# Vidofludimus Calcium Activity on Nurr1 in Preclinical Models: A Potential Neuroprotective Function in Multiple Sclerosis

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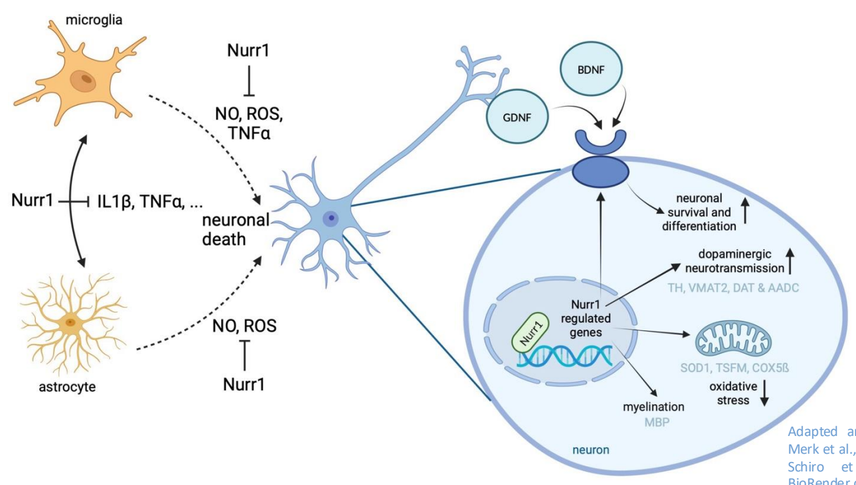
## Background

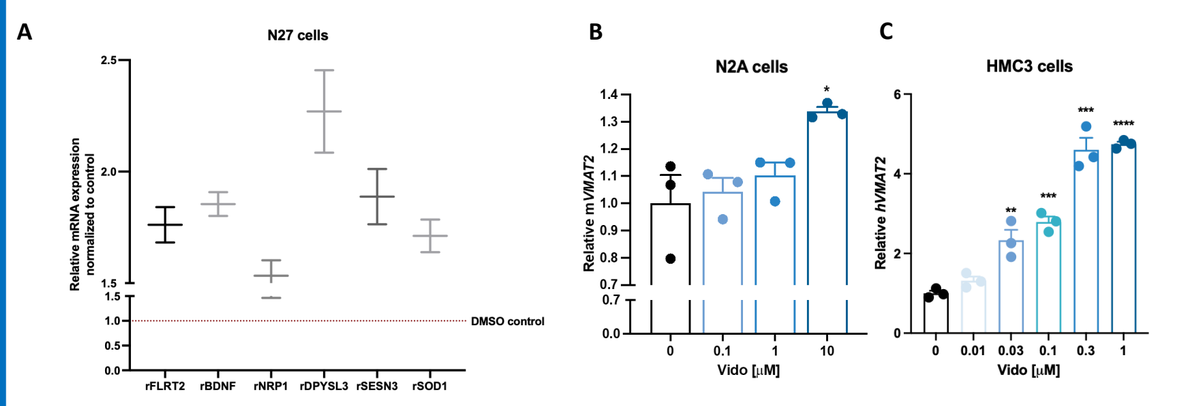
Vidofludimus calcium (VidoCa) is an orally bioavailable potent nuclear receptor related 1 (Nurr1) activator and dihydroorotate dehydrogenase inhibitor[1] that is currently being tested in phase 2 and 3 clinical trials for the treatment of progressive and relapsing MS, respectively.

Nurr1 is mainly expressed in neurons, astrocytes, and microglia. It regulates the expression of genes that support neuronal survival and mitigates production of neurotoxic mediators[1,2,3]. The potential neuroprotective effects of VidoCa as a Nurr1 agonist *in vitro* and *in vivo* is investigated here.




Adapted and modified from Willems, Merk et al., 2022, Vietor et al., 2023 and Schiro et al., 2022. Created in BioRender.com

