

## Comparison of Post-Mortem and Non-human Primate Brain

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### INTRODUCTION

- A controlled Phase 2 trial of AAV2-neurturin (CERE-120), injected stereotactically into the putamen of moderately advanced Parkinson's patients, was completed in late 2008.
- Protocol-prescribed analyses failed to show a difference on the primary endpoint (UPDRS motor off at 12 mos), though:
  - (a) several secondary endpoints at 12 mos suggested modest improvement (while no measure favored the control group) and
  - (b) blinded analyses of longer time points (15 & 18 mos) revealed even greater benefits, including UPDRS motor off (see Marks et al. this session)
- Two patients died from events unrelated to the treatment and their brains were donated for study.
- The present study compared the bioactivity of CERÉ-120 in PD versus several nonhuman primate models (using IHC peroxidase)
- The findings from this analysis were instrumental in understanding the prior CERÉ-120 Phase 2 data and in redesigning the current Phase 1/2 trial. They also have implications for other surgically-based treatments for PD.

### MATERIALS AND METHODS

**Study Subjects:** Each patient received 5.4X10<sup>11</sup>vg of CERÉ-120, distributed in four, 10 µL deposits bilaterally within each putamen, equally distributed.

**Subject 1904:** 73 year old male (one hemisphere) Day 47: fatal MI. Time from dose to death = 1.5 mo (NRTN expression time) Time from death to brain fixation = 13 hours (autolysis time)

**Subject 1802:** 59 year old male (two hemispheres) Day 91: inf. vena cava thrombosis followed by renal failure and fatal pulmonary embolism. Time from dose to death = 3 mo (NRTN expression time) Time from death to brain fixation = 6 hours (autolysis time)

**Non-human primate comparisons:** Ten monkeys, from approximately 50 that were administered CERÉ-120, were selected for this comparison with PD, specifically selecting monkeys given a range of CERÉ-120 doses equivalent to, or well below, those administered to the PD patients (based on relative striatal volumes).

**Young monkeys:** Two young, healthy monkeys were administered 0.5x10<sup>11</sup>vg of CERÉ-120 per hemisphere in a single deposit of 25 µL within the striatum and euthanized 1 month later.

**Aged monkeys:** Three aged monkeys (>20 years old) were administered 3x10<sup>11</sup>vg of CERÉ-120, unilaterally, via 5 equally spaced 30 µL deposits within the striatum and euthanized 8 months later.

**MPTP monkeys:** Five monkeys first received unilateral, intracarotid infusions of MPTP. Following clear evidence of motor dysfunction 4 days later, they then received infusions of 1.5x10<sup>11</sup>vg of CERÉ-120, via 5 equally spaced 15 µL deposits within the striatum, as well as a single, small 0.2x10<sup>11</sup>vg dose in the nigra. They were euthanized at 10 months.

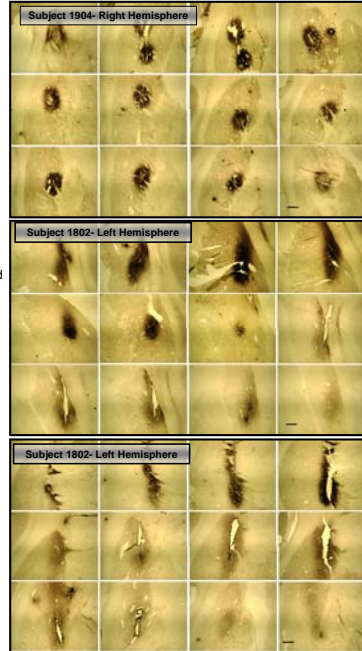
### RESULTS

**NRTN EXPRESSION IN PD:** Two independent analyses were performed at Rush and Ceregene. Both estimated the volume of NRTN within the putamen using the Cavalieri method.

- These data provide first evidence for expression of a neurotrophic factor in PD brain, with both analyses estimated roughly 15% of entire putamen covered with NRTN.

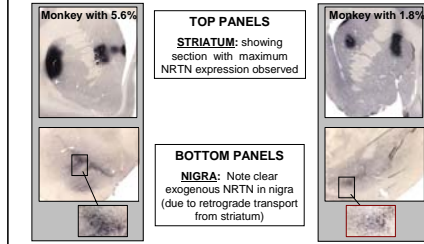
Subject #	RUSH	Ceregene	$\bar{x}$ of 2 methods
1802 - Left	23%	15.1%	19%
1802 - Right	15.8%	15%	15.4%
1904 - Left	8%	14.5%	11.3%
$\bar{x}$ of 3 hemispheres	15.6%	14.9%	15.2%

### NEURTURIN (NRTN) IN PD PUTAMEN

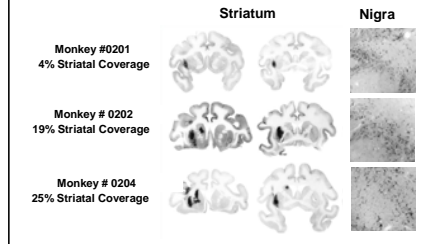


### NRTN IN NON-HUMAN PRIMATE STRIATUM AND NIGRA

**Young Monkeys:** Two of 46 young monkeys given CERÉ-120 to striatum. Note even very low dose produces clear NRTN in both striatum and nigra (i.e., NRTN transported to nigra despite very low, 2% and 6% NRTN coverage of striatum and relatively short, 1 mo post-CERÉ-120 exposure).

