Potential Neuroprotective Activity by Vidofludimus Calcium in *In Vivo* Models of Multiple Sclerosis

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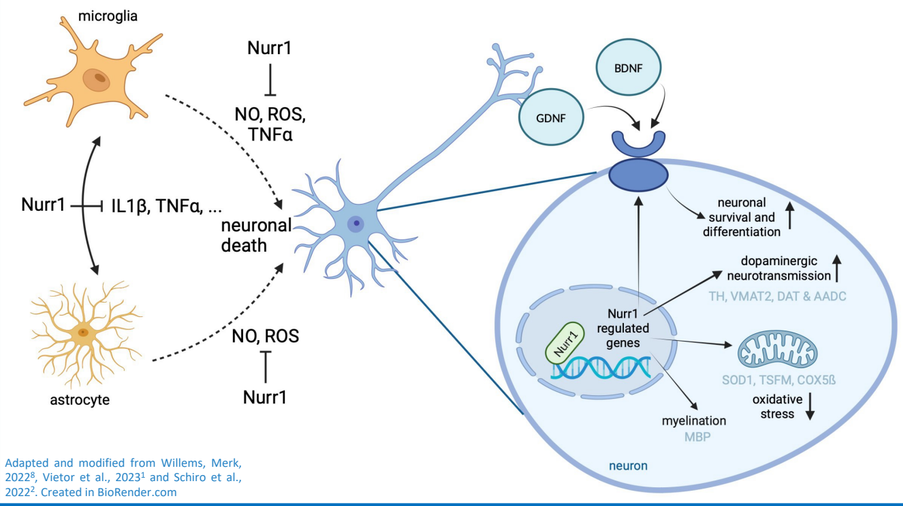
1. Background

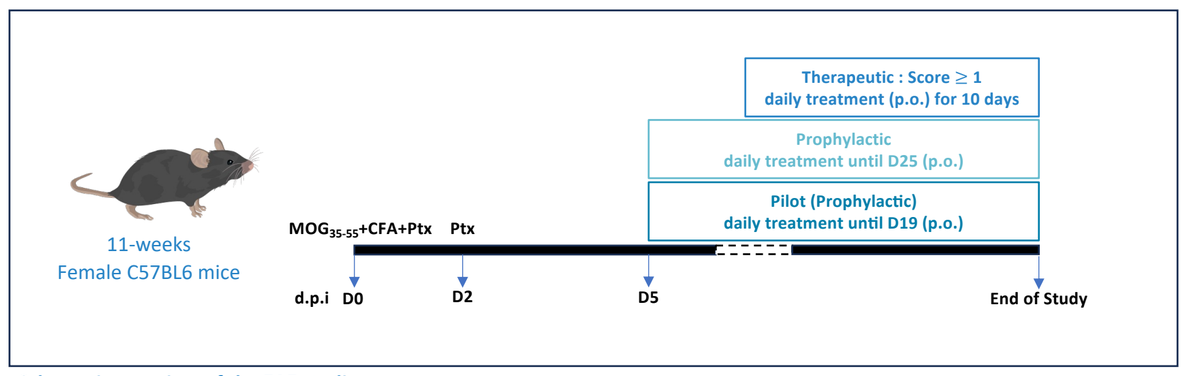
Nuclear receptor-related 1 (Nurr1), a transcription factor, regulates genes that enhance neuronal survival and reduces neurotoxic mediators produced by microglia and astrocytes[1-3]. Growing evidence supports its protective role in neurodegenerative diseases[4]. In persons with multiple sclerosis (PwMS), Nurr1 gene expression in blood was reduced[5]. Fingolimod is the sole disease modifying therapy tested that showed increased Nurr1 mRNA levels in peripheral immune cells of PwMS[6,7]. However, this increase was only observed after 2 years of treatment[7]. Here, the potential Nurr1 activity of vidofludimus calcium (VidoCa), currently in phase 2 and 3 clinical trials for progressive and relapsing MS, respectively, was assessed *in vivo*. VidoCa has a dual mode of action, functioning both as as a potent Nurr1 activator and a next generation DHODH inhibitor.



Adapted and modified from Willems, Merk, 2022[8], Vietor et al., 2023[1] and Schiro et al., 2022[2]. Created in BioRender.com

AADC = Aromatic L-amino acid decarboxylase; BDNF = brain derived neurotrophic factor; COX5b = cytochrome c oxidase subunit 5B; DAT = dopamine transporter; DHODH = dihydroorotate dehydrogenase; GDNF = Glial cell line-derived neurotrophic factor; IL-1β = interleukin 1 beta; MBP = myelin basic protein; NO = Nitric oxide; ROS = Reactive oxygen species; SOD1 = superoxide dismutase 1; Th = tyrosine hydroxylase; TNFα = Tumor necrosis factor alpha; TSFM = Ts translation elongation factor, mitochondrial; VMAT2 = vesicular monoamine transporter 2

