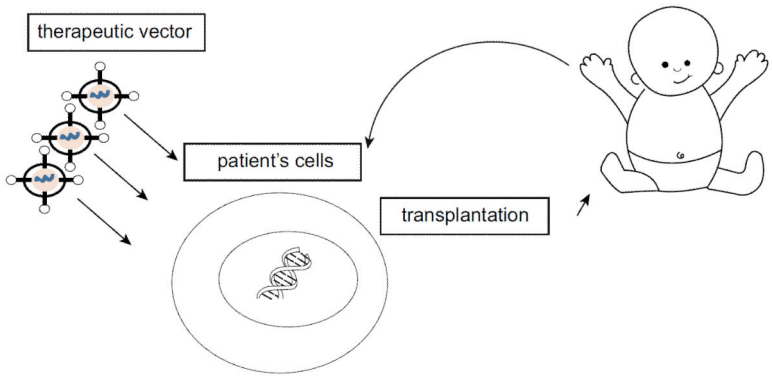

Figure 2.1.

Ex vivo Gene Transfer Using a Retroviral Vector. Hematopoietic stem cells isolated from a patient's bone marrow are transduced *ex vivo* with lentiviral vector. Transduced cells then reinfused back to the patient expressing the therapeutic protein.

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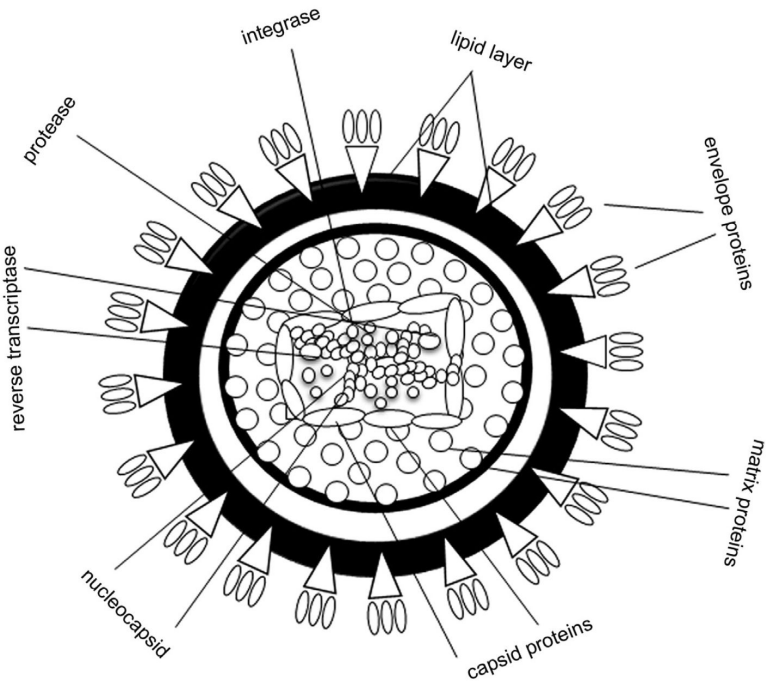

Figure 2.2.

HIV-1 Structure. HIV-1 is a complex retrovirus that contains two copies of RNA molecules embedded into nucleocapsid proteins (small open beads at the center). protease (*Pr*), integrase (*Int*), and reverse transcriptase (*RT*) enzymes are shown surrounded by the viral capsid (oval-shaped beads). Matrix proteins (shown as circles) enclosed by the viral envelopes consist of two components: *transmembrane*, gp41 (closed triangle), and surface, gp120 (ovals), embedded into the lipid membrane.