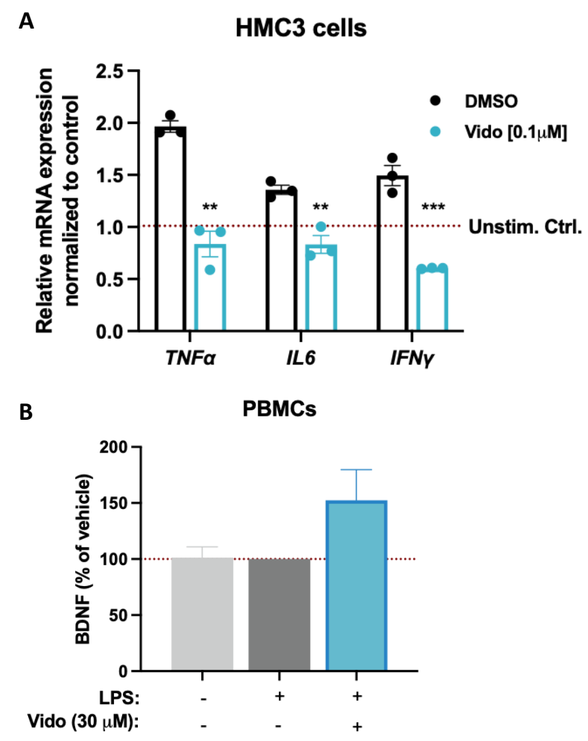
## VidoCa enhances expression of Nurr1 target genes




**VidoCa activates Nurr1-regulated genes in cell lines that represent brain resident cells.** (A) Rat dopaminergic neurons (N27) were treated with 1 µM VidoCa for 21 h. (B) murine neuroblastoma cells (N2A), and (C) human microglia (HMC3) were treated with different VidoCa concentration for 24 h. Gene expression of different Nurr1-regulated genes, critical for neuronal growth and survival as well as dopamine synthesis and regulation, was assessed. Next to VMAT2 also TH was assessed in HMC3 and N2A cells and gave similar results (not shown).

DMSO = dimethylsulfoxide; FLRT2 = fibronectin leucine rich transmembrane protein; BDNF = brain derived neurotrophic factor; NRP1 = neurophilin-1; DPYSL3= dihydropyrimidinase-like 3; SESN3 = sestrin 3; SOD1 = superoxide dismutase type 1; VMAT2 = Vesicular monoamine transporter 2; TH = tyrosine hydroxylase; Vido = vidofludimus calcium (VidoCa). * p<0.05; ** p<0.01.

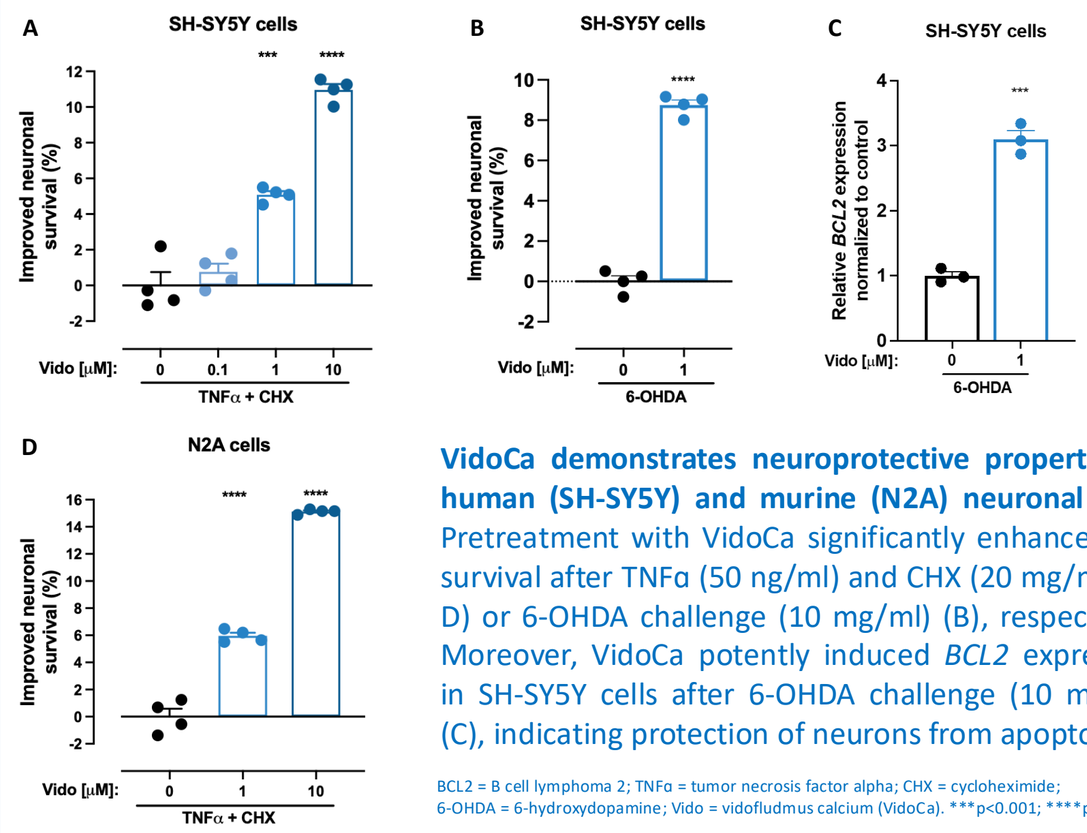
## VidoCa reduces the neurotoxic environment




