# Vidofludimus Calcium, an Orally Available DHODH Inhibitor in Phase 3 Clinical Trials for Multiple Sclerosis, Potently Activates NURR1

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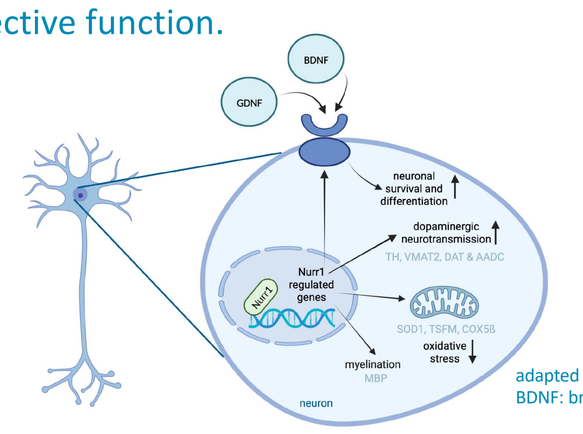
## Background[1-3]

The orally available dihydroorotate dehydrogenase (DHODH) inhibitory small molecule, vidofludimus calcium (VidoCa), being developed for the treatment of multiple sclerosis (MS) is currently tested in phase 2 and 3 clinical trials for progressive (P)MS and relapsing (R)MS, respectively.

Nuclear receptor related 1 (NURR1, *NR4A2*) is a nuclear receptor (NR) that is highly expressed in, among other, neurons and thought to have a neuroprotective function.




adapted from Willems, et al [1]
BDNF: brain-derived neurotrophic factor

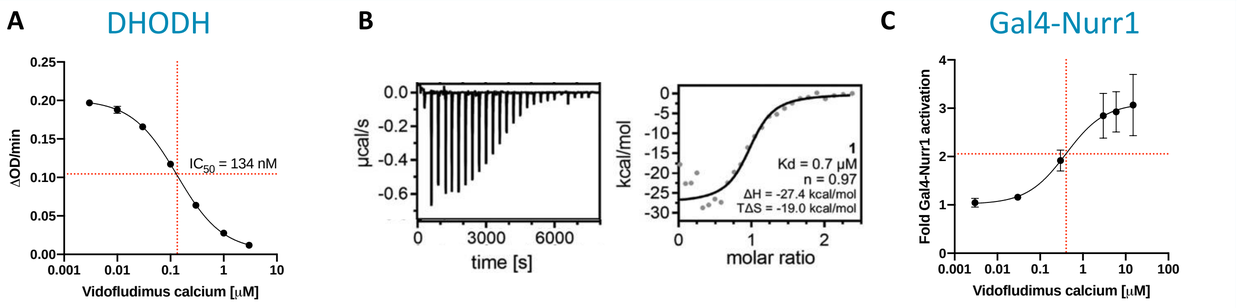
Also, anti-inflammatory roles of NURR1 in peripheral and brain resident immune cells have been described.

Altered NURR1 expression levels have been detected in Parkinson's and MS patients and heterozygous NURR1 knockout in experimental autoimmune encephalomyelitis (EAE) mice caused earlier disease onset. Here, we investigated the potential of VidoCa to act as NURR1 agonist.

