**Abstract**

Background: There is an unmet need for remyelinating therapies for diseases like multiple sclerosis (MS). Remyelination depends upon the ability of oligodendrocyte progenitor cells (OPCs) to differentiate into mature oligodendrocytes that can then repair the damaged myelin sheath. NDC-1308 is an analog of estradiol (E2) that was previously shown in culture to cause 3-fold or more mouse OPCs to differentiate into mature myelinating oligodendrocytes compared to vehicle. E2 and estriol do not possess this myelinating activity. Side-by-side comparison of NDC-1308 and E2 in the cuprizone mouse model of demyelination showed that only NDC-1308 dramatically increases the level of remyelination (up to 44%) in the hippocampus.

Objectives: We investigated how NDC-1308 has gained the function to remyelinate axons, but lost the deleterious side-effects commonly associated with estrogens.

Methods: In silico modeling was used to compare the orientation of NDC-1308 in the estrogen receptor (ER) ligand binding domain to E2. The affinity of NDC-1308 for different ER subtypes was characterized using a fluorescence polarization-based assay. Intracellular pathway activation of NDC-1308 was compared to E2 by real-time PCR (qPCR) in several human cell lines. We determined whether NDC-1308 is estrogenic, a potential safety concern for treating MS patients. Estrogenicity was directly measured in a mouse uterotropic assay since E2 is known to cause a rapid and dramatic increase in uterine weight in this assay.

Results: In silico modeling studies suggest that NDC-1308 interacts with diverse regions in the ER ligand binding domain compared to E2, thereby eliciting a distinct pattern of gene expression that is beneficial for myelin repair. Indeed, NDC-1308 and E2 are both ER agonists, the unique remyelinating activity of NDC-1308 can be traced back to its ability to significantly up-regulate key genes (OLIG2, OLIG1, MOG, and MBP) for oligodendrocyte differentiation and remyelination. Real-time qPCR analysis showed these same genes are up-regulated in human PIMBa treated with NDC-1308, suggesting they could be a new therapeutic biomarker. Unlike E2, NDC-1308 was not found to be estrogenic in the uterotropic assay. Further testing revealed that NDC-1308 is non-mutagenic and not genotoxic.

Conclusions: Because of its unique mechanism of action, its potent remyelinating activity and its demonstrated lack of harmful side-effects, NDC-1308 possesses many desirable attributes of an effective MS therapy.

**Remyelination Background**

Remyelination Background

**NDC-1308 regulates oligodendrogenesis genes distinctly from estradiol**

- Human cell line Panc-1 or SK-DMV-3 cells were treated with NDC-1308 (10 µM), 5.36 µg/mL estradiol (E2), or 10.9 µg/mL estradiol. (E2, 10 µM) or vehicle for 72 hrs.
- Real-time PCR of key genes for OPC differentiation and myelin synthesis were significantly up-regulated (3 biological replicates, duplicate RT-PCR).

**NDC-1308 is rapidly absorbed into CNS tissues**

- Female C57/BL6 mice (N = 5) each with single s.c. injection of 5 µg/mL of NDC-1308 formulated in DMSO, 5 days later.
- Brain, spinal cord and plasma were collected at termination.
- NDC-1308 has high affinity to ERs (IC50 = 913 ± 4 nM).
- NDC-1308 has high CNS absorption (~ 2%).

**NDC-1308 is not estrogenic**

- Female C57/BL6 mice were dosed with either estradiol or NDC-1308 once daily for 4 days.
- Uterus were measured 6 hours after last dose.
- Each uterus was removed, uncircumcised and dried by distillation.

**Conclusion**

1. NDC-1308 is a small, lipophilic molecule that is systemically administered and absorbed into CNS tissues in amounts sufficient for inducing remyelination of cortical and hippocampal brain regions.
2. In the mouse cuprizone model, the remyelinating activity of NDC-1308 is associated with a functional improvement in forelimb grip strength.
3. In vivo, NDC-1308 can induce OPCs to differentiate into mature, myelinating oligodendrocytes.
4. NDC-1308 appears to override inhibitors of OPC differentiation leading to formation of mature, myelinating oligodendrocytes that express MBP, a key component of the myelin sheath.
5. Mechanistically, NDC-1308 is an estrogen receptor antagonist that up-regulates several key genes which drive oligodendrogenesis.
6. The activity of NDC-1308 is in strong contrast to that of estradiol. NDC-1308 has gained the function of remyelination compared to estradiol, but lost commonly associated side-effects, such as estrogenicity.

**Favorable kinetics for NDC-1308 in dog**

**NDC-1308 is not mutagenic or genotoxic**

**Conclusions**

- NDC-1308 is a small, lipophilic molecule that is systemically administered and absorbed into CNS tissues in amounts sufficient for inducing remyelination of cortical and hippocampal brain regions.
- In the mouse cuprizone model, the remyelinating activity of NDC-1308 is associated with a functional improvement in forelimb grip strength.
- In vivo, NDC-1308 can induce OPCs to differentiate into mature, myelinating oligodendrocytes.
- NDC-1308 appears to override inhibitors of OPC differentiation leading to formation of mature, myelinating oligodendrocytes that express MBP, a key component of the myelin sheath.
- Mechanistically, NDC-1308 is an estrogen receptor antagonist that up-regulates several key genes which drive oligodendrogenesis.
- The activity of NDC-1308 is in strong contrast to that of estradiol. NDC-1308 has gained the function of remyelination compared to estradiol, but lost commonly associated side-effects, such as estrogenicity.

**References**: BBM and Y2F are shareholders of ENDECE Neural. Funding: This work was funded in part by a Fast Forward grant from the National MS Society.

---

**Oligodendrogenesis via a small molecule therapy for treating multiple sclerosis patients**

Steven H. Nye and James G. Yarger, ENDECE Neural, LLC, Mequon, WI USA