**VidoCa reduces the neurotoxic environment upon LPS stimulation in human microglia and primary monocytes.** (A) Pre-treatment of human microglia (HMC3) with 0.1 µM VidoCa for 4h significantly reduced the expression of pro-inflammatory cytokines *TNFα*, *IL6*, and *IFNγ* after 24 h stimulation with 1 µg/ml LPS. (B) Also, VidoCa increased BDNF protein levels in human PBMCs as a surrogate for microglia after 14 h challenge with 0.1 µg/ml LPS.

BDNF = brain neurotrophic factor; Ctrl = control; DMSO = dimethylsulfoxide (vehicle); IFNγ = interferon gamma; IL6 = interleukin 6; LPS = Lipopolysaccharide; PBMCs = peripheral blood mononuclear cells; TNFα = tumor necrosis factor alpha; Unstim. = unstimulated; Vido = vidofludimus calcium (VidoCa). **p<0.01; ***p<0.001

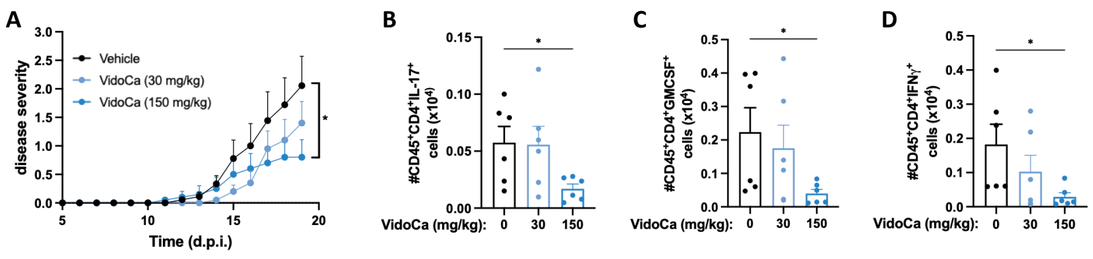
## VidoCa protects neurons under pro-apoptotic conditions




**VidoCa demonstrates neuroprotective properties in human (SH-SY5Y) and murine (N2A) neuronal cells.** Pretreatment with VidoCa significantly enhanced cell survival after TNFα (50 ng/ml) and CHX (20 mg/ml) (A, D) or 6-OHDA challenge (10 mg/ml) (B), respectively. Moreover, VidoCa potently induced *BCL2* expression in SH-SY5Y cells after 6-OHDA challenge (10 mg/ml) (C), indicating protection of neurons from apoptosis.

BCL2 = B cell lymphoma 2; TNFα = tumor necrosis factor alpha; CHX = cycloheximide; 6-OHDA = 6-hydroxydopamine; Vido = vidofludimus calcium (VidoCa). ***p<0.001; ****p<0.0001

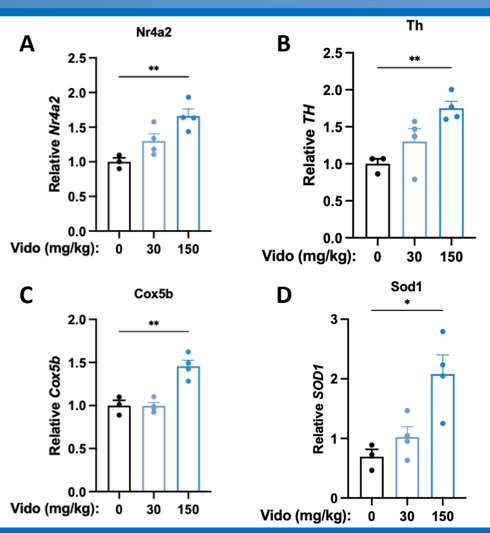
## VidoCa attenuates disease severity in an EAE model




**VidoCa attenuates disease severity in a $MOG_{35-55}$ murine EAE model.** VidoCa dose-dependently reduced disease severity (A) and total numbers of infiltrating pathogenic IL-17⁺ (B), GMCSF⁺ (C), and IFNγ⁺ CD4⁺ T cells into the CNS at study end.

d.p.i. = days post immunization; EAE = experimental autoimmune encephalomyelitis; GMCSF = Granulocyte-macrophage colony-stimulating factor; IFNγ = interferon gamma; IL-17 = interleukin 17; study end = 19 & 25 d.p.i.; vehicle = PEG400; Vido = vidofludimus calcium (VidoCa); CNS = central nervous system; *p<0.05. B-D: grouped data from two different timepoints within the same experiment.