## Methods[2,4-6]

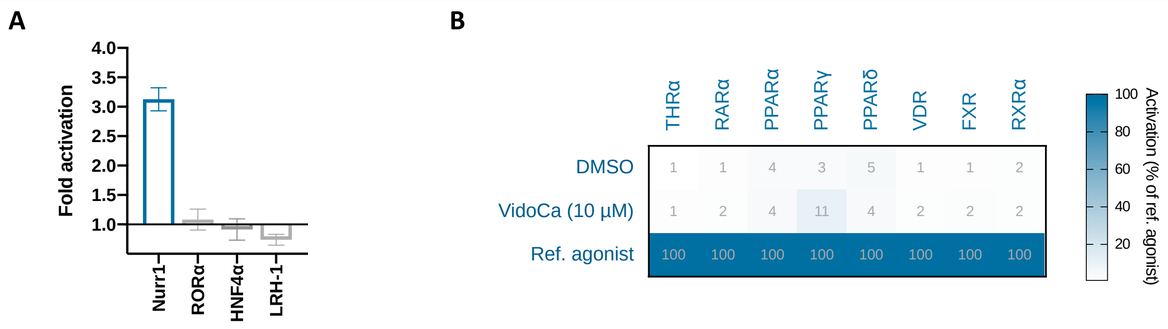
- **Cell free DHODH inhibition assay.** DHODH inhibition was measured on N-terminally truncated recombinant human DHODH enzyme as previously described[4,5]. Four biological independent assays were performed with each data point in triplicates.
- **Isothermal Titration Calorimetry (ITC).** ITC experiments were conducted on an Affinity ITC instrument (TA Instruments, New Castle, DE) as previously described[2].
- **Hybrid Reporter Gene Assays.** Nurr1 modulation was determined in a Gal4-hybrid, a full-length monomer (NBRE) and a full-length heterodimer (Nurr1/RXR, DR5) reporter gene assays in HEK293T cells as previously described[2]. A similar setup was used for screening the other nuclear receptors (NR). Five hours after transfection, the cells were incubated with VidoCa or respective agonists for 16h before luciferase activity was measured. Relative light units (RLUs) were calculated to normalize for transfection efficiency and cell growth. All samples were tested in at least three biologically independent.
- **Functional biological assays.** After 16h incubation with 0.1, 0.3 and 1 µM VidoCa, T98G (glioblastoma cell line) were harvested and processed for qPCR. Expression of the NURR1-regulated genes (tyrosine hydroxylase [TH], vesicular amino acid transporter 2 [VMAT2]) relative to the house keeping gene GAPDH were assessed. In another model, human peripheral blood mononuclear cells (PBMC) were stimulated with LPS (100 ng/ml) with or without 30 µM VidoCa. After 14h, supernatant was harvested to assess brain derived-neurotrophic factor [BDNF] secretion by ELISA (Invitrogen, Thermo Fisher Scientific).
- **Experimental autoimmune encephalomyelitis (EAE) model[6].** EAE was induced in 11-week-old dark agouti female rats (n = 8/group). The rats were immunized by subcutaneous administration of rat spinal cord homogenate. Once an animal reached an individual clinical score ≥ 1, it was randomly assigned to one of the five groups, and treatment was started by once daily p.o. application. Clinical scoring was assessed from day 7 after disease induction until the end of the study.

