Characterisation of Dual Nurr1 Activator/DHODH Inhibitor Vidofludimus Calcium and Development Towards a Nurr1 Selective Tool Compound

C. Gege [1], H. Wu [2], J. Vietor [3], T. Wulff [1], M. Jafari [1], E. Peelen [1], T. Stiller [3], R. Busch [3], J. A. Marschner [3], D. Merk [3], Z. Sun [2], H. Kohlhof [1], D. Vitt [1]

[1] Immunic AG, Gräfelfing, Germany

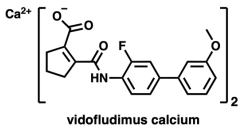
[2] Department of Immunology & Theranostics, Arthur Riggs Diabetes & Metabolism Research Institute, Beckman Research Institute of the City of Hope, Duarte, CA 91010, USA

[3] Department of Pharmacy, Ludwig-Maximilians-Universität (LMU) München, 81377 Munich, Germany

Background

Nurr1 Activator

- Protecting neurons from cell death
- Continuous effect independent from focal inflammation



vidofludimus calcium

DHODH Inhibitor

- Selective anti-inflammatory effect reduces focal inflammation
- Antiviral effect prevents reactivation of EBV and could stop cross reactive immune responses

Fig. 1: The immunomodulatory and antiviral activity of the Dihydroorotate Dehydrogenase (DHODH) inhibitor **vidofludimus calcium** (**VidoCa**) is well known [1]. DHODH catalyzes the rate-limiting step of *de novo* pyrimidine synthesis. We recently found that **VidoCa** also activates the neuroprotective transcription factor Nuclear Receptor Related 1 (Nurr1), which is an emerging target in neurodegenerative diseases like Parkinson's disease (PD), Alzheimer's disease (AD) or multiple sclerosis (MS) [2].

VidoCa is a potent Nurr1 agonist

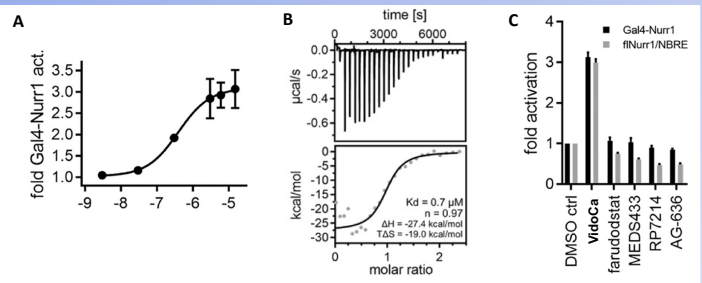


Fig. 2: **(a) VidoCa** activates Gal4-Nurr1 with an EC_{50} value of 0.4±0.2 µM with an 3.1±0.4-fold maximal activation. Mean±SEM, n ≥3. **(b)** Binding of **VidoCa** to the Nurr1 ligand binding domain was confirmed by isothermal titration calorimetry with a K_d value of 0.7 µM. **(c)** Activity of other DHODH inhibitors related to **VidoCa** on Nurr1 in the hybrid Gal4-Nurr1 and a full-length Nurr1 (NBRE) reporter gene assay tested at 10 µM. Mean±SEM, n ≥3.

VidoCa is effective in a mouse neuronal cell line

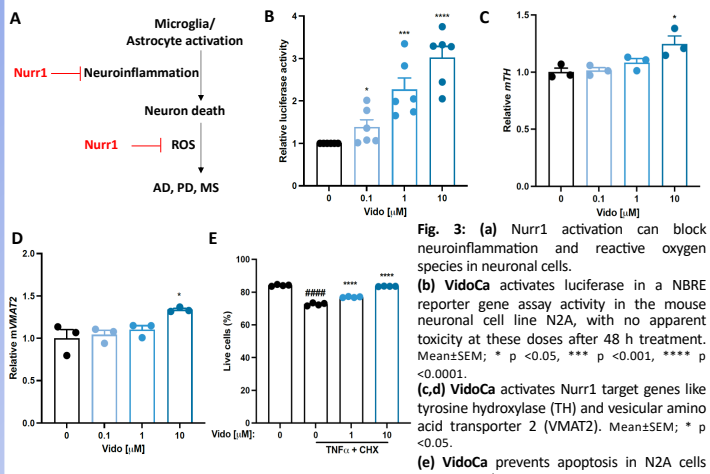


Fig. 3: (a) Nurr1 activation can block neuroinflammation and reactive oxygen species in neuronal cells.
(b) VidoCa activates luciferase in a NBRE reporter gene assay activity in the mouse neuronal cell line N2A, with no apparent toxicity at these doses after 48 h treatment. Mean±SEM; * p <0.05, *** p <0.001, **** p <0.0001.
(c,d) VidoCa activates Nurr1 target genes like tyrosine hydroxylase (TH) and vesicular amino acid transporter 2 (VMAT2). Mean±SEM; * p <0.05.
(e) VidoCa prevents apoptosis in N2A cells after TNFα/cycloheximide (CHX) stimulation. #### p <0.0001 vs. non-treated, **** p <0.0001 vs. treated DMSO.

