CERE-120 (AAV-Neurturin) for Parkinson’s Disease

NIH OBA Protocol # 0501-689
A Phase I, Open-Label Study of CERE-120 (Adeno-Associated Virus Serotype 2 [AAV2]-Neurturin [NTN] to Assess the Safety and Tolerability of Intrastriatal Delivery to Subjects with Idiopathic Parkinson's Disease

CERE-120 Program Overview
Raymond T. Bartus, Ph.D.
Senior V.P and COO, Ceregene, Inc.
AAV-NTN: opportunity for innovative therapy for Parkinson’s disease

- **Target dopamine nigrostriatal neurons:** degeneration implicated as key pathogenic event in disease
- **Provide constant supply of neurotrophic factor**
  - enhancing condition and function of neurons
  - strengthening their ability to withstand degeneration
Targeted neurotrophic factors should offer two benefits to Parkinson’s patients:

1) Improve disease symptoms
2) Retard disease progression

Most experts acknowledge that if these goals could be achieved…

…it would revolutionize the treatment of PD
AAV-NTN (CERE-120) produces functional improvement in MPTP lesioned monkeys over time.
Potential advantages of AAV-NTN gene transfer for PD

• Employ **same AAV vector currently used in other CNS gene transfer trials**, including AAV-NGF (CERE-110) for AD

• Administer relatively **small quantities** of vector and transgene **directly** to target

• **Avoid significant systemic exposure** of vector and transgene
Controlling potential risks with innovative gene transfer for PD

- **Leverage** much of prior *experience* with delivering *neurotrophic factors* to the brains of animals and *humans*

- Deliver **NTN** gene, which is structurally and functionally *similar to GDNF*, which in turn, has been well-characterized and administered into human brain for years

- Execute **comprehensive safety/toxicology** program with high dose multiples
Overview: CERE-120
Nonclinical program, part 1

19 total studies (7 monkey and 12 rat studies, examining 45 monkeys and 384 rats) involving three different types of studies:

- Pharmacology
- Efficacy
- Safety/toxicology

- **Pharmacology** (established expression kinetics, volume of distribution & dosing relationships of CERE-120)
  - 4 Rat studies (82 rats)
  - 1 monkey study (3 monkeys)
Overview: CERE-120
Nonclinical program, part 2

- **Efficacy** (established *bioactivity/efficacy and dose-response* of NTN)
  - 3 Rat studies (109 rats)
  - 2 monkey studies (13 monkeys, including 3 aged)

- **Safety/toxicology** (established *wide safety margin* of CERE-120)
  - 5 rat studies (193 rats, including 25 aged)
  - 4 monkey studies (29 monkeys)
CERE-120 Safety Profile, part 1

- Large dose multiples were tested:
  - Efficacious in rats at 125 fold lower than highest (safe) toxicology dose
  - Safe in monkeys at >100 and 400 fold higher than proposed human doses

- No overt, adverse effects: body weight, appearance, general health and general behavior (rats: 12mos; monkeys: ~8mos)

- No adverse effects on formal neurological or behavioral assessments
CERE-120 Safety Profile, part 2

• No functional impairments on the targeted nigrostriatal system

• No histopathological changes in targeted nigrostriatal system or cerebrum, cerebellum, brain stem, spinal cord or any peripheral organ

• No adverse effect on blood clinical chemistry or hematology

Bottom line: No sign of any toxicity of any kind, at very large dose multiples, over many months in rats and monkeys
Key RAC review points

1) Questions regarding efficacy of CERE-120
2) Kinetics and accumulation of NTN in brain
3) ‘Multiple brain regions’ targeted and spread of protein to non-targeted brain regions
4) The use of non-regulatable vector
5) Question of ‘rescue strategy’
6) Cerebellar toxicity reported in select, GDNF protein-treated monkeys
7) Rationale for dosing schedule in humans
Issue #1: Questions regarding efficacy of CERE-120
CERE-120 produces functional improvement in MPTP lesioned monkeys over time

Mean Clinical Rating Score

CONTROL (n=5) vs CERE-120 (n=5)

