NDC-1308, a small molecule with remyelinating activity for treatment of secondary progressive multiple sclerosis patients

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Abstract

Background: Current therapeutics for MS patient's impact the immune mechanisms of the disease, rather than the elusive remyelination activity needed to repair the damaged myelin sheath. NDC-1308 is known to activate intracellular pathways for oligodendrocyte progenitor cell (OPC) differentiation. NDC-1308 induces mouse OPCs to differentiate into mature, myelinating oligodendrocytes *in vitro* and significantly increases remyelination of axons *in vivo*. These studies extend the *in vivo* remyelination results while addressing the safety and exposure of NDC-1308.

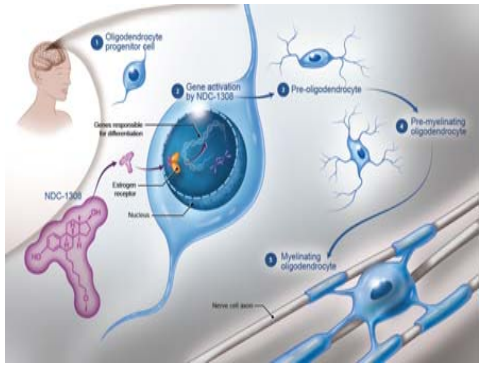
Objectives: A main goal was to correlate the ability of NDC-1308 to repair the damaged myelin sheath, in the cuprizone mouse model of demyelination, with a functional improvement. Intended outcomes also included an initial safety assessment for NDC-1308 and determining the dosing parameters for non-clinical IND-enabling studies.

Methods: Male mice were treated for 12-weeks with cuprizone and rapamycin to cause demyelination of white and gray matter regions of the brain. The demyelinated mice were administered NDC-1308 (68 mg/Kg, i.p., q.d.) for up to 6 weeks. Blood was collected at termination for clinical chemistry analysis, along with reproductive tracts for pathology, and brain regions for assessing the level of NDC-1308 remyelination. The OPC population in different brain regions was evaluated by immunohistochemistry using PDGFRα antibodies. The degree of mutagenicity and genotoxicity of NDC-1308 was measured by a bacterial reverse mutation assay and a mammalian cell micronucleus screening assay, respectively.

Results: NDC-1308 rapidly crosses the blood brain barrier and is absorbed into CNS tissues at levels exceeding the EC50 required for OPC differentiation *in vitro*. NDC-1308 is eliminated from the CNS and periphery after 24 hours. Following chronic treatment of demyelinated mice with NDC-1308, remyelination was significantly increased 18% (P<0.01) in cortical regions and 44% (P<0.0001) in hippocampal regions. A significant increase in grip strength was measured in the animals treated with NDC-1308. Observed animal behavior and clinical chemistries were normal and the OPC population remained intact. NDC-1308 was found to be non-mutagenic and non-genotoxic.

Conclusions: These results suggest NDC-1308 can be delivered to the CNS tissues in amounts sufficient for inducing OPCs to differentiate into mature, myelinating oligodendroctyes that can then repair the myelin sheath. NDC-1308 appears to be a potentially safe and effective therapeutic for treating SPMS patients. Non-clinical IND-enabling studies and a first-in-human Phase 1 study are planned.

Remyelination Background



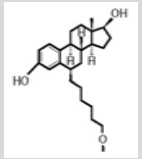
Normal myelin production
Myelin sheaths are derived from OPCs (1), which differentiate (2,3,4) into mature, myelinating oligodendrocytes (5).

Myelin block in SPMS patients
OPCs are present but quiescent in SPMS patients; no drugs exist that induce OPC differentiation to repair the damaged myelin sheath.

How NDC-1308 works
Induces OPCs to become mature, myelinating oligodendrocytes leading to a new myelin sheath.