2. Methods



Schematic overview of the EAE studies

Treatment

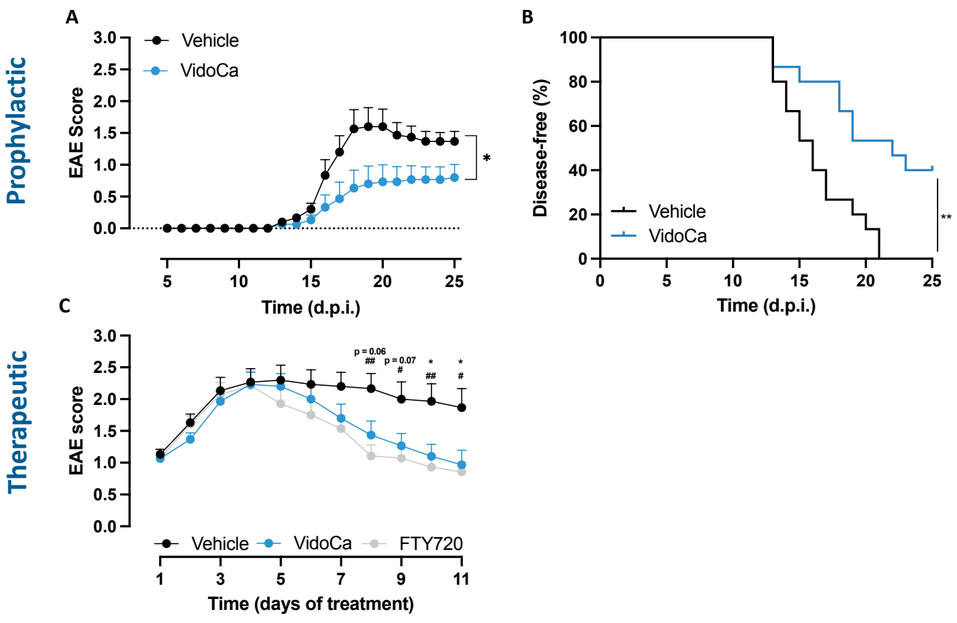
Treatment	Dose	Model
Vehicle (PEG400)	-	all
VidoCa	150 mg/kg	all
Reference (FTY720)	5 mg/kg	Therapeutic

Readouts (end of study)

Readouts	Pilot	Prophylactic	Therapeutic
Disease (EAE) score	✓ (n = 4)	✓ (n = 15)	✓ (n = 14/15)
FACS		✓ (n = 8)	✓ (n = 7/8)
RT-qPCR (brain, SC)		✓ (n = 7)	✓ (n = 7)
Plasma NfL		✓ (n = 15)	
Heart Plasma BDNF		✓ (n = 15)	
Histology	✓ (n = 4)		

BDNF = brain derived neurotrophic factor; CFA = Complete Freund's Adjuvant; d.p.i. = days post immunization; EAE = experimental autoimmune encephalomyelitis; FACS = fluorescence-activated cell sorting; FTY720 = fingolimod; MOG = myelin oligodendrocyte glycoprotein; NfL = neurofilament light chain; p.o. = per os (oral gavage); Ptx = pertussis toxin; SC = spinal cord

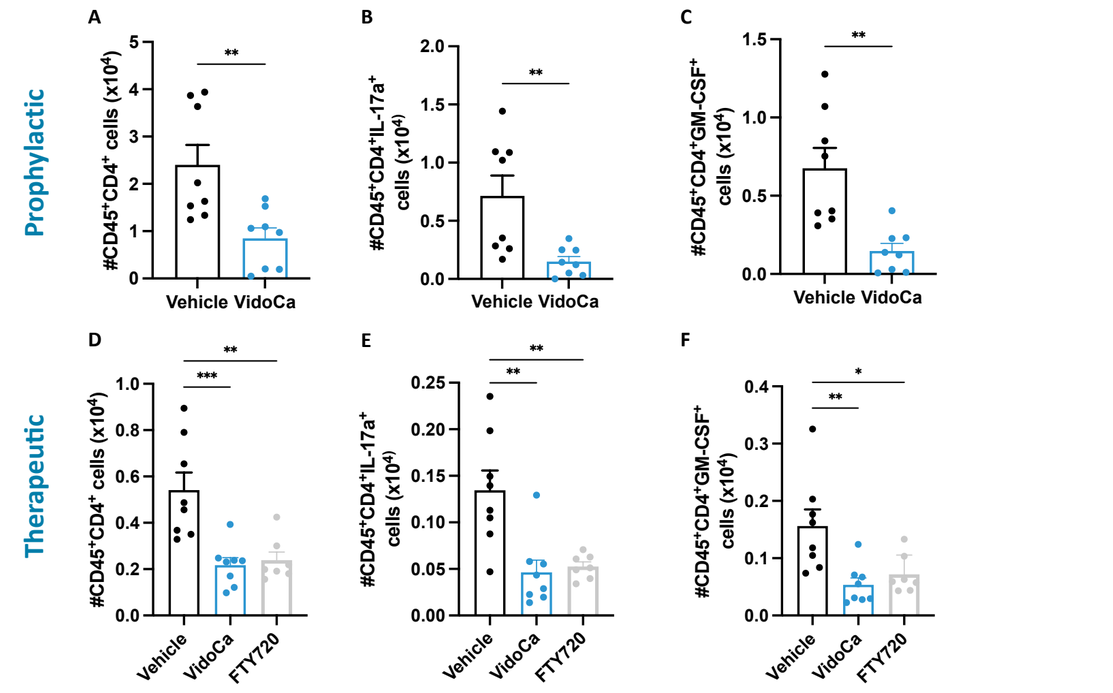
3. VidoCa Attenuates Disease Severity in EAE Models