* p<0.05

Months 0 to 6
CERE-120 Enhances $^{18}$F-Dopa in Striatum of Aged Monkey via PET

**$K_{occ}$ values:**

<table>
<thead>
<tr>
<th>Monkey #</th>
<th>Treated hemisphere</th>
<th>Untreated hemisphere</th>
<th>% difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>0201</td>
<td>0.056</td>
<td>0.046</td>
<td>+19.6%</td>
</tr>
<tr>
<td>0202</td>
<td>0.065</td>
<td>0.055</td>
<td>+17.4%</td>
</tr>
<tr>
<td>0204</td>
<td>0.047</td>
<td>0.037</td>
<td>+26.6%</td>
</tr>
<tr>
<td>Mean</td>
<td>0.056±0.005</td>
<td>0.046±0.005</td>
<td>+21.2%</td>
</tr>
</tbody>
</table>

$t(2)=39.74$, $p<0.001$
CERE-120: multiple, mutually corroborating evidence of bioactivity/efficacy

- Young, healthy monkeys
  - Enhanced nigrostriatal TH staining
  - Enhanced activation of pERK signaling
- 6-OHDA rat model of PD
  - Protection of nigral cells at multiple time points (up to 7 mos)
  - Protection of nigral cells over range of doses, including fraction of dose shown to be safe (i.e. 1/125)
  - Functional (behavioral) benefit
- MPTP monkey model of PD
  - long-lasting improvement in motor performance
- Aged monkeys
  - Enhanced $^{18}$F-Dopa PET update in striatum
- Aged rats (New since filing App. M)
  - ‘Classic’ neurotrophic-induced hypertrophy: dopamine nigra neurons
Issue #1: Questions regarding efficacy of CERE-120

Synopsis:
CERE-120 provides clear and consistent neurotrophic support for nigrostriatal neurons in multiple rat and monkey studies, including ‘best models of PD’
Issue #2: Kinetics and Accumulation of NTN in Brain
Summary: CERE-120 Pharmacology

• **NTN** is **expressed** in the rat striatum
  – as **early as 2 days**
  – approaches **asymptote** at approximately **4 weeks**
  – shows **no significant increases thereafter** (up to seven months)

• **NTN** volume of **distribution**
  – **controlled via dose** of CERE-120
  – shows **no further accumulation** over range of doses
NTN expression seen soon after CERE-120 administration

![Graph showing volume of striatal NTN (mm3) 25 days following CERE-120 injection.]

Days Following CERE-120 Injection

Volume of Striatal NTN (mm3)
NTN volume of distribution is stable over time following AAV-NTN treatment in rats

<table>
<thead>
<tr>
<th>Months: post CERE-120 injection</th>
<th>Volume of NTN distribution (mm$^3$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Striatum</td>
</tr>
<tr>
<td>1</td>
<td>14.84 ± 1.16</td>
</tr>
<tr>
<td>3</td>
<td>12.79 ± 1.12</td>
</tr>
<tr>
<td>6</td>
<td>11.08 ± 1.15</td>
</tr>
<tr>
<td>7</td>
<td>14.43 ± 1.47</td>
</tr>
</tbody>
</table>

NOTE:
- **Total** reflects sum of all NTN staining, including striatum and all surrounding areas, particularly the globus pallidus and cortex around needle track (only).
- **ND**: The total volume of NTN spread at 7 mo. was not determined.
- **Data** are derived from several separate experiments.
Issue #2: Kinetics and Accumulation of NTN in Brain

Synopsis:

• **Onset of NTN expression:** rapid

• **Volume of expression:** reaches steady state levels at about 4 weeks and then shows no significant, further increase

• **No accumulation** during many months, over range of doses
Issue #3: ‘Multiple Brain Regions’
Targeted and Spread of Protein to
Non-targeted Brain Regions
Dose-related NTN distribution in monkey striatum

Cd = caudate
ic = internal capsule
Pt = putamen

FB

CERE-120 Low Dose (3x10^{10} vg)
CERE-120 Mid Dose (1x10^{11} vg)
CERE-120 High Dose (3x10^{11} vg)

Doses: vg/hemisphere
Distribution of NTN in monkey following highest possible CERE-120 dose (1.75 x 10^{12} vg/hemi.)
## Primate brain regions expressing NTN protein following CERE-120

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6E10</td>
</tr>
<tr>
<td>Striatum</td>
<td>yes</td>
</tr>
<tr>
<td>Substantia Nigra</td>
<td>yes</td>
</tr>
<tr>
<td>Globus pallidus (neuropil/fibers only)</td>
<td>(yes)</td>
</tr>
<tr>
<td>VTA</td>
<td>NO</td>
</tr>
<tr>
<td>Thalamus</td>
<td>NO</td>
</tr>
<tr>
<td>Cortex (excluding track)</td>
<td>NO</td>
</tr>
<tr>
<td>Remainder of brain</td>
<td>NO</td>
</tr>
</tbody>
</table>
Issue #3: ‘Multiple Brain Regions’ Targeted and Spread of Protein to Non-targeted Brain Regions