Conflicts: SHN and JGY are shareholders of ENDECE Neural. Funding: This work was funded in part by a Fast Forward grant from the National MS Society

NDC-1308 Characterization



NDC-1308

- **Analog of estradiol with alkoxyalkyl tail at C-6.**
- **Conceived by *in silico* modeling.**
- **Highly lipophilic (crosses blood brain barrier).**
- **Binds to estrogen receptors with similar affinity.**
- **Agonist for ER-α and ER-β.**
- **GMP-manufacturing process.**
- **Stably formulated with cyclodextrin.**
- **Possesses activity to remyelinate axons.**
- **Has lost side-effects associated with estradiol.**

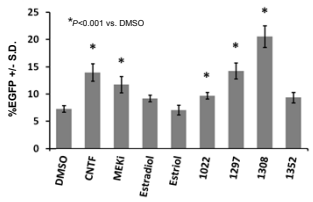
NDC-1308 up-regulates genes in pathways for OPC differentiation and myelin synthesis

- 3 human cell lines were treated with NDC-1308 (10 μM, 24 hrs).
- Microarray analysis identified common genes to all 3 cell lines.
- Genes for OPC differentiation and myelin synthesis were significantly up-regulated.

Gene	Gene Expression Increase
Delta/notch-like EFG repeat	75
Oligodendrocyte lineage transcription factor 2	72
Myelin basic protein (variant 7)	65
Myelin oligodendrocyte glycoprotein (variant alpha 4)	38

NDC-1308 induces OPC differentiation

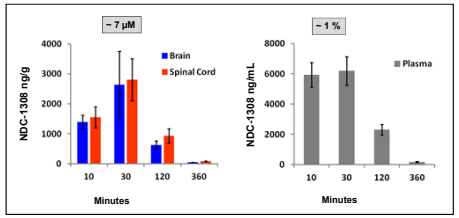
- OPCs were isolated from PLP-EGFP transgenic mice.
- OPCs were treated with10 μM of test agents for 5 days.
- Mature oligodendrocytes (EGFP expressing) were detected by luminescence



- NDC-1308 (EC_{50} = 1.0 μM) is the most potent of ENDECE Neural's analogs for inducing OPCs to differentiate into mature myelinating oligodendrocytes.
- Estradiol and estriol *do not* induce OPCs to differentiate into mature oligodendrocytes.

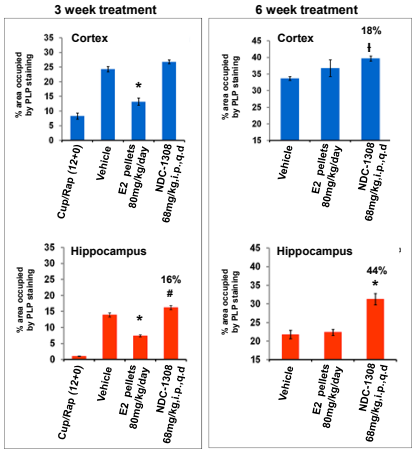
NDC-1308 is rapidly absorbed into CNS tissues

- Single injection.
- C57BL/6 mice (N=5).
- 75 mg/Kg of NDC-1308 formulated in SBE-β-cyclodextrin.
- Brain, spinal cord and plasma were collected at termination.
- NDC-1308 (+/-S.D.) measured by mass spectrometry
- ***CNS absorption: i.v. >> s.c. ≥ i.p. >> p.o.***



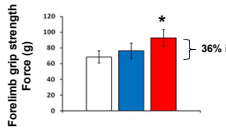
NDC-1308 induces remyelination in a cuprizone mouse model of demyelination

- Mice were demyelinated with a cuprizone/rapamycin treatment for 12 weeks.
- NDC-1308, estradiol (E2) or vehicle was administered for 3 or 6 weeks.
- Myelin synthesis in hippocampus and cortex was detected by PLP staining.
- N=7-12 per group; Average %PLP +/- SEM.
- $^{\#}P < 0.05$ vs. veh; $^{\dagger}P < 0.01$ vs. veh; $^{*}P < 0.0001$ vs. veh.



