Vidofludimus Calcium Shows a Potential Neuroprotective Function in Multiple Sclerosis through its Activity on Nurr1 in Preclinical Models

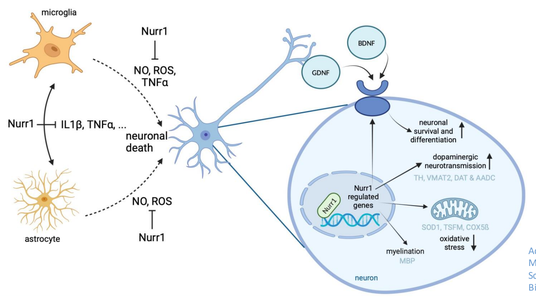
E. Peelen[1], H. Wu[2], A. Herrmann[1], T. Wulff[1], M. Jafari[1], C. Gege[1], A. Muehler[1], D. Vitt[1], Z. Sun[2], H. Kohlhof[1]

[1] Immunic AG, Graefelfing, Germany; [2] Beckman Research Institute of City of Hope, Department of Immunology & Theranostics, Duarte, United States

1. Background

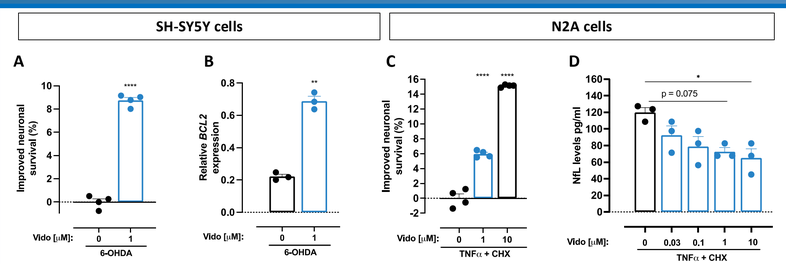
Vidofludimus calcium (VidoCa) is an orally bioavailable potent activator of nuclear receptor-related 1 (Nurr1) and dihydroorotate dehydrogenase inhibitor[1]. It is currently undergoing phase 2 and 3 clinical trials for the treatment of progressive and relapsing multiple sclerosis (MS), respectively.

Nurr1, a transcription factor, is expressed in various neurons, astrocytes, microglia and other cell types. It regulates genes that support neuronal development, function and survival, and mitigates neurotoxic mediators produced by microglia and astrocytes[1,2,3]. A study has also suggested a role of Nurr1 in the post-mortem motor cortex of progressive MS, where its nuclear localization correlated with both neuron numbers and disease severity[4]. This study presents further evidence supporting the neuroprotective effects of VidoCa through Nurr1 activation.



Adapted and modified from Willems, Merk et al., 2022, Vietor et al., 2023 and Schiro et al., 2022.[2,3,5] Created in BioRender.com

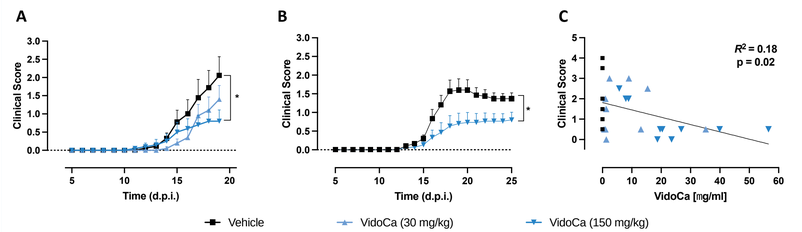
2. VidoCa enhances neuronal survival



VidoCa displays neuroprotective properties in human (SH-SY5Y) and murine (N2A) neuronal cells. (A, B) Four-hour pretreatment with VidoCa (**A**) significantly enhanced SH-SY5Y cell survival and (**B**) induced *BCL2* gene expression after apoptosis induction by the neurotoxic agent 6-OHDA (10 µg/ml) for 16 hours. **(C, D)** One-hour pretreatment with VidoCa (**C**) significantly improved N2A cell survival and (**D**) dose-dependently reduced NfL levels in the supernatant after 6 hours of apoptosis induction by TNFα (50 ng/ml) and CHX (20 mg/ml). These data indicate that VidoCa protects neurons from apoptosis.

6-OHDA = 6-hydroxydopamine; BCL2 = B cell lymphoma 2; CHX = cycloheximide; NfL = neurofilament light chain; TNFα = tumor necrosis factor alpha; Vido = vidofludimus calcium (VidoCa); .***p<0.001; ****p<0.0001. Cell viability was assessed by flow cytometry, *BCL2* gene expression by PCR and NfL by ELISA.. Data are shown as mean + SEM. Statistics: two-tailed t-test.

