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Challenges for Gene Therapy of CNS Disorders and Implications for Parkinson's Disease Therapies

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The CNS poses significant challenges for effective gene therapy, including the presence of the blood–brain barrier, which prevents the entry of large molecules. Adeno-associated viral (AAV) vectors have been developed that demonstrate efficient and stable transgene expression in the CNS and are the most advanced vector class in clinical application, but limitations still manifest. One of them is the difficulty to achieve extensive transduction volumes. Bearing this in mind, anti-parkinsonian therapies with relatively restricted targets are particularly suited for initial clinical attempts. In this issue of *Human Gene Therapy* a long-term follow-up of one such AAV Parkinson's disease (PD) clinical trial is presented (Mittermeyer *et al.*, 2012, this issue). Several important implications of this work are discussed, including the need for more widespread transduction to achieve a clinical benefit. Results obtained with AAV9 demonstrating blood–brain barrier crossing and extensive CNS transduction have raised hopes for noninvasive delivery of viral vectors to wide CNS targets. A second study in this issue of *Human Gene Therapy* explores AAV9 efficiency in nonhuman primates and underscores the importance of delivery route, preexisting antibody response, and vector tropism (Samaranch *et al.*, 2012, this issue). Taken together, these two studies showcase progress and current challenges in clinical and nonhuman primate CNS gene therapy.

Degeneration of the substantia nigra pars compacta and subsequent loss of striatal dopamine content is believed to underpin the cardinal motor symptoms of PD, namely tremor, rigidity, and bradykinesia. Although current pharmacotherapies are initially effective, they are associated with a decline in efficacy as the disease progresses and have a number of side effects, including hallucinations and uncontrollable motor movements (dyskinesias), that may effectively limit the dose of L-DOPA patients can tolerate (Obeso *et al.*, 2000). Hence the search for alternative treatment, which needs to be safe and ideally requires a single administration, provides effective symptomatic relief, and even potentially halts or reverses the disease process. One way in which this may be achieved is through the use of gene therapy. The majority of current gene therapy approaches for the treatment of CNS disorders have focused on the use of AAV vectors, as they offer stable, long-term gene expression (McCown, 2011). Such vectors have been administered directly into the target sites of the CNS through stereotaxic surgery (Christine *et al.*, 2009; Marks *et al.*, 2010). However, this invasive approach requires specialist surgical facilities and accounts for some of the undesirable side effects of gene therapy reported in the literature (e.g., intracranial hemorrhage and edema; Christine *et al.*, 2009).

Nevertheless, localized infusions of vector can efficiently target specific brain regions, with the associated reduced risk of adverse events not directly related to vector delivery. The results of several phase I/II gene therapy trials for Parkinson's have thus far been encouraging, with vectors showing good safety profiles and being well tolerated in patients. Current trials can be subdivided into three main strategies: increasing striatal dopamine content, using aromatic L-amino acid decarboxylase (rAAV2-hAADC; Genzyme, Cambridge, MA) alone or a combination of hAADC, tyrosine hydroxylase, and guanosine 5′-triphosphate cyclohydrolase I (carried by equine infectious anemia virus-derived lentiviral vector ProSavin; Oxford BioMedica, Oxford, UK); changing basal ganglia circuitry by inhibiting the subthalamic nucleus, using the gene for glutamic acid decarboxylase (AAV-GAD; Neurologix, Fort Lee, NJ); or a trophic factor (neurturin) approach aiming to improve the nigrostriatal pathway (AAV2-NTN, CERE-120; Ceregene, San Diego, CA) (Witt and Marks, 2011).

In this issue of *Human Gene Therapy*, Mittermeyer and colleagues report a long-term evaluation of a phase I study of AADC gene therapy for PD (Mittermeyer *et al.*, 2012, this issue). AADC is the rate-limiting enzyme for the conversion of L-DOPA to dopamine, and loss of AADC may be associated with the wearing off of L-DOPA responsiveness in patients (Ichinose *et al.*, 1994). Thus, restoration of AADC capacity within the putamen should result in elevated dopamine levels in response to exogenous L-DOPA. This study is a continuation of previous work by this group, who initially reported findings based on a 6-month follow-up of 10 patients who received either a low dose (9×10^{10} vector genome copies [VG]) or a high dose (3×10^{11} VG) of AADC

