1353

Effects of NE3107 Anti-Inflammatory Treatment on Motor Activity and Neurodegenerative Features of Parkinson's Disease in a Marmoset Monkey Model

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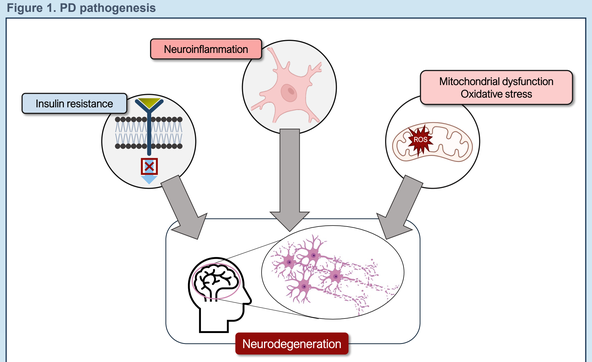
BACKGROUND

Figure 1. PD pathogenesis



For more information see Athauda D, Foltynie T, 2016[1]; and Dias V, Junn E, Mouradian MM, 2013[3]
ROS, reactive oxygen species.

- Chronic neuroinflammation, aberrant insulin signaling, and oxidative stress are key processes that drive neurodegeneration in Parkinson's disease (PD; **Figure 1**)[1,2]
- Levodopa, a dopamine precursor, is an effective first-line treatment for PD and improves motor function, but it lacks disease-modifying potential, and long-term exposure can lead to diminished efficacy and involuntary movements called levodopa-induced dyskinesia (LID), due to dopamine hypersensitivity[3-5]
  - There is a great need for non-dopaminergic, safe, and effective therapeutic alternatives to levodopa that can slow/prevent the progression of PD
- NE3107 is an oral, blood-brain–permeable, anti-inflammatory, and insulin-sensitizing molecule that binds to the inflammatory mediator ERK and inhibits inflammation-specific ERK and NF-κB activation and TNF-α signaling, without affecting homeostasis[6]
  - In the context of PD, NE3107 may downregulate microglia to reduce purine- and glutamate-stimulated inhibition of dopamine receptors, thereby improving motor function[7]
- NE3107 has been shown to have a favorable safety profile and demonstrated anti-inflammatory, insulin-sensitizing, pro-motoric, and neuroprotective effects in pre-clinical studies using rodents[4,6,8,9]
- To bolster the rationale for investigating NE3107 in human PD clinical trials, we used an MPTP-induced marmoset model of PD and studied the potential effects of NE3107 on disease pathology and motor function

OBJECTIVES

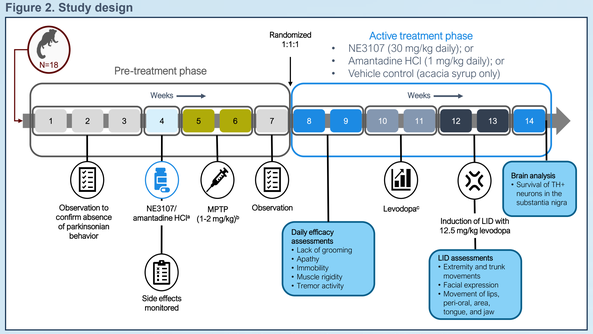
- The overall objectives of this pre-clinical study were to evaluate the safety, tolerability, and the extent of the anti-inflammatory effects of NE3107 treatment, specifically on the major features of PD, in marmosets with Parkinson's-like disease

METHODS

Study Design

- In this 14-week study, Parkinson's-like disease and LID were induced in marmosets (*Callithrix jacchus*) aged 2-5 years, and the effects of NE3107 (30 mg/kg daily), amantadine HCl (1 mg/kg daily), or vehicle (placebo; acacia syrup) were evaluated (**Figure 2**)
  - This study was conducted in a series of three sequential independent cohorts of 6 marmosets
  - Week 4: single dose of NE3107 or amantadine HCl to ensure the absence of behavioral side effects
  - Weeks 5-6: subcutaneous injections of 1-2 mg/kg MPTP on days 3-5 of week 5 and days 1 and 3 of week 6
  - Week 7: at the end of week 7, animals were randomized to receive NE3107, amantadine HCl, or control
  - Weeks 8-9: treatment effects against parkinsonian behavior were evaluated
  - Weeks 10-11: escalating doses of levodopa (5, 7.5, 10, and 12.5 mg/kg) were administered along with treatment, and effects on parkinsonian behavior were evaluated
  - Weeks 12-13: LID was induced in all animals with 12.5 mg/kg levodopa BID along with treatment, and the severity of LID was measured using Abnormal Involuntary Movement Score (AIMS)
  - Week 14: treatment was administered without levodopa, and animals were euthanized for brain immunohistochemical analyses

Figure 2. Study design



[a]Single dose. [b]Administered subcutaneously on days 3-5 of week 5 and days 1 and 3 of week 6 for a total of 6.5 mg/kg. [c]Single escalating doses of 5, 7.5, 10, and 12.5 mg/kg.
LID, levodopa-induced dyskinesia; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; TH, tyrosine hydroxylase.