Functional improvement following NDC-1308 treatment of demyelinated mice

- 3-week NDC-1308 (68 mg/Kg, i.p., q.d.) treatment of cuprizone demyelinated mice.
- Forelimb grip strength assessment with 10 trials per animal.
- N=12 per treatment group.
- Force (g) +/- S.D.
- *$^{*}P$<0.005 vs. Veh

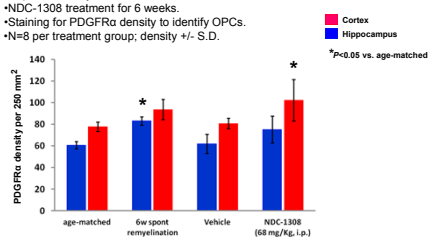


NDC-1308 treated animals have significantly higher forelimb grip strength compared to vehicle.

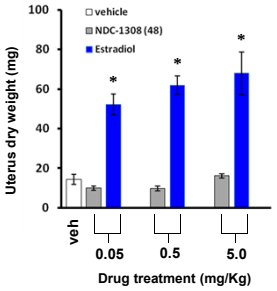
This test could potentially be used to assess improvement of function in Secondary Progressive MS patients during clinical trials.

OPC pool remains intact after NDC-1308 treatment in the cuprizone model

- Cuprizone demyelinated mice.
- NDC-1308 treatment for 6 weeks.
- Staining for PDGFRα density to identify OPCs.
- N=8 per treatment group; density +/- S.D.



NDC-1308 is not estrogenic



- Ovariectomized C57BL/6 mice were dosed with either estradiol or NDC-1308 once daily for 4 days.
- Mice were euthanized 6 hours after last dose.
- Each uterus was removed, punctured and dried by blotting.

N=6
*P < .0001 vs. veh

Enhanced CNS absorption of NDC-1308 in dog

Species	NDC-1308 injected (mg/Kg)	NDC-1308 Detected		NDC-1308 Brain:Plasma
		Brain (mg)	Plasma (mg)	
Mouse	68.00	$^{\pm}$0.0008	*0.012	0.066
Dog	2.81	$^{\#}$0.432	†1.422	0.304

±Assumes ave brain wt = 0.4 g in a 20 g mouse
#Assumes ave brain wt = 72 g in a 10 Kg dog
*Assumes 1 mL plasma in a 20 g mouse
†Assumes 450 mL plasma in a 10 Kg dog

Phase 1 trial design for NDC-1308

	Phase 1A	Phase 1B
Study Description	Single Ascending Dose (SAD)	Multiple Ascending Dose (MAD)
Patient Population	Healthy Volunteers	Healthy Volunteers
Placebo Included	Yes	Yes
Enrollment	48	48
Dosing Schedule	1 qd (iv)	14 qd (iv)
Follow-Up	1, 7, 30 and 90 days	14, 30, 90 and 180 days
Objectives	• Standard safety & tolerability assessment in healthy volunteers over 1 month • Standard safety & tolerability assessment in healthy volunteers over 3 months • Determined plasma pharmacokinetic levels	• Standard safety & tolerability assessment of 14 infusions of NDC-1308 in healthy volunteers • Standard safety & tolerability assessment in healthy volunteers over 6 months • Determine plasma pharmacokinetic levels

Conclusions

1. **NDC-1308 has desirable qualities for an MS therapeutic. It is a small molecule that rapidly crosses the blood brain barrier and is absorbed into CNS tissues following systemic administration.**
2. **NDC-1308 has a potent activity *in vitro* to induce OPCs to differentiate into mature, myelinating oligodendrocytes.**
3. **In the cuprizone mouse model of demyelination, NDC-1308 induces significant remyelination of demyelinated axons in cortical and hippocampal regions.**
4. **The remyelinating activity of NDC-1308 is associated with a functional improvement in forelimb grip strength.**
5. **The biological activity of NDC-1308 is distinct from estradiol. NDC-1308 has gained the function of remyelination, but lost commonly associated side-effects of estradiol, such as estrogenicity.**
6. **NDC-1308 is being advanced to the clinic as a therapy for treating patients with Secondary Progressive MS.**