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CERE-120

Antiparkinsonian Drug
Gene Therapy

AAV-hNGF-hNTN
AAV2-NTN

Adeno-associated virus type 2 vector encoding a modified human neurturin (*NRTN*) gene that carries the prepro domain of human nerve growth factor β (NGF)

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Abstract

Parkinson's disease is a progressive neurodegenerative disorder for which there is no known cure. Agents have been developed to alleviate symptoms, reduce functional disability and slow or arrest progression of the disease, resulting in a near-normal life expectancy and satisfactory quality of life. Dopaminergic agents, which are the mainstay of antiparkinsonian therapy, are effective only during the early phases of the disease and their efficacy is reduced with complications. A novel and promising approach to the treatment of Parkinson's disease is gene therapy, including viral vector-mediated transfer of candidate genes to enhance dopamine production or the survival of dopaminergic neurons. CERE-120 (AAV-hNGF-hNTN) is a novel gene therapy product that delivers a modified human neurturin (*NRTN* or *NTN*) gene (*i.e.*, prepro sequence of human neurturin replaced with the prepro domain of human nerve growth factor β [NGFβ]) via an adeno-associated virus type 2 (AAV2) vector under the control of the CAG promoter. CERE-120 has been shown to be efficiently secreted from human cells and to be safety delivered to target areas, exerting biological activity *in vivo*. Moreover, CERE-120 demonstrated promise in a phase I study in patients with Parkinson's disease.

Background

Parkinson's disease is a progressive and incurable neurodegenerative disorder that afflicts an estimated 5-24 per 10,000 population worldwide. Approximately 1-1.5 million individuals in the U.S. alone have been diagnosed with the disease and an estimated 35-42% of cases of the disorder are thought to be undiagnosed. Parkinson's disease is characterized by bradykinesia, rigidity, tremor at rest and postural instability. In a healthy individual, control of movement and muscle tone is achieved through the extrapyramidal system, with particular regulation by the striatum. The activity of the striatum is finely controlled via glutamatergic pathways in the cortex and dopaminergic pathways in the substantia nigra. The activity of the striatum includes inhibitory GABAergic, enkephalinergic and substance P-mediated signals to the thalamus, subthalamic nuclei, substantia nigra and globus pallidus. Pathologically, the symptoms of Parkinson's disease are primarily due to the gradual loss of dopaminergic cells in the substantia nigra and degeneration of the nigrostriatal pathway. A pronounced inhibition of the thalamus by the substantia nigra is evident, resulting in an inability to control muscle tone and movement. The presence of Lewy bodies in the substantia nigra, and possibly in the cortex, amygdala, locus coeruleus, vagal nucleus and peripheral autonomic nervous system, is another pathological feature of Parkinson's disease and is thought to contribute to the numerous nonmotor symptoms of the disease (1, 2).

To date, no cure for Parkinson's disease has been discovered. Agents are available to alleviate symptoms, reduce functional disability and slow or arrest progression of the disease, resulting in a near-normal life expectancy and satisfactory quality of life. Dopaminergic agents, particularly levodopa and direct or indirect dopamine agonists, are the mainstay of treatment for Parkinson's disease. However, although this form of treatment is effective in the early phases of the disease, its benefits decrease with disease progression and when problems such as dyskinesia and on-off phenomena begin to manifest. Thus, researchers continue to search for novel agents to manage and possibly prevent this disease (1-3).

One of the most promising and safe approaches to the treatment of Parkinson's disease is gene therapy. Viral vector-mediated transfer of candidate genes may be used to boost dopamine production, such as those encoding tyrosine hydroxylase, guanosine triphosphate

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cyclohydrolase I and aromatic L-amino acid decarboxylase, or to promote the survival of dopaminergic neurons, such as glial cell line-derived neurotrophic factor (GDNF) (1, 4-7). Gene therapies under development for the treatment of Parkinson's disease are shown in Table I.

Neurturin is a member of the GDNF family of ligands that has been shown to exert neuroprotective and restorative effects on nigrostriatal dopaminergic neurons. It is secreted from producer cells following cleavage at an RXXR site of a precursor protein and has been identified as an attractive candidate protein for the gene therapy of Parkinson's disease. CERE-120 (AAV-hNGF-hNTN) is a novel gene therapy product that shows particular promise. CERE-120 delivers a modified human neurturin (*NRTN* or *NTN*) gene in which the prepro sequence of human neurturin cDNA is replaced with the prepro domain of human nerve growth factor β (NGFβ) via an adeno-associated virus type 2 (AAV2) vector under the control of the CAG promoter. The result is a gene product that is efficiently secreted from human cells with potent biological activity. CERE-120 was chosen for further development for the treatment of Parkinson's disease (8).