Synopsis:
Targeting of CERE-120 is limited to nigrostriatal system and NTN expression is mostly limited to this system, as well
Issue #4: The Use of Non-regulatable Vector
Perspectives on the general use of non-regulatable vectors for neurotrophic factors

• Several **human trials** already **delivered neurotrophic factors into CNS** (up to several years); risks appear well characterized and likely related to non-targeted delivery

• Several **gene therapy trials** previously approved by RAC for **heart and CNS** indications, delivered growth factors via non **regulatable vectors**

• **Regulatable vectors** have their own **potential risks** (e.g., unnatural, transcriptional **proteins** are persistently expressed **without regulation** and can generate **immune reaction**; i.e., the **regulator is unregulated**

• Unknown **risks** associated with small molecule regulator (i.e., systems inherently require additional ‘**regulator drug**’)

• **No regulatable vector** has **yet been tested in humans** and a full assessment of efficacy & risks is still several years away
Justification for using a non-regulatable vector specifically to deliver NTN for PD

- **CERE-120** produces no apparent toxicity, including none of the empirically-defined effects of poorly targeted growth factors

- **Doses hundreds of times higher** than those proposed for this human trial were tested in animals, demonstrating that:
  - Expression of protein is mostly restricted to nigrostriatal system
  - No significant increase in volume of expression occurs after 4 weeks
  - No adverse effects are observed anywhere in the CNS
  - No adverse effects are seen anywhere systemically
CERE-120 dose multiples

• Rat dose multiple
  – Rat efficacious dose versus dose shown to be safe: **250 times**

• Rat to human dose multiple (via brain weight)
  – Dose shown to be safe versus proposed human doses: **50 and 200 times**

• Monkey to human dose multiple (brain weight)
  – Dose shown to be safe versus proposed human doses: **100 and 400 times**
Conclusion: Data support CERE-120 as a non-regulatable vector to deliver NTN for PD

- **Wide safety margin** of CERE-120 established, without regulation:
  - expression of protein is controlled
  - is stable from 1 month to > 7 months
  - is safe at large dose multiples

- **Arguments against a regulatable vector:**
  - an **appropriate risk: benefit** ratio established
  - regulatable vector *could* conceivably **increase risk** due to unknown aspects of more complicated, ‘first in human’ construct
  - **No prior studies required** a regulatable vector and the data with CERE-120 reveal no reason for greater concern
Issue #4: The Use of Non-regulatable Vector

Synopsis:
While concerns about unregulated expression of NTN may seem understandable, they are not supported by CERE-120 safety/distribution data and nature of proposed protocol.
Issue #5: ‘Rescue Strategy’ Employed
Addressing Possible Adverse Events (AEs) to CERE-120

1) We carefully considered possible and hypothetical AEs, based on collective past experience with growth factors, nuances of Parkinson’s disease & comprehensive review of literature

2) Are providing clear information regarding all potential & hypothetical risks to each subject via “informed consent”

3) Will continuously and carefully monitor subjects for AEs and manage with available therapy
## Hypothetical AE’s and Treatment Strategies

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Setting / Liability</th>
<th>Treatment Options</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nausea &amp; vomiting</td>
<td>Only seen with ICV GDNF; delivery problem</td>
<td>Antiemetics (e.g., ondansetron; trimethobenzamide; domperidone)</td>
</tr>
</tbody>
</table>
| Anorexia & weight loss    | Only seen with ICV GDNF; delivery problem                | • Clinical monitoring & diet change  
• Appetite enhancers       |
| Parethesias / Pain        | More severe with ICV GDNF; less with intraputaminal infusion; delivery problem | • NSAIDS & acetaminophen  
• Tricyclic antidepressants  
• Gabapentin &/or tizanidine |
| Lhermitte’s sign          | More severe with ICV GDNF; less with intraputaminal infusion | Pharmacological Rx (mexiletine; gabapentin; tizanidine; tricyclics)               |
| Hyponatremia              | Only seen with ICV GDNF; delivery problem                | • Limit free water intake  
• Demeclocycline & lithium carbonate                                          |
| GI Disturbances           | Only seen with ICV GDNF; delivery problem                | • Adjust diet; add bulking agents  
• RX: laxatives/soften stool; diarrhea                                           |
| Dsykinesias               | Hypothetical AE: GDNF actually appears to reduce dyskinesias | • Adjust antidopaminergic therapy  
• Add amantadine  
• Deep brain stimulation                                                       |
| Psychotic symptoms        | Hypothetical AE: No empirical data                       | • Adjust dopaminergic therapy  
• Add atypical anti-psychotics                                                   |
Issue #5: ‘Rescue Strategy’