## 1. Vidofludimus calcium does not only inhibit DHODH, but also binds and activates the nuclear receptor Nurr1



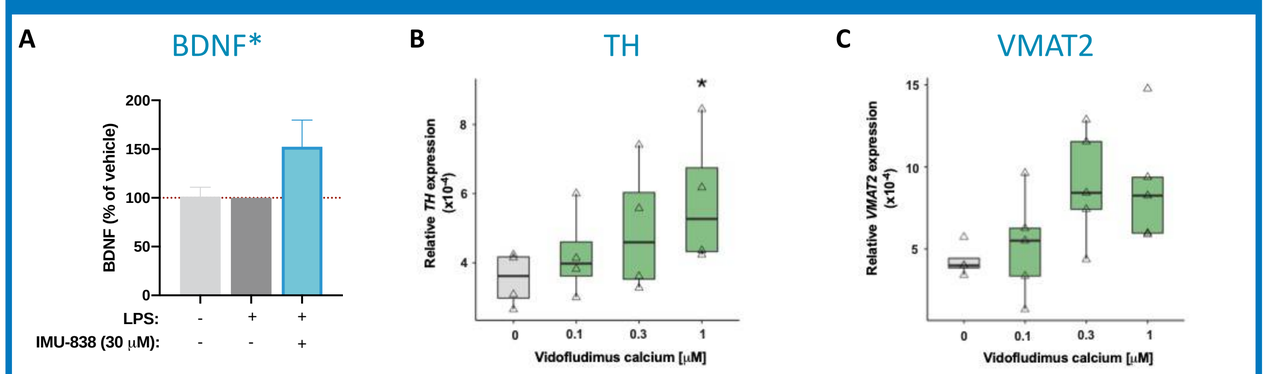

VidoCa is an established DHODH inhibitor with an average $IC_{50}$ of 160 nM. (A) shows a representative graph from 4 independent DHODH inhibition experiments. Besides DHODH, VidoCa binds also strongly to Nurr1 (Kd = 0.7 µM, B). This results in a strong activation of Nurr1 as shown in the reporter assay containing the Nurr1 ligand binding domain (C, n = 3, $EC_{50}$ = 0.4 ± 0.2 µM). Also, VidoCa potently activates full-length Nurr1 using the NBRE and DR5 reporter assays with an $EC_{50}$ of 0.3 ± 0.1 µM and 0.4 ± 0.2 µM, resp. and a ~3 and 5-fold activation above it's basal activity level, resp.

## 2. Vidofludimus calcium is selective for Nurr1 over other nuclear receptors



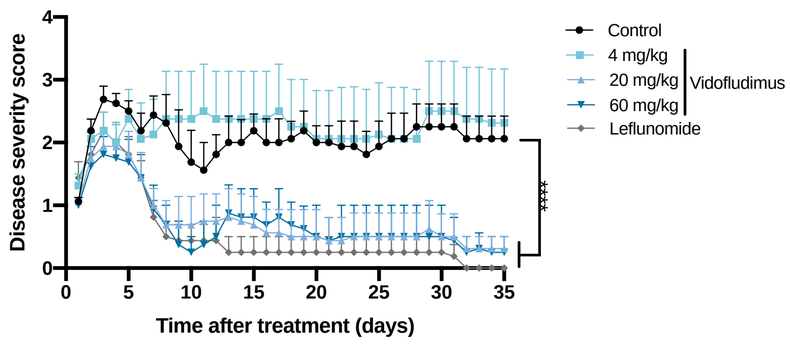

VidoCa is selective for Nurr1 compared to other nuclear receptors (NR). First VidoCa was tested against the closely related NRs, NOR1 ($EC_{50}$ = 2.9 ± 0.9 µM) and Nur77 ($EC_{50}$ = 3.1 ± 0.7 µM) and thus showing a > 7-fold higher potency for Nurr1. Next, 10 µM VidoCa was tested against other constitutively active NR and showed no activity (A). Finally, 10 µM VidoCa was examined against other NR and its potency was compared to the activation of the respective agonists (100% activity). VidoCa only showed a weak activation on PPAR$\gamma$, while having no effect on the other receptors (B, heatmap).

## 3. Vidofludimus calcium upregulates Nurr1 related genes in monocytes as well as in brain derived T98G cells




VidoCa was further tested for functional readouts regarding Nurr1 activation. Since Nurr1 has several different target genes, 3 of them were assessed. Preliminary data show that 30 µM VidoCa (IMU-838) increases the secretion of BDNF from human PBMC stimulated with LPS. In another assay using T98G cells, a dose dependent effect of VidoCa was seen on the expression of genes related to dopamine metabolism, tyrosine hydroxylase (TH, B) and vesicular monoamine transporter 2 (VMAT2, C).

## 4. Vidofludimus reduces disease severity in EAE




Therapeutic treatment of a rat EAE model. Rats treated with different doses of vidofludimus, negative control or positive control (leflunomide) show that 20 and 60 mg/kg Vido potently reduced disease activity, similar to the positive control. In addition, 20 and 60 mg/kg Vido completely ameliorated the disease in 5/8 and 7/8 rats, resp. at the end of the study compared to 0/8 and 3/8 in the control and 4 mg/kg group, resp.

## Conclusions

- Vidofludimus calcium potently induces Nurr1 activity
- Induction of Nurr1 activity by vidofludimus calcium is selective over other nuclear receptors, even a >7- fold difference between two closely related NR4A-family members
- Vidofludimus calcium increases the expression of Nurr1 related genes, indicating functional relevance of increased Nurr1 activity in a cellular system
- Potential neuroprotective role for vidofludimus calcium via its newly found target Nurr1, which could be a possible explanation for the lower frequency of patients showing confirmed disability progression on active treatment in the EMPhASIS study

**References**

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