Preclinical Pharmacology

The efficacy of CERE-120 was demonstrated *in vivo* in a rat 6-hydroxydopamine (6-OHDA)-induced model of Parkinson's disease. The agent (1×10^{12} vector genomes/ml; 4×10^9 total vector genomes/rat in 2 injections of 2 μl) was injected into the striatum 2 weeks before a single intrastriatal dose of 6-OHDA (20 μg). Analysis of the striatum and substantia nigra of animals sacrificed 2 weeks later revealed marked immunohistochemical signals for neurturin and a significant sparing (62-68%) of tyrosine hydroxylase-positive nigral neurons. In further experiments, striatal neurturin expression was found to be sustained even at 28 weeks postdosing and treatment resulted in significant benefits in amphetamine-induced rotation tests (9-11).

When administered intrastriatally to both intact aged rats and intact young rats, CERE-120 induced robust striatal neurturin expression in all animals, which remained stable from 4 weeks to over 12 months postdosing, as well as a trophic effect on tyrosine hydroxylase-positive cells. Intrastriatal delivery of the agent even at doses 125 times higher than the lowest effective dose tested was concluded to be safe and well tolerated (11).

CERE-120 also induced significant behavioral benefits in a rat 3-nitropropionic acid (3-NP)-induced model of Huntington's disease. Administration of 3-NP (20 mg/kg s.c. b.i.d. for 6 days) 4 weeks after dosing with CERE-120 resulted in rapid weight loss, impairment in the platform and rotarod tests and high scores on the qualitative neurological scale (QNS). All animals regained the weight lost by week 10. During week 11, CERE-120/3-NP-treated rats performed significantly better on the QNS and significantly improved on the platform test (*i.e.*, remaining a full 20 s) as compared to 3-NP-treated control rats. In addition, by weeks 13-14, CERE-120-treated animals performed significantly better on the rotarod test (12).

The effects of CERE-120 (6×10^{10}-6×10^{11} total vector genomes) delivered into the caudate, putamen or substantia nigra pars compacta of naïve macaque monkeys were investigated. Treatment was well tolerated and no neurological deterioration was seen. Neurturin immunoreactivity was observed in the striatum and substantia nigra pars compacta; robust neurturin expression was observed at the highest dose. Low and high doses resulted in increased striatal tyrosine hydroxylase staining in the gene delivery area. Retrograde transport of the protein was suggested in the substantia nigra pars compacta, since many neurturin-positive cells were detected in this region. Anterograde transport of the protein was suggested in the striatonigral and striatopallidal systems, since neurturin-positive fibers and puncta were detected in the globus pallidus and substantia nigra reticulata (13).

CERE-120 administered as five unilateral injections (3×10^{11} vector genomes/animal) was shown to be safe and well tolerated in an aged (> 25 years) rhesus monkey model of nigrostriatal degeneration. None of the animals exhibited abnormalities in appearance or behavior and no alterations were observed in the brain following histological examination. Significant increases in [^{18}F]-Dopa uptake in the injected striatal hemisphere as compared to

Table I: Gene therapies under development for Parkinson's disease (from Prous Science Integrity®).

Gene therapy	Description	Source	Phase
AV-201	Adeno-associated virus (AAV) vector containing the gene that encodes the enzyme L-amino acid decarboxylase (AADC)	Genzyme/Avigen	I/II
CERE-120	AAV type 2 vector encoding a modified human neurturin (NRTN) gene that carries the preprodomain of human nerve growth factor β (NGF)	Ceregene	I
NLX-P101	Recombinant AAV vector comprising the human glutamate decarboxylase 65 (GAD65) coding sequence and the neuron-specific enolase (NSE) promoter	Neurologix	I
ProSavin®	Tricistonic lentiviral vector encoding human tyrosine hydroxylase, aromatic AADC and GTP cyclohydrolase 1	Oxford BioMedica	Preclinical
TREAT-HGF	Naked plasmid encoding human HGF	AnGes	Preclinical

the untreated control hemisphere were observed during imaging at 4 months postdosing, and these increases were sustained for at least 8 months. Robust striatal neurturin expression and increased striatal tyrosine hydroxylase immunoreactivity were also reported, as well as an increase in the number of tyrosine hydroxylase-positive cells in the ipsilateral substantia nigra and a unilateral increase in cystolic phosphorylated ERK (pERK) in the substantia nigra of the CERE-120-treated hemisphere. These results confirmed robust trophic responses and enhanced functional activity observed in the nigrostriatal system following neurturin expression (14, 15).

Treatment with CERE-120 (2×10^{11} total vector genomes) improved MPTP-induced motor disability in rhesus monkeys. While control animals displayed sustained hemiparkinsonian motor deficits for more than 8 months following treatment with MPTP, treatment with CERE-120 on day 3 after intracarotid MPTP injection resulted in significant and sustained improvements, consistent with trophic affects of neurturin within the nigrostriatal system. Of the 5 animals receiving CERE-120, 4 completely recovered by 8 months postdosing (16).