Employed

Synopsis:
Rescue strategies do exist to deal with hypothetical risks of CERE-120
Issue #6: Cerebellar Toxicity Reported in Select, GDNF-treated Monkeys
Synopsis of GDNF-induced ‘Cerebellar Toxicity’

• Toxicity reported: **focal cell loss in cerebellum** of some of ‘6mo highest dose- 3 mo recovery’ monkeys (only)

• If link between GDNF and cytotoxicity is proven, data suggests it was **likely caused** by deficiencies in delivery system (i.e., **leakage of protein from indwelling cannula**); supported by:
  – Clear evidence of ‘classic’ changes near meninges (e.g, pia thickening, Schwann cell hyperplasia and sympathetic in growth; Boyd & Hovland, 2004) in mid and high dose monkeys
  – Confirmation of I^{125}-GDNF in occipital cortex and cerebellum in monkeys infused using similar pump/cannula system (Gash et al, 2005)
Synopsis of GDNF-induced ‘Cerebellar Toxicity’: part 2

• Evidence of leakage (and possible toxicity) reinforces need for improved delivery method
  – No evidence for protein leakage or cerebellar cytotoxicity following very high doses of CERE-120 in rats or monkeys

• Subjects in GDNF studies: no apparent cerebellar AEs
  – Initial autopsy subject from Gill et al study revealed no cerebellar toxicity
Issue #6: Cerebellar Toxicity Reported in Select, GDNF-treated Monkeys

Synopsis:
Putative GDNF toxicity most likely reflects untargeted delivery in monkeys and not inherent limitation of protein—this argues FOR, NOT AGAINST use of gene transfer for this application.
Issue #7: Rationale for Dosing Schedule in Humans
Schematic of Proposed Dosing Schedule for CERE-120

* DSMB Cumulative Data Review

Month 1    Month 2    Month 3    Month 4    Month 5    Month 6    Month 7    Month 8

Low Dose

S#1
S#2
S#3
S#4
S#5
S#6

High Dose

S#7
S#8
S#9
S#10
S#11
S#12
Rationale for CERE-120 Dosing Schedule in Advanced PD subjects

- Purpose of this study is to evaluate safety of CERE-120
  - **Volume of expression** of NTN occurs early and has reached steady state by **four weeks**
  - The **nonclinical package** reveals an ‘uneventful’ **safety/tox profile**, at very **high dose multiples**, following **many months** of treatment in both rats and monkeys
    - No evidence of toxicity observed and no evidence of greater risk over time
Rationale for CERE-120 Dosing Schedule in Advanced PD subjects (cont.)

• Protocol ‘leverages’ decades of **experience** gained with **growth factors** in animals and **humans** (scores of patients dosed up to several years each)

• Those studies suggest **greatest risk** for toxic effects (humans and animals) is **untargeted delivery** and these effects typically appear within days to < four weeks

• **Hypothetical risk** with gene transfer: **uncontrolled spread** of protein to ventricles. **Data** for CERE-120 (volume of distribution with large dose multiples over many months) convincingly argue that this is extremely unlikely with proposed human doses
Issue #7: Rationale for Dosing Schedule in Humans

Synopsis:

• Proposed dosing schedule supported by:
  – Data generated for CERE-120, as well as, that for neurotrophic factors, generally
  – Need to find more effective treatments for advanced PD patients
  – Careful safety monitoring proposed
Specific issues raised in RAC review deserving special comment

1) Evidence of efficacy of CERE-120
2) Kinetics and accumulation of NTN in brain
3) ‘Multiple brain regions’ targeted and spread of protein to non-targeted brain regions
4) The use of non-regulatable vector
5) Question of ‘rescue strategy’
6) Cerebellar toxicity reported in select, GDNF-treated monkeys
7) Rationale for dosing schedule in humans