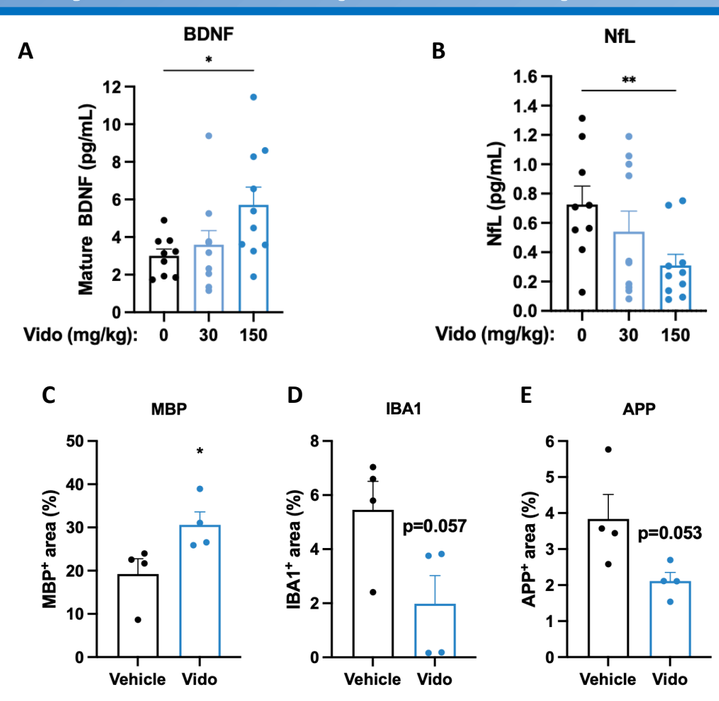
## VidoCa increases expression of Nurr1 target genes *in vivo*




**VidoCa treatment affects mRNA levels of Nurr1-regulated genes in the CNS of EAE mice**. The study revealed significantly higher Nurr1 (*Nr4a2*; A), *Th* (B), and *Cox5b* (C) levels in the brain and higher *Sod1* (D) levels in the spinal cord of mice receiving 150 mg/kg VidoCa daily at study end.

Cox5b = cytochrome c oxidase subunit 5B; EAE = experimental autoimmune encephalomyelitis; Nr4a2 = nuclear receptor subfamily 4 group A member 2; Nurr1 = nuclear receptor 1; SOD1 = superoxide dismutase 1; TH = tyrosine hydroxylase; Vido = vidofludimus calcium (VidoCa); *p<0.05; **p<0.01. Grouped data from two different experiments with different endpoints are depicted.

## VidoCa may have neuroprotective potential in EAE




**VidoCa displays neuroprotective activity *in vivo* in an EAE model**. Treatment with 150 mg/kg VidoCa significantly enhanced plasma BDNF levels (A), a potential peripheral biomarker for Nurr1 activation, and reduced plasma NfL levels (B), a biomarker for axonal damage and neurodegeneration at study end. Moreover, in a small pilot study a higher myelin (MBP, C) content in the spinal cord of EAE mice treated with VidoCa has been observed as well as a tendency towards lower microglial activation (IBA1, D) and axonal injury (APP, E).

APP = amyloid precursor protein; BDNF = brain derived neurotrophic factor; EAE = experimental autoimmune encephalomyelitis; IBA = ionized calcium-binding adaptor molecule 1; MBP = myelin basic protein; Vido = vidofludimus calcium (VidoCa); *p<0.05; **p<0.01

## Summary and conclusions

- **VidoCa activates Nurr1 *in vitro* and *in vivo***
- **VidoCa reduces neuronal loss and injury by decreasing microglial activation and pro-inflammatory neurotoxic environment**
- **VidoCa displays neuroprotective properties *in vitro* and *in vivo*, most likely mediated by Nurr1-driven regulation of survival signals**




Image reference: https://www.nih.gov/news-events/news-releases/new-videos-show-nih-studies-communication-between-brain-cells

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## References

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