Assessments

Treatment effects on parkinsonian behavior – weeks 8-11; scored on a 0 (normal) – 4 (most severe) scale

- Lack of grooming
- Apathy
- Immobility
- Muscle rigidity
- Tremor activity

Severity of LID – weeks 12-13; measured by AIMS (adapted to the primate model)

- Assessment of:
  - Extremity and trunk movements
  - Facial expression
  - Movement of the lips, peri-oral area, tongue, and jaw
- Rating scale: 0 (normal), 1 (extreme normal), 2 (mild), 3 (moderate) to 4 (severe)

Brain immunohistochemical analysis – end of week 14

- DA-positive neurons within the SNpc using TH-IR staining

RESULTS

Tolerability

- NE3107 treatment was well tolerated
- One monkey randomized to amantadine HCl and one randomized to NE3107 died prior to treatment

Immobility analysis

- Mean immobility scores with NE3107 monotherapy during weeks 8 and 9 were significantly lower (indicating improved mobility) compared with amantadine HCl treatment or vehicle only (**Table 1**)
- Immobility scores significantly improved with NE3107 in combination with levodopa doses ≥7.5 mg/kg/day during weeks 10-11, compared with NE3107 monotherapy (**Figure 3**)

Table 1. Mean immobility scores with monotherapy

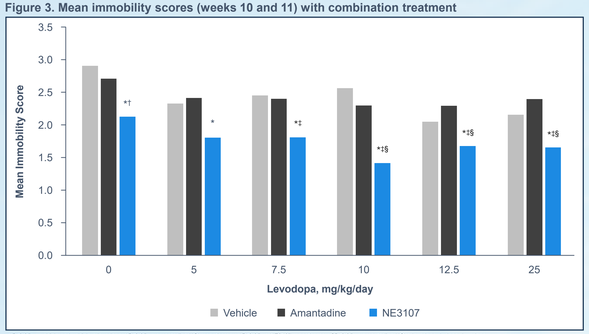
Week	Vehicle	Amantadine HCl	NE3107	*P* (vehicle vs amantadine)	*P* (NE3107 vs vehicle)	*P* (NE3107 vs amantadine)
7[a]	2.71	2.59	2.72	0.58	0.86	0.433
8	3.0	2.74	2.26	0.104	<0.0001	0.0048
9	2.8	2.72	2.10	0.75	0.00088	0.0044

[a]Represents scores at the end of disease induction and prior to initiation of therapy.

Effect of treatment on LID (evaluated using AIMS)

- During week 12, all animals developed LID following administration of 12.5 mg/kg levodopa BID
- NE3107 + levodopa combination treatment was associated with significantly lower AIMS compared with vehicle + levodopa treatment (mean: 2.1 vs 7.2; *P*<0.0001) and amantadine HCl + levodopa treatment (mean: 2.1 vs 5.4; *P*<0.0001) (**Table 2**)
  - NE3107 + levodopa combination treatment was associated with fewer LID observations compared with either vehicle or amantadine + levodopa combination treatments (**Figure 4**)

Figure 3. Mean immobility scores (weeks 10 and 11) with combination treatment



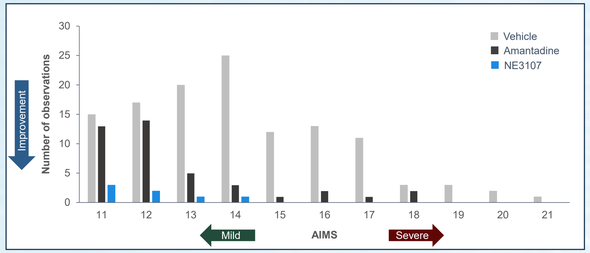
[a]*P*<0.05 vs vehicle and vehicle + levodopa; [b]*P*<0.05 vs amantadine HCl monotherapy; [c]*P*<0.05 vs NE3107 monotherapy; [d]*P*<0.05 vs amantadine HCl + levodopa. L-dopa, levodopa.