The rate of natural pre-existing humoral immunity to wild-type AAV2 is about 50-90% in humans, with approximately 30% developing neutralizing antibodies. An *in vivo* study examined the safety and efficacy of CERE-120 (3.5×10^{11} vector genomes/striatum) in 3 adult rhesus monkeys with pre-existing neutralizing antibody titers of 1/20-1/640 acquired during natural infection. At 3 months posttreatment, robust neurturin expression was detected in the striatum and related areas, such as the substantia nigra. In addition, qualitative increases in pERK were observed in treated animals. No changes in body weight, food consumption, neurobehavioral evaluations, organ histopathology, clinical chemistry, hematology or brain inflammatory markers (*e.g.*, GFAP, CD68, CD45) were seen in CERE-120-treated animals. It was concluded that treatment did not cause neurotoxicity, since the number of tyrosine hydroxylase-positive cells in the nigra was unaltered in treated animals. AAV2-reactive antibody titers increased 3-9-fold in treated animals, with 2 animals also exhibiting an increase in neutralizing antibody titers. These increases were not, however, associated with clinical alterations, local inflammation or neurotoxicity. There were no antibody responses against neurturin. Thus, the presence of AAV2-reactive antibodies did not affect the successful delivery of CERE-120 to target brain regions in nonhuman primates (17).

Pharmacokinetics and Metabolism

The biodistribution of CERE-120 (8×10^9 or 4×10^{10} vector genomes/animal) was examined in rats. Analysis of tissue at 3, 28 and 90 days postdosing indicated that most of the vector DNA remained localized at the target injection site. Dose-dependent mean vector loads were measured at the injection site, which decreased over time. In contrast, stable transgene expression was sustained long term. Low levels of CERE-120 DNA were found in the brainstem and cerebellum, with transgene expression detected in the brainstem possibly due to the close proximity of the target area (substantia nigra) to this region. Vector was found 3 days postdosing in the liver and spleen, although levels disappeared by 28 days postdosing; vector was also detected in cervical lymph nodes in animals receiving the high dose, although no transgene expression was observed in this tissue. Animals receiving the higher dose also showed weak to moderate serum AAV2- and neurturin-reactive antibody responses at 28 and 90 days postdosing. No morbidity, mortality or treatment-related organ toxicity, serum chemistry or hematological changes were observed with treatment (18).

Similar results were obtained in rats and monkeys administered intrastriatal CERE-120. Treatment was safe and well tolerated. Approximately 99% of the vector remained in the injection region, with only low levels detected in cervical lymph nodes, liver and spleen; peripheral vector was cleared between 3 and 90 days. Steady-state vector-derived neurturin expression, first observed within 2 days postdosing, was achieved at about 4 weeks and was sustained for over a year (19).

Clinical Studies

An ongoing phase I trial in 12 patients with Parkinson's disease (Hoehn/Yahr stage 3 or greater) and motor fluctuations is examining the safety, tolerability and efficacy of CERE-120 (2×10^{11} or 8×10^{11} vector genomes injected intraputaminally along 4 trajectories/hemisphere). Dosing with the lower dose has been completed, while the higher dose cohort is ongoing. No surgical complications or serious adverse events have been reported and no treatment-related adverse events have been seen at 2-17 weeks of follow-up. Analysis is ongoing (20, 21).

Source

Ceregene, Inc. (US).

References

1. Prous Science Drug R&D Backgrounders: *Parkinson's disease (online publication)*. Updated May 8, 2006.

2. Samii, A., Nutt, J.G., Ransom, B.R. *Parkinson's disease*. Lancet 2004, 363: 1783-93.

3. Tolosa, E., Wenning, G., Poewe, W. *The diagnosis of Parkinson's disease*. Lancet Neurol 2006, 5(1): 75-86.

4. Kordower, J.H., Emborg, M.E., Bloch, J. et al. *Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease*. Science 2000, 290: 767-73.

5. Chen, Q., He, Y., Yang, K. *Gene therapy for Parkinson's disease: Progress and challenges*. Curr Gene Ther 2005, 5(1): 71-80.

6. Carlsson, T., Winkler, C., Burger, C. et al. *Reversal of dyskinesias in an animal model of Parkinson's disease by continuous*

L-DOPA delivery using rAAV vectors. Brain 2005, 128(3): 559-69.

7. Eslamboli, A., Georgievska, B., Ridley, R.M. et al. *Continuous low-level glial cell line-derived neurotrophic factor delivery using recombinant adeno-associated viral vectors provides neuroprotection and induces behavioral recovery in a primate model of Parkinson's disease*. J Neurosci 2005, 25(4): 769-77.