VidoCa attenuates disease severity in both prophylactic and therapeutic murine EAE models. In the prophylactic EAE model, mice treated with VidoCa showed lower disease severity as well as a 0.22-fold lower relative risk (HR) of developing symptoms compared to mice treated vehicle. Reducing disease severity by VidoCa was also shown in a therapeutic model, to a similar extent as FTY720 treatment.

d.p.i. = days post immunization; FTY720 = fingolimod (reference); HR = hazard ratio; Data in the graphs are shown as mean + SEM. Statistics: (**A,C**) two-way repeated measures ANOVA and (**B**) Log-rank (Mantel-Cox) test and for the HR the Mantel-Haenszel. #/*p<0.05; ##/**p<0.01 *VidoCa vs. vehicle and # FTY720 vs. vehicle.

4. VidoCa Reduces T Lymphocyte Infiltration into the CNS



VidoCa reduces T helper (Th) cell infiltration into the CNS in murine EAE models. In the therapeutic model VidoCa shows similar activity in reducing Th cell infiltration as the reference treatment (FTY720).

CNS = central nervous system;. Data in the graphs are shown as mean + SEM. Statistics: (**A-C**) two-tailed t-test, (**D-E**) one-way ANOVA followed by Dunnett's multiple comparisons test. *p<0.05; **p<0.01

5. VidoCa Increases Expression of Nurr1 Target Genes *In Vivo*

VidoCa treatment augments Nurr1-regulated gene expression in the CNS in both prophylactic and therapeutic EAE models. The regulation of these genes seems different compared to the reference treatment (FTY720). These data support the potential activation of Nurr1 by VidoCa *in vivo*.

FTY720 = fingolimod; Nr4a2 = nuclear receptor subfamily 4 group A member 2; Nurr1 = nuclear receptor 1; SC = spinal cord; Sod1 = superoxide dismutase 1; Th = tyrosine hydroxylase; Data in the graphs are shown as mean + SEM. Statistics: (**A-C**) two-tailed t-test; (**D-F**) one-way ANOVA followed by Dunnett's multiple comparisons test. *p<0.05; **p<0.01

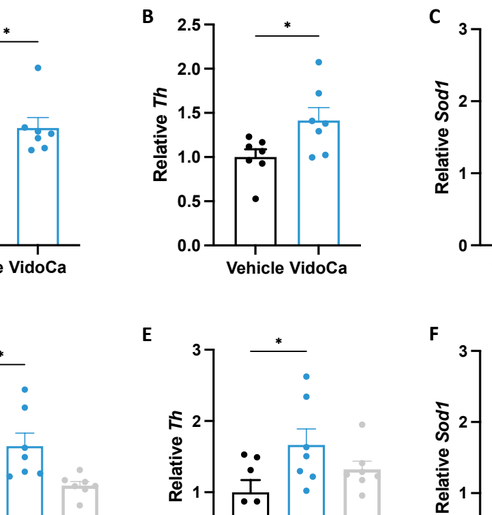
6. VidoCa Displays Neuroprotective Potential in EAE

VidoCa displays neuroprotective activity *in vivo* in an EAE model. Prophylactic treatment with VidoCa increased plasma BDNF levels, a potential peripheral biomarker for Nurr1 activation, and reduced plasma NfL levels, a biomarker for axonal damage and neurodegeneration. These data indicate that VidoCa activates Nurr1 *in vivo* and support its potential neuroprotective activity. This is further supported by a higher myelin (MBP) content, a tendency towards lower microglial activation (IBA1) and axonal injury (APP) in the spinal cord in a small prophylactic pilot EAE study.

APP = amyloid precursor protein; BDNF = brain derived neurotrophic factor; IBA1 = ionized calcium-binding adaptor molecule 1; MBP = myelin basic protein; Statistics: two-tailed t-test. *p<0.05; **p<0.01

7. Overview and Conclusions

Nurr1 Activation



Reduces activation of microglia and production of neurotoxic mediators[3,9]

Improves survival of neurons in a neurotoxic environment[9-11]

VidoCa

- **Reduces disease severity and immune cell infiltration into the CNS in EAE**
- **Activates Nurr1 *in vitro* and *in vivo***
- **Displays neuroprotective effects *in vitro* and *in vivo*, most likely mediated by Nurr1-driven regulation of survival signals**
- **Offers the potential to treat both relapsing and progressive forms of MS, due to its dual mode of action**

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Disclosure

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