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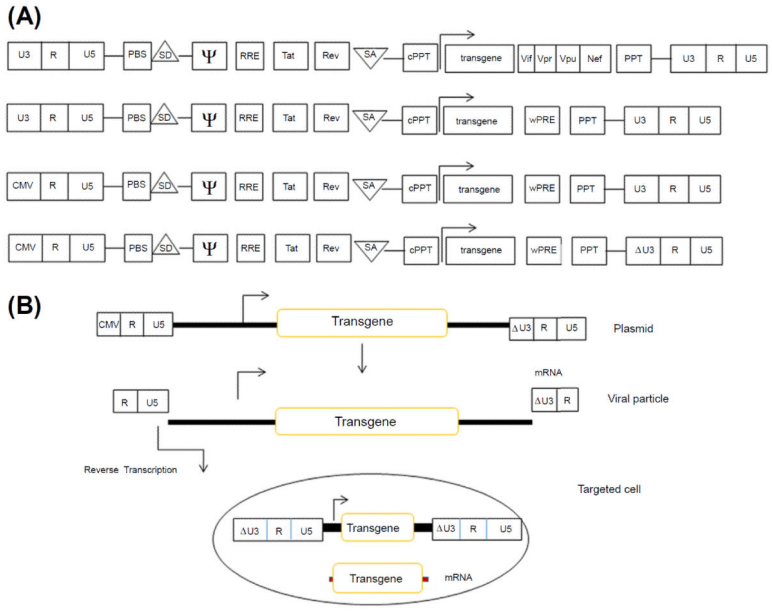

Figure 2.3.

Generations of Lentivirus Cassettes. The first generation of the lentivirus (expression) cassette carried all accessory genes, *vif*, *vpu*, *vpr*, and *nef*, and regulatory genes, *tat* and *rev*, flanked by unmodified 5′- and 3′- LTRs. In addition, it harbors *cis*-acting elements, including a primer binding site (PBS); a splice donor (SD) and acceptor (SA); central polypurine tract (cPPT) and polypurine tract (PPT); Rev response element (RRE); and a packaging signal, psi (Y). Second generation retroviral vectors are characterized by a deletion of all accessory genes of HIV. The woodchuck hepatitis virus posttranscriptional regulatory element (WPRE) is incorporated into the viral cassette. In a third generation, the cytomegalovirus (CMV) promoter replaced a parental HIV promoter located in the 5′-LTR. The self-inactivated (SIN) vector represents a fourth generation. SIN is completely devoid of HIV-parental enhancer/promoter sequences, located in the U3′ of the 3′-LTR (deletion is shown). (B) A deletion introduced in the 3′LTR translocated to the 5′LTR during reverse transcription as shown (see also in the text).

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Table 2.1

CNS Gene Therapy Trials Using Retroviral Vectors

ID/References	Diseases	Vector	Title	Transgene	Phase
US-0322	Alzheimer	Retrovirus	A Phase I study of NGF ex vivo gene therapy for Alzheimer's disease	Nerve growth factor	I
US-0851	Multiple sclerosis	Retrovirus	Cell-based gene therapy using MRC-MBP for treatment of multiple sclerosis	Myelin basic protein	I/II
FR-0041	Parkinson's	Lentivirus	Study of the safety, efficacy, and dose evaluation of ProSavin for the treatment of bilateral idiopathic Parkinson's disease	Tyrosinase GTP Cyclohydrolase 1 DOPA decarboxylase	I/II
UK-0191	Parkinson's	Lentivirus	Study of the safety, and dose evaluation of ProSavin, administered using stereotaxic injection to the striatum of patients with idiopathic Parkinson's disease	Tyrosinase GTP Cyclohydrolase 1 DOPA decarboxylase	I/II
Cartier et al. (2009)	X-linked adrenoleukodystrophy	Lentivirus	Hematopoietic stem cell gene therapy with a lentiviral vector in X-linked adrenoleukodystrophy	ABCD1	I/II
Biffi et al. (2013)	Metachromatic leukodystrophy	Lentivirus	Lentiviral hematopoietic stem cell gene therapy benefits metachromatic leukodystrophy	ARSA	I/II
Aiuti et al. (2013)	Wiskott–Aldrich syndrome	Lentivirus	Lentiviral hematopoietic stem cell gene therapy in patients with Wiskott–Aldrich syndrome	WASP	I/II

ARSA, arylsulfatase A

Adv Genet. Author manuscript; available in PMC 2015 July 29.