Table 2. Mean AIMS for week 12

	Vehicle	Amantadine HCl	NE3107
AIMS	7.2	5.4	2.1
P[a] (vs vehicle)	-	0.094	<0.0001
P[a] (vs amantadine HCl)	0.094	-	<0.0001

[a]Student's t-Test (two-sided assuming equal variance). AIMS, Abnormal Involuntary Movement Score.

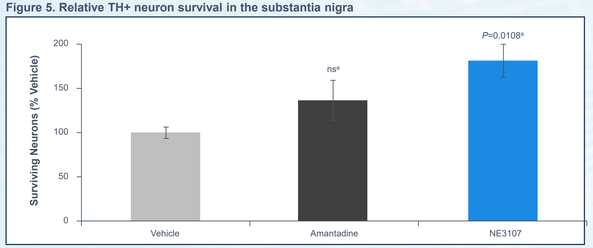
Figure 4. Effect of combination treatment on LID (AIMS)



Brain immunohistochemical analysis

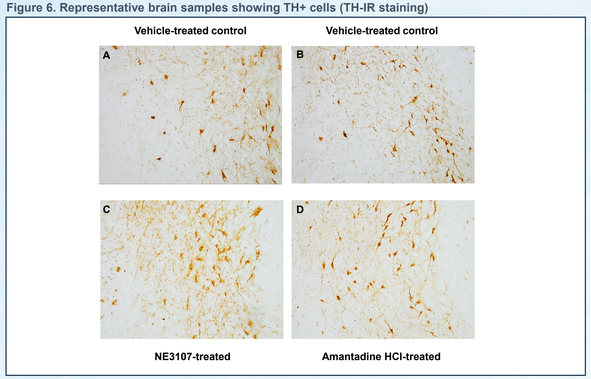
- Monkeys treated with NE3107, but not amantadine HCl, had significantly more surviving TH+ neurons compared with those treated with vehicle control (*P*=0.0108; **Figures 5** and **6**)

Figure 5. Relative TH+ neuron survival in the substantia nigra



[a]vs vehicle. ns, non-significant; TH, tyrosine hydroxylase.

Figure 6. Representative brain samples showing TH+ cells (TH-IR staining)



TH, tyrosine hydroxylase; TH-IR, tyrosine hydroxylase–immune reactive.

CONCLUSIONS

- In this 14-week pre-clinical trial of 18 marmoset monkeys with MPTP-induced Parkinson's-like disease, NE3107 monotherapy was associated with significantly lower immobility scores than treatment with either amantadine HCl or vehicle alone
- Improvements in mobility were observed within 24 hours of initiating NE3107 monotherapy, suggesting a direct influence on neuro-motor signaling, as opposed to an effect that increased with time of treatment, which might imply a link to neuroprotection
- NE3107 was significantly more effective in alleviating symptoms of LID in monkeys, compared with treatment with either amantadine HCl or vehicle
  - Importantly, the reduction in LID was achieved simultaneously with improvements in mobility
  - Key mediators of neuroinflammation such as ERK, NF-κB, and TNF-α have been implicated in the pathogenesis of LID[10-12]
  - The risk of LID represents an important unmet medical need in PD as patients are often unable to benefit fully from levodopa due to its potential to cause dyskinesia[5]
- Monkeys treated with NE3107, but not amantadine HCl, had significantly more surviving TH+ neurons in the SNpc, indicating decreased neurodegeneration
  - TH is an essential enzyme in the biosynthesis of dopamine, and its deficiency is integral to PD progression[13]
  - Neuroinflammation and aberrant insulin signaling are thought to contribute to neurodegeneration in PD, and inhibition of key neuroinflammatory mediators may have neuroprotective effects[1]
- Our findings corroborate the involvement of chronic neuroinflammation and insulin resistance in PD clinical symptoms, in addition to neurodegenerative pathways, and support the continued investigation of nondopaminergic therapies
- A recently completed phase 2, double-blind, placebo-controlled trial (NCT05083260) evaluated the safety and efficacy of NE3107 in 45 levodopa-treated patients with PD

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DISCLOSURES

CA and **CLR** are employees of BioVie Inc. **IHCHMP** has nothing to disclose.

BID, twice per day; DA, dopamine; ERK, extracellular signal-regulated kinase; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; ns, non-significant; SNpc, substantia nigra pars compacta; TH, tyrosine hydroxylase; TH-IR, tyrosine hydroxylase immune reactive; TNF-α, tumor necrosis factor alpha.

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