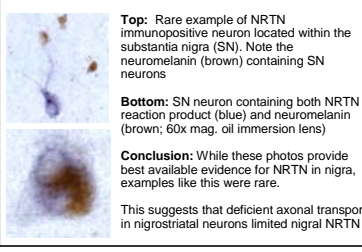


**Aged Monkeys:** CERÉ-120 injections into striatum produce NRTN in striatum as well as in nigra (note clear NRTN in nigra despite varying degrees of NRTN coverage of striatum; 4 to 25%).

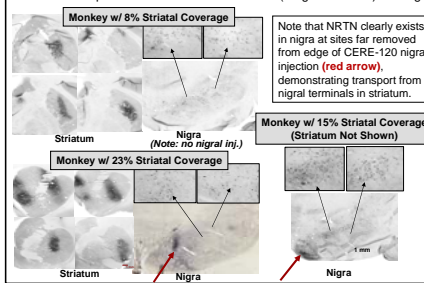


### LACK OF NRTN IN PARKINSON'S NIGRA

Despite clear & intense NRTN expression in putamen, only rarely was evidence of NRTN seen in substantia nigra in PD



**MPTP Monkeys:** 3 of 5 of the MPTP monkeys injected with CERÉ-120 in striatum that produced NRTN in both striatum (range: 8 to 23%) and nigra.



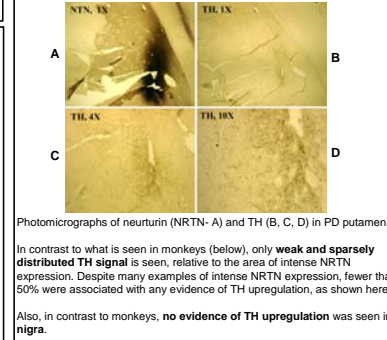
### SUMMARY: NRTN Expression in Striatum and Nigra of Monkey versus Parkinson's Brain

**Monkey studies:** Delivery of CERÉ-120 to striatum produced: (1) expression of NRTN in striatum as well as (2) substantia nigra (pars compacta) over a range of volumes of striatal coverage. - Provides clear evidence for transport of vector and/or NRTN from striatum to nigra

**Parkinson's cases:** Delivery of CERÉ-120 to striatum also produced clear expression of NRTN in striatum (within range or exceeding that of monkeys). However, in stark contrast to non-human primates, **only rarely was evidence for NRTN seen in nigra** - These data suggest significant deficiency in axonal transport in degenerating nigrostriatal neurons in advanced PD

### TYROSINE HYDROXYLASE INDUCTION

#### PD Putamen: Neurturin & Tyrosine Hydroxylase



In contrast to what is seen in monkeys (below), only **weak and sparsely distributed TH signal** is seen, relative to the area of intense NRTN expression. Despite many examples of intense NRTN expression, fewer than 50% were associated with any evidence of TH upregulation, as shown here. Also, in contrast to monkeys, **no evidence of TH upregulation** was seen in nigra.

### SUMMARY: TH in Monkeys versus PD Nigrostriatum

**Non-human primates:** clear and consistent enhancement of TH occurs in terminal fields of striatum and nigra cell bodies following CERÉ-120 treatment; increased TH is linked to NRTN protein in both brain regions

**Parkinson's brains:** no evidence for TH induction in nigra; in striatum, only a very weak enhancement is observed, restricted to small area within most intense sites of NRTN expression (and even this weak signal is observed less than 50% of time) - Lack of robust TH response is consistent with lack of significant NRTN protein in nigra - Without neurotrophic factor in cell body, induction of genes to repair degenerating nigrostriatal neurons cannot occur

### SYNOPSIS: CERÉ-120 Bioactivity Chain of Events

CERÉ-120 (into striatum)	Neurturin Expression: STRIATUM	Neurturin Protein: NIGRA	TH Induction: STRIATUM	TH Induction: STRIATUM
→	→	→	→	→
All monkeys (MPTP, aged and young)	+/+++	+/-+++	++	++
Advanced PD Cases	++	-/?	-/?	(+)--

**In monkeys:** CERÉ-120 into striatum initiates a cascade that provides NRTN in striatum and substantia nigra and TH induction in both regions.

**In advanced Parkinson's disease cases:** CERÉ-120 into striatum produces clear NRTN expression in striatum, but little evidence for NRTN in nigra and thus very little TH induction (for genes to repair neurons not likely activated).

### SUMMARY & CONCLUSIONS

- Clear evidence for NRTN expression was observed in the targeted PD putamen following CERÉ-120 administration (~15% of entire putamen covered).
- This provides first evidence for successful transgene expression of a neurotrophic factor in PD brain.
- In contrast to all non-human primate studies, very little evidence for NRTN was seen in nigra cell bodies, consistent with impaired axonal transport.
- Furthermore, in putamen NRTN staining occasionally (<50%) was associated with a sparse increase in TH-immunoreactivity, encompassing **only a small subregion** of the intense NRTN signal; no evidence for enhanced TH was seen in nigra.
- This also stands in stark contrast to monkey results, where the breadth of TH upregulation in striatum **exceeded** that of NRTN signal and consistently TH enhancement seen in nigra.
- Lack of significant TH signal likely due to lack of NRTN in nigra- an event essential for inducing genes to repair nigrostriatal neurons.

### IMPLICATIONS

- To maximize bioactive effects of CERÉ-120 in advanced PD, need to add 'nigra target' to assure adequate NRTN is expressed in cell body
- Therefore, revised plan will both target nigra directly as well as increase dose to putamen

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