Vidofludimus Calcium (IMU-838), a Small Molecule DHODH Inhibitor in Phase 2 Clinical Trial for Ulcerative Colitis, Shows Potent Anti-Inflammatory Activity in Cell-Culture-Based Systems

Immunic THERAPEUTICS

Evelyn Peelen[1], Melanie Eschborn[2], Andreas Muehler[1], Luisa Klotz[2], Daniel Vitt[1], Hella Kohlhof[1]

[1]Immunic AG, Gräfelfing, Germany

[2]University Hospital Münster, Department of Neurology with Institute of Translational Neurology, Münster, Germany

Background

Vidofludimus calcium (VidoCa, IMU-838) is a well-tolerated and orally available small molecule inhibitor of dihydroorotate dehydrogenase (DHODH) and is currently in phase 2 clinical development for ulcerative colitis (UC) and in phase 3 for MS.

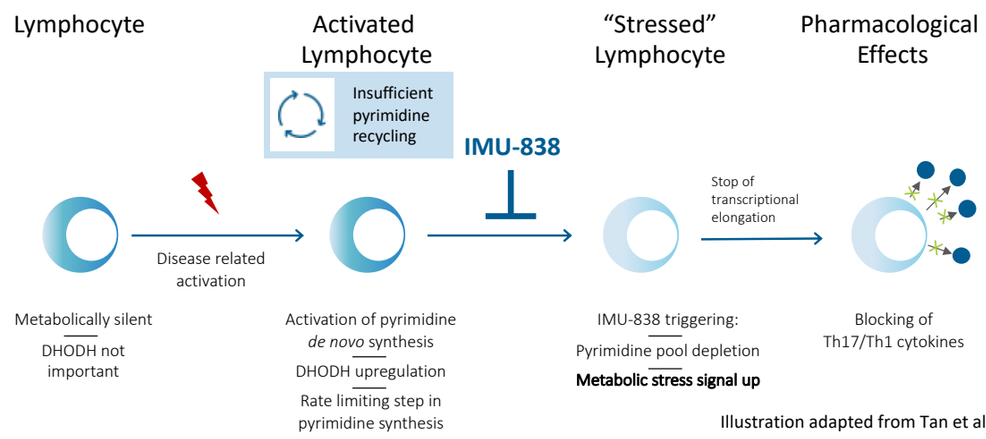


Illustration adapted from Tan et al., 2016[1]

In inflammatory bowel disease (IBD), the innate and adaptive immune systems have been implicated in the pathogenesis. Anti-TNFs are a treatment option for IBD and it has been shown that they induce regulatory macrophages (Mregs) that are implicated in immune regulation and mucosal healing in responders.[2] Next to macrophages, T lymphocytes have also been implicated in this disease. Literature indicates that highly metabolically active and high affinity T cells might be driving autoimmune disease.[3,4] Therefore, we investigated the effect of VidoCa on 1) Mregs induction (alone or in combination with anti-TNF) and 2) analyzed the mechanism of action of VidoCa in more detail on high affinity T cells and T cell metabolism.

Methods

Mixed Lymphocyte Reaction (MLR)[5]

performed by Prof. Wildenberg Amsterdam Medical Center, The Netherlands

PBMC of two individual healthy donors were mixed in equal numbers and were plated (n=3-6)

- After 48 hours: DMSO or VidoCa (1, 3, 10, 30 μM) with and without anti-TNF (infliximab or adalimumab, both 10 μg/ml) were added
- After 4 days:
  - Supernatant: Harvest and stored → cytokine detection (CBA, BD Biosciences)
  - Cells: flow cytometry for CD14 and CD206

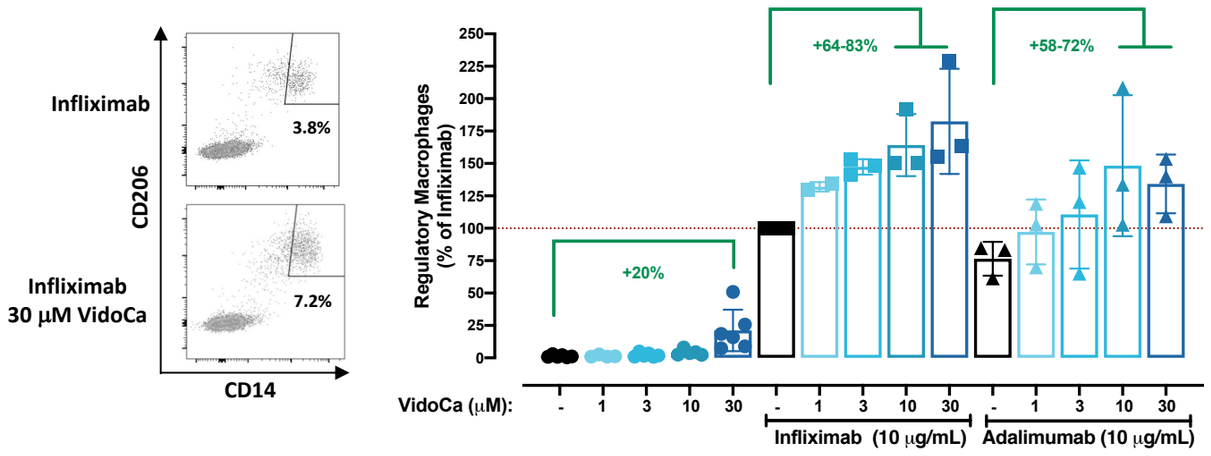
Affinity