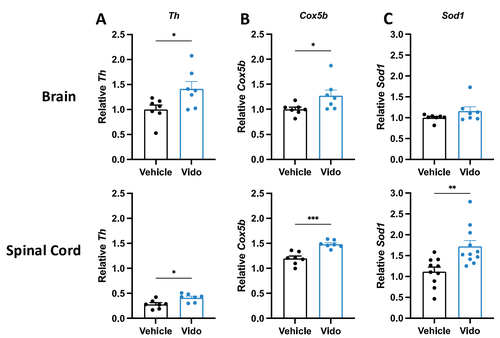
3. VidoCa attenuates disease severity in an EAE model



VidoCa attenuates disease severity in a prophylactic MOG_{35-55} murine EAE model. EAE was induced in 11-weeks old female C57BL/6 mice by immunization with MOG_{35-55}. Daily treatment per oral gavage with VidoCa or vehicle was started at 5 d.p.i.. (**A**) VidoCa dose-dependently reduced disease severity (clinical score) up to 19 d.p.i.. (**B**) The model was repeated with the highest dose and confirmed that VidoCa reduced disease severity. (**C**) A weak but significant negative correlation between plasma VidoCa concentrations and clinical score was observed in this cohort that combines data from two different "end of study" time points. Disease severity and VidoCa plasma levels were determined at study end (19 or 25 d.p.i.).

d.p.i. = days post immunization; EAE = experimental autoimmune encephalomyelitis; MOG = myelin oligodendrocyte glycoprotein; vehicle = PEG400; VidoCa = vidofludimus calcium; *p<0.05. Data in the graphs are shown as mean + SEM. A & C grouped data from two different timepoints within the same experiment. Statistics: (**A**) t-test, (**B**) two-way repeated measures ANOVA and (**C**) simple linear regression

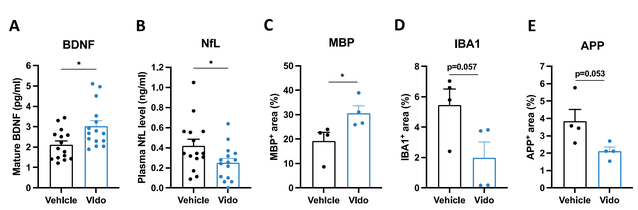
4. VidoCa enhances Nurr1 target gene expression in the CNS



In a prophylactic MOG_{35-55} murine EAE model, VidoCa treatment augments Nurr1-regulated gene expression in the CNS. Brain and spinal cord samples were derived from the model in the previous figure (box 3, B) on 25 d.p.i.. The study revealed significantly higher (**A**) *Th*, and (**B**) *Cox5b* levels in brain and spinal cord as well as (**C**) higher *Sod1* levels in the spinal cord of mice receiving 150 mg/kg VidoCa. These data confirm our previous pilot EAE model and indicate that VidoCa likewise activates Nurr1 *in vivo*.

Cox5b = cytochrome c oxidase subunit 5B; d.p.i. = days post immunization, EAE = experimental autoimmune encephalomyelitis; Nurr1 = nuclear receptor-related 1; Sod1 = superoxide dismutase 1; Th = tyrosine hydroxylase; Vido = vidofludimus calcium (VidoCa); . *p<0.05; **p<0.01; ***p<0.001. Data in the graphs are shown as mean + SEM. Statistics: two-tailed t-test.

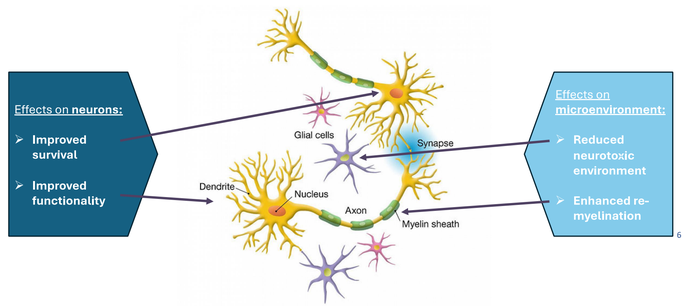
5. Potential neuroprotective activity of VidoCa *in vivo*



VidoCa displays neuroprotective activity *in vivo* in an EAE model. The EAE model was performed as described before. (**A**) The potential peripheral Nurr1 activation biomarker, BDNF, is augmented and (**B**) the biomarker for axonal damage and neurodegeneration, NfL, is reduced in the plasma of mice treated with 150 mg/kg VidoCa at study end (25 d.p.i.). These data confirm the results of our previous pilot EAE model indicating that VidoCa also activates Nurr1 *in vivo* and that VidoCa has potential *in vivo* neuroprotective activity. This is further supported by a small pilot study for histology showing (**C**) a higher myelin (MBP) content in the spinal cord of EAE mice treated with 150 mg/kg VidoCa as well as a tendency towards (**D**) lower microglial activation (IBA1) and (**E**) axonal injury (APP).

APP = amyloid precursor protein; BDNF = brain derived neurotrophic factor; d.p.i. = days post immunization; EAE = experimental autoimmune encephalomyelitis; IBA = ionized calcium-binding adaptor molecule 1; NfL = neurofilament light chain; MBP = myelin basic protein; Vido = vidofludimus calcium (VidoCa); *p<0.05. Data in the graphs are shown as mean + SEM. Statistics: two-tailed t-test.

6. Summary and conclusion



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

Contact: evelyn.peelen@imux.com

References

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

Disclosures

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