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(Christine *et al.*, 2009). Bilateral putaminal convection-enhanced delivery of AAV-2 vector encoding human AADC, expected to transduce striatal interneurons that do not degenerate in idiopathic PD, resulted in about 30% improvement in mean scores, based on the Unified Parkinson's Disease Rating Scale (UPDRS), both on and off medication, without associated dyskinesias. This clinical improvement was accompanied by robust gene expression, measured by positron emission tomography (PET) scans using the AADC tracer [^{18}F]fluoro-L-*m*-methyltyrosine (FMT). These PET scans confirmed localized improvements within the putamen, which were dose dependent (higher signals were observed in the high-dose group). Although the procedure was well tolerated, there were hemorrhages in three patients (asymptomatic in two) that were related to the surgical procedures in administering vectors. It is noteworthy that this study excluded patients on the basis of elevated antibody titers to AAV2 (Christine *et al.*, 2009), in keeping with the reported effect of the immune system abrogating the benefits after gene transfer (Manno *et al.*, 2006).

In the latest long-term follow-up study, the elevated PET signal induced by AAV2-AADC therapy was observed to persist over 4 years in both dose groups compared with baseline and was accompanied by UPDRS improvements in patients both on and off medication over the first 12 months, with a slow worsening of symptoms over the remainder of the study. After 12 months there were no differences in UPDRS scores or PET signals between the high- and low-dose groups, which may reflect the unrelenting neurodegeneration seen in PD. The low overall intensity of PET signal may reflect a need for larger amounts of vector (both volume and dose) for increased transduction of the putamen (Mittermeyer *et al.*, 2012, this issue).

This important study has revealed safe, efficient, and essentially permanent gene transfer to cells within the CNS. To our knowledge this is the longest follow-up study of CNS gene therapy and is an important milestone in trials for PD. However, the apparent improvement in symptoms may be due to the powerful placebo effect seen particularly in patients with PD. It has previously been reported that a positive placebo effect was observed in approximately 16% of patients with PD, with increasing prevalence in those trials involving surgery (Goetz *et al.*, 2008). The mechanism for this response appears to be the involvement of cortical pathways implicated in the expectation of improvement, and subsequent dopamine release within the striatum resulting in improved motor symptoms of PD (Diederich and Goetz, 2008). As such, open-label studies may overemphasize the positive results of gene therapy trials, which are then not reproducible when investigated in double-blind, sham-surgery controlled randomized trials. This was observed in the initially positive phase I open-label trial of AAV-NTN, where significant improvements in UPDRS scores were observed (Marks *et al.*, 2008). However, a phase II multicenter, double-blind, randomized controlled trial of AAV-NTN concluded that this approach was not superior to sham surgery with respect to the primary outcome measure, a change in UPDRS III (motor) score in the off-medication state (Marks *et al.*, 2010).

Other potential broader issues needing to be dealt with by ongoing gene therapy trials for PD include arresting the underlying disease progression and addressing the nonmotor symptoms of PD, which are receiving increasing attention with regard to quality-of-life issues for patients (Martinez-Martin, 2011). It is also becoming clearer that PD is a multiorgan, multicellular disorder that may benefit from wider application of therapeutic vectors than solely to the striatum or substantia nigra (Jellinger, 2012). However, the prospect for eventual gene therapy to treat PD is promising, with recent AAV2-GAD gene therapy being effective in a double-blind, sham-surgery controlled randomized trial of 45 patients (LeWitt *et al.*, 2011). As such, it appears that gene therapy trials are turning the corner and may soon offer a valuable weapon in the battle against PD.

With the caveats highlighted by the Parkinson clinical gene therapy trials in mind, considerable scientific excitement surrounded the first reported AAV serotype able to cross the blood–brain barrier and efficiently transduce cells of the nervous system, AAV9 (Duque *et al.*, 2009; Foust *et al.*, 2009; Manfredsson *et al.*, 2009). The implications of these reports were that direct surgical targeting may no longer be required. Instead, a single intravenous injection could deliver the therapeutic gene throughout the CNS. Encouragingly, it also appeared that intravenous AAV9 could be detargeted away from the liver, thus potentially enhancing vector available for CNS transduction and preventing any hepatotoxic effects (Pulicherla *et al.*, 2011). These results were tempered by the realization that, similar to other paradigms, the immune system plays an important role and circulating neutralizing antibodies against AAV9 can prevent efficient CNS transduction (Gray *et al.*, 2011b). This is noteworthy, because approximately 30% of adults are positive for AAV9 antibodies at sufficiently high titers to possibly prevent their routine clinical use (Boutin *et al.*, 2010). Concerns were also raised about the high doses of vector required for efficient CNS transduction: some 1×10^{13} VG/kg/mouse. If this were to be scaled up to humans (approximately 1×10^{15} VG), this may represent a significant technical challenge to achieve sufficient vector for therapies (Forsayeth and Bankiewicz, 2011). Furthermore, in comparison with the neuronal expression observed in mice (Duque *et al.*, 2009) there are significant intra- and interspecies differences in vector cell tropism. For example, in the nonhuman primate astrocytes were reported to be the cell type preferentially transduced by AAV9 (Gray *et al.*, 2011b). With these three issues in mind—antibodies, dose, and cellular tropism—Samaranch and colleagues, in the current issue of *Human Gene Therapy*, report the effects of differing routes of administration of AAV9 in the nonhuman primate (Samaranch *et al.*, 2012, this issue).

In this report, the authors investigated the effects of intraarterial (via the internal carotid artery) or intra-cerebrospinal fluid (CSF; via the cisterna magna, CM) self-complementary AAV9 vector administration, in contrast to intravenous injections, which require large amounts of vector and convey body-wide transduction. The authors report that intraarterial injections gave similar efficacy compared with intravenous administration, with animals expressing the green fluorescent protein (GFP) reporter gene in the CNS in a dose-dependent manner. CM injections resulted in many more GFP-positive cells and greater intensity of GFP expression in the CNS. The CM-injected monkeys showed much reduced GFP expression in peripheral organs such as the liver and spleen. Within the brain, most transduction occurred in astrocytes, regardless of route of administration, although there were some γ-aminobutyric acid (GABA)-ergic cortical interneurons transduced in the

CM group. Strikingly, the effects of preexisting AAV9 immunity were confirmed in nonhuman primates, with both high antibody titers (>1:200) and moderate antibody titers (1:200) preventing transduction, even when GFP or hAADC vectors were administered directly into the CSF (Samaranch *et al.*, 2012, this issue).

These results have important implications for systemic and intra-CSF AAV9 gene transfer for adult CNS disorders. The high dose of AAV9 vectors required for efficient transduction remains a technical challenge, although one that may be overcome by more advanced production methods. However, it should be considered that higher doses of vector may present with an increased incidence of unwanted side effects, as currently witnessed with pharmacotherapies. More problematic issues with the use of AAV9 vectors are the effects of preexisting immunity and cellular tropism. Encouragingly, low titers of anti-AAV9 antibodies have been reported in children (Calcedo *et al.*, 2011), and as such, inherited CNS diseases may offer the most viable targets for current AAV9-based therapies. This suggestion is supported by the work of Mattar and colleagues, who described efficient neuronal transduction after intrauterine gene therapy (Mattar *et al.*, 2012). Furthermore, site-directed injections have revealed efficient neuronal expression of AAV9, at least in adult pigs (Federici *et al.*, 2011). Alternatively, patient groups could be stratified on the basis of their levels of preexisting immunity. The issue of cell type specificity may be overcome with targeted promoters such as the human synapsin promoter, or a fragment of the mouse methyl-CpG-binding protein-2 (MeCP2) promoter, to convey neuronal specificity (Kugler *et al.*, 2003; Gray *et al.*, 2011a). However, astrocytic expression per se may not preclude AAV9 for use in some neurodegenerative disorders, including PD, as these may be viable targets for neurotrophic factor expression (Drinkut *et al.*, 2011). An alternative approach would be to use RNA interference to prevent transgene expression in nontarget cell populations, an approach already being investigated with AAV9 vectors (Xie *et al.*, 2011). Alternatively, directed evolution of AAV may allow preferential targeting, for instance, work in the 1-methy-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated primate may reveal additional chimeric vectors suitable for the treatment of PD (Gray *et al.*, 2010; Asokan *et al.*, 2012). Given that AAV9 binding is mediated by nonsialylated cell surface glycan receptors, it may be possible to increase CNS penetrance through enhanced receptor expression or pharmacological treatments that can enhance AAV receptor function, for example, recombinant sialidase (Bell *et al.*, 2011; Shen *et al.*, 2011). Even if AAV9 does not live up to the initial excitement, one report has suggested that other recombinant AAV vectors are at least as good as AAV9 in crossing the blood–brain barrier in neonatal mice (Zhang *et al.*, 2011). Although interspecies differences in cell tropism have yet to be described for these agents, novel engineered vectors may have reduced issues with preexisting immunity and may therefore offer further options for the treatment of CNS disorders. The floodgates have now opened, and we eagerly await further developments in this field.

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