8. Ramirez, A., Hao, J., Kethchum, E., Kwok, K., Vacante, D., Bartus, R., Gasmi, M. *Design and production of AAV2 vector encoding a chimeric cDNA allowing efficient secretion of functional human neurturin from human cells*. 7th Annu Meet Am Soc Gene Ther (ASGT) (June 2-6, Minneapolis) 2004, Abst 536.

9. Herzog, C.D., Gasmi, M., Cunningham, J.J. et al. *Anatomical and behavioral effects of AAV2-mediated delivery of neurturin to the rat striatum prior to 6-hydroxydopamine induced nigrostiatal degeneration*. 7th Annu Meet Am Soc Gene Ther (ASGT) (June 2-6, Minneapolis) 2004, Abst 227.

10. Herzog, C.D., Gasmi, M., Cunningham, J.J. et al. *Design of an AAV2 vector encoding chimeric cDNA to promote secretion of human neurturin and characterization of its anatomical and functional effects using the 6-hydroxydopamine model of nigrostriatal degeneration*. 34th Annu Meet Soc Neurosci (Nov 12-16, Washington, D.C.) 2004, Abst 791.8.

11. Bartus, R.T., Herzog, C.D., Cunningham, J.J. et al. *Biological activity of CERE-120, an AAV2 vector encoding human neurturin, in the rat 6-hydroxydopamine model of nigrostriatal degeneration and in the young intact and aged rat*. 35th Annu Meet Soc Neurosci (Nov 12-16, Washington, D.C.) 2005, Abst 545.1.

12. Ramaswamy, S., McBride, J.L., Brandon, E.P. et al. *Gene therapy using CERE-120 (AAV-neurturin) in a 3NP rat model of Huntington's disease*. 35th Annu Meet Soc Neurosci (Nov 12-16, Washington, D.C.) 2005, Abst 90.1.

13. Dass, B., Herzog, C.D., Gasmi, M. et al. *Adeno-associated virus mediated gene delivery of neurturin (CERE-120) in nonhuman primates*. 34th Annu Meet Soc Neurosci (Oct 23-27, San Diego) 2004, Abst 790.19.

14. Herzog, C.D., Holden, J.E., Bakay, R.A. et al. *Enhanced 18F-DOPA uptake in the striatum of aged rhesus monkeys following striatal delivery of CERE-120, an AAV2 vector encoding human neurturin*. 35th Annu Meet Soc Neurosci (Nov 12-16, Washington, D.C.) 2005, Abst 545.7.

15. Dass, B., Herzog, C.D., Holden, J.E., Bakay, R.., Bartus, R.T., Kordower, J.H. *Striatal administration of an AAV-2 vector encoding human neurturin (CERE-120) reverses the age-related decline in markers of nigrostriatal function in rhesus monkeys*. Mov Disord 2006, 21(Suppl. 13): Abst P28.

16. Dass, B., Herzog, C.D., Bakay, R.A.E. et al. *Adeno-associated virus mediated gene delivery of neurturin (CERE-120) prevents MPTP-induced motor disability following injection into the striatum of nonhuman primates*. 35th Annu Meet Soc Neurosci (Nov 12-16, Washington, D.C.) 2005, Abst 545.6.

17. Printz, M.A., Herzog, C.D., Wilson, A. et al. *Effects of natural pre-existing humoral immunity to AAV on administration of AAV2-NTN to the brain of non-human primates*. 8th Annu Meet Am Soc Gene Ther (ASGT) (June 1-5, St. Louis) 2005, Abst 947.

18. Landers, M., Kwok, K., Ketchum, E. et al. *Biodistribution and safety profile of CERE-120 (AAV2-NTN) delivered to the rodent brain*. 8th Annu Meet Am Soc Gene Ther (ASGT) (June 1-5, St. Louis) 2005, Abst 950.

19. Brandon, E.P., Herzog, C.D., Wilson, A. et al. *Controlled distribution of vector and neurturin following striatal administration of CERE-120, an AAV2-based vector being developed for Parkinson's disease*. 35th Annu Meet Soc Neurosci (Nov 12-16, Washington, D.C.) 2005, Abst 545.2.

20. Marks, W.J. Jr., Verhagen, L.A., Starr, P.A. et al. *Phase I study of putaminal gene transfer with adeno-associated virus serotype 2 [AAV2]-neurturin [NTN] (CERE-120) for Parkinson's disease: Preliminary observations*. Neurology [58th Annu Meet Am Acad Neurol (April 1-8, San Diego)] 2006, 66(5, Suppl. 2): Abst S23.006.

21. *Safety of CERE-120 (AAV2-NTN) in subjects with idiopathic Parkinson's disease (NCT00252850)*. ClinicalTrials.gov Web site 2006.