VidoCa is effective in human cell lines

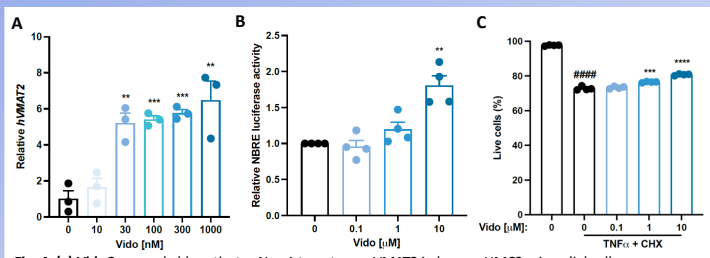


Fig. 4: (a) VidoCa remarkably activates Nurr1 target gene VMAT2 in human HMC3 microglial cells. Mean±SEM; ** p <0.01, *** p <0.001. **(b) VidoCa** activates NBRE luciferase activity in the human SH-SY5Y neuronal cell line (with no apparent toxicity at these doses after 48 h treatment). Mean±SEM; ** p <0.01. **(c) VidoCa** prevents apoptosis in SH-SY5Y cells after TNFα/cycloheximide (CHX) stimulation. #### p <0.0001 vs. non-treated, *** p <0.001, **** p <0.0001 vs. treated DMSO.

VidoCa shows Nurr1 related activity in mouse EAE

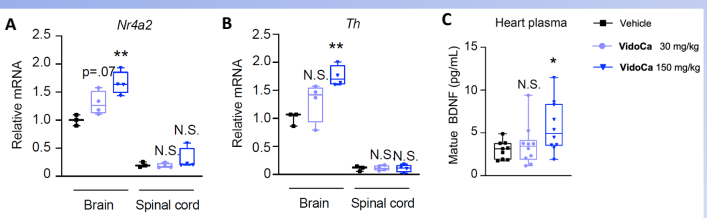
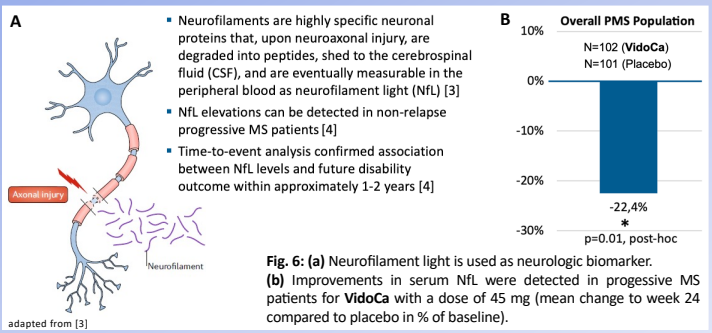


Fig. 5: In a pilot experimental autoimmune encephalomyelitis model (female C57BL/6 mice; 30 or 150 mg/kg **VidoCa** p.o./p.d.) mRNA levels were determined: **(a)** Increased Nurr1 levels were measured in brain tissue upon **VidoCa** treatment in a dose dependent manner. **(b)** A significant increase of Nurr1 target genes TH (mRNA, in brain) and **(c)** brain-derived neurotrophic factor (BDNF) (protein, in heart plasma) were detected upon **VidoCa** treatment.

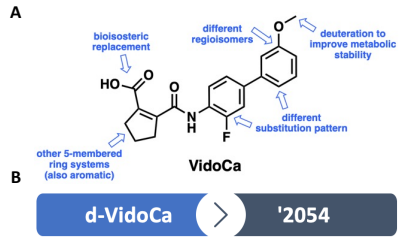
VidoCa shows neuroprotective effects in MS patients



- Neurofilaments are highly specific neuronal proteins that, upon neuroaxonal injury, are degraded into peptides, shed to the cerebrospinal fluid (CSF), and are eventually measurable in the peripheral blood as neurofilament light (NfL) [3]
- NfL elevations can be detected in non-relapse progressive MS patients [4]
- Time-to-event analysis confirmed association between NfL levels and future disability outcome within approximately 1-2 years [4]

Fig. 6: (a) Neurofilament light is used as neurologic biomarker.
(b) Improvements in serum NfL were detected in progessive MS patients for **VidoCa** with a dose of 45 mg (mean change to week 24 compared to placebo in % of baseline).

Optimization towards a Nurr1 selective tool compound



B

d-VidoCa		'2054
79 nM	EC_{50} Nurr1	12 nM
2.6-fold	max. activation	1.9-fold
400 nM	IC_{50} DHODH	9500 nM
5.1	selectivity index (IC_{50} DHODH/EC_{50} Nurr1)	790
2.2-fold	ratio brain exposure over EC_{50} Nurr1 mouse (♀) 20 mpk @2 h	27.2-fold

Fig. 7: Compound optimization of deuterated VidoCa results in '2054 with improved selectivity towards Nurr1.
(a) Pharmacokinetic properties (e.g. brain exposure) and target engagement of **d-VidoCa** were further improved during a lead-optimization process, resulting in a large chemical space with new IP.
(b) *In vitro* enzyme assays revealed an improved selectivity towards Nurr1 and improved brain penetration for tool compound **'2054**.

Conclusions and outlook

Conclusion:

- Identified neuroprotective transcription factor Nurr1 as new target for our drug candidate **VidoCa**
- **VidoCa** binds to and activates Nurr1
- **VidoCa** is effective in mouse and human neuronal and microglial cell lines
- **VidoCa** shows beneficial effects in a murine EAE model, indicating Nurr1 target engagement *in vivo*
- Nurr1 activation might explain the beneficial neuroprotective effects seen in MS patients
- Lead optimization yielded Nurr1 agonist **'2054** with improved potency, lack of DHODH inhibitor activity and optimized brain penetration properties

Next steps:

- Additional profiling, e.g. selectivity towards related nuclear receptors
- Use **'2054** or related compounds as chemical tools to dissect the DHODH effects from the Nurr1 effects *in vitro* and *in vivo*
- Find improved compounds within the new chemical space
- Elucidate the utility of Nurr1 in other diseases beyond multiple sclerosis

References:
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Contact: christian.gege@imux.com