

Parkinson's Disease, Primates, and Gene Therapy: Vive la Différence?

Travis B. Lewis, BA and David G. Standaert, MD, PhD*

Department of Neurology, Center for Neurodegeneration and Experimental Therapeutics, The University of Alabama at Birmingham, Birmingham, Alabama, USA

Clinical studies of neurotrophic factor therapy for the treatment of Parkinson's disease (PD) have progressed rapidly in less than a decade. From early trials using direct protein infusion through to recent studies with targeted gene therapy, many practical obstacles have been overcome.¹⁻⁴ The next major hurdle is a convincing demonstration of therapeutic efficacy in human PD, together with an acceptable safety profile. Bartus et al. report herein the first histological descriptions of gene transfer and expression in an efficacy study in humans—a milestone in the development and refinement of neurotrophic factor gene therapy clinical trials. This unplanned opportunity came about when two patients enrolled in an AAV2-NRTN (adeno-associated virus serotype 2 expressing the growth factor neurturin; trade name CERE-120, a product of Ceregene, Inc.) clinical trial died of unrelated events 1.5- and 3-months into the 12-month study. They have studied these cases carefully, and correlated the histologic data from humans with studies of viral gene delivery in non-human primate models of PD.

This study provides proof that gene therapy with AAV2-NRTN results in functional infection and transgene expression in target cells in humans. The AAV2-NRTN was injected into the putamen. They found that in each of the human cases, NRTN transgene expression was the strongest adjacent to the sites of injection. Eight separate deposits of vector were made in each putamen, resulting in transduction of putamenal neurons and expression of NRTN that appeared to occupy about 15% of the putamenal volume. This degree of NRTN transduction is similar to that seen

in non-human primate models in which AAV-NRTN has been studied. Despite this congruence, the observed effectiveness in the preclinical models versus clinical application was quite different. In non-human primate models of PD (induced with the neurotoxin 1-Methyl-phenyl-1,2,3,6-tetrahydropyridine [MPTP]) and in aged monkeys this degree of AAV-NRTN transduction has been associated with clear-cut neuroprotective and neurorestorative effects.^{5,6} In the clinical trial in which the human subjects participated, there was no benefit, at least as measured by the primary endpoint of Unified Parkinson's Disease Rating Scale (UPDRS) in the "off" state at 12 months, although there were hopeful signals in the subgroup with longer follow-up and in secondary measures.⁷ This disparity in efficacy was supported by assessment of tyrosine hydroxylase (TH) immunoreactivity in the striatum, a surrogate marker for functional enhancement of degenerating dopaminergic neurons, which revealed minimal recovery in humans as compared to the robust TH recovery seen in non-human primate studies.

These data make a compelling case that the delivery of NRTN to the SNc is important for functional recovery of dopaminergic neurons, and open the door to speculation regarding the fundamental reason(s) behind the discrepancy observed between humans and non-human primate models. We see two primary lines of reasoning that would explain this. The first, proposed by the authors, is that human PD is associated with an underlying defect in axonal transport such that there is no effective delivery of NRTN from the source in the putamen to the dopaminergic neurons in the SNc. This is plausible, and as the authors have noted there is evidence for abnormalities of axonal transport in other neurodegenerative diseases.⁸ If this is indeed the reason for impaired NRTN delivery, their observations do suggest the transport defect in PD is widespread in the brain: they noted not only deficient retrograde transport to the SNc, but also absence of anterograde transport to the SN pars reticulata, suggesting that the disease produces defects in transport in dopaminergic and nondopaminergic neurons. An alternate hypothesis, unrelated to PD, is

*Correspondence to: Dr. David G. Standaert, John and Juanelle Strain Professor of Neurology, Director, Center for Neurodegeneration and Experimental Therapeutics, Director, Division of Movement Disorders, University of Alabama at Birmingham (UAB), 1719 6th Ave. South, CIRC 516, Birmingham, AL 35294-0021; dstandaert@uab.edu

Relevant conflicts of interest: Nothing to report.
Full financial disclosures and author roles may be found in the online version of this article.

Received: 1 November 2010; **Accepted:** 2 November 2010
Published online in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/mds.23544

that monkeys are not humans, and that there are species-specific differences in the capacity for anterograde and retrograde transport of NRTN. This is difficult to refute without empirical data. These uncertainties reflect a more general problem in PD research, in that none of the existing animals models has established power to predict the outcome of a human neuroprotective trial.⁹

Regardless of the mechanism, the fact remains that neurotrophic factor delivery to the putamen of PD patients was insufficient to alter the clinical endpoint in this trial, leading to the quandary of what to do next. The proposed solution is to circumvent the axonal transport issue completely by direct delivery of CERE-120 to the SNc in PD patients. As the AAV2 vector used in this trial has good tropism for dopaminergic neurons, there is every reason to believe this approach will succeed in delivering NRTN to the SNc neurons.^{10,11} The question then becomes whether direct nigral infusion will lead to new and unforeseen adverse events. Clearly, there are potential surgical issues with additional injections to deeper brain regions. There has also been the suggestion that such injections would lead to undesirable effects through transduction of adjacent regions, such as the weight loss seen in rodents and monkeys after glial cell-line derived neurotrophic factor delivery directly to the SNc.^{12,13} This is a valid concern, but it is important to note that has not been recapitulated with NRTN protein itself, and that the distribution of expression in human brain may differ from that in these smaller animals.

As one of the few therapies immediately available that may not only slow PD progression, but also improve outcomes, we feel that the potential benefits of a clinically successful CERE-120 treatment cannot be ignored. The data in this article describe a new barrier to gene therapy, and point to the need for further study into axonal transport in PD. However, this barrier does not demand a dramatic rethinking of neurotrophic gene therapy in general. As is the case in any

experimental therapy, caution is paramount, but balanced against the potential for a transformative therapeutic breakthrough, it seems right to proceed with further clinical studies.

References

1. Slevin JT, Gash DM, Smith CD, et al. Unilateral intraputamenal glial cell line-derived neurotrophic factor in patients with Parkinson disease: response to 1 year of treatment and 1 year of withdrawal. *J Neurosurg* 2007;106:614–620.
2. Nutt JG, Burchiel KJ, Comella CL, et al. Randomized, double-blind trial of glial cell line-derived neurotrophic factor (GDNF) in PD. *Neurology* 2003;60:69–73.
3. Marks WJ, Jr., Ostrem JL, Verhagen L, et al. Safety and tolerability of intraputamenal delivery of CERE-120 (adeno-associated virus serotype 2-neurturin) to patients with idiopathic Parkinson's disease: an open-label, phase I trial. *Lancet Neurol* 2008;7:400–408.
4. Lewis TB, Standaert DG. Design of clinical trials of gene therapy in Parkinson disease. *Exp Neurol* 2008;209:41–47.
5. Kordower JH, Herzog CD, Dass B, et al. Delivery of neurturin by AAV2 (CERE-120)-mediated gene transfer provides structural and functional neuroprotection and neurorestoration in MPTP-treated monkeys. *Ann Neurol* 2006;60:706–715.
6. Herzog CD, Brown L, Gammon D, et al. Expression, bioactivity, and safety 1 year after adeno-associated viral vector type 2-mediated delivery of neurturin to the monkey nigrostriatal system support cere-120 for Parkinson's disease. *Neurosurgery* 2009;64:602–612; discussion 612–603.
7. Marks WJ, Jr., Bartus RT, Siffert J, et al. Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet Neurol* 2010;9:1164–1172.
8. Morfini GA, Burns M, Binder LI, et al. Axonal transport defects in neurodegenerative diseases. *J Neurosci* 2009;29:12776–12786.
9. Standaert DG, Yacoubian TA. Target validation: the Parkinson disease perspective. *Dis Model Mech* 3:259–262.
10. Lehtonen E, Bonnaud F, Melas C, et al. AAV2 vectors mediate efficient and sustained transduction of rat embryonic ventral mesencephalon. *Neuroreport* 2002;13:1503–1507.
11. St Martin JL, Klucken J, Outeiro TF, et al. Dopaminergic neuron loss and up-regulation of chaperone protein mRNA induced by targeted over-expression of alpha-synuclein in mouse substantia nigra. *J Neurochem* 2007;100:1449–1457.
12. Su X, Kells AP, Huang EJ, et al. Safety evaluation of AAV2-GDNF gene transfer into the dopaminergic nigrostriatal pathway in aged and parkinsonian rhesus monkeys. *Hum Gene Ther* 2009;20:1627–1640.
13. Manfredsson FP, Tumer N, Erdos B, et al. Nigrostriatal rAAV-mediated GDNF overexpression induces robust weight loss in a rat model of age-related obesity. *Mol Ther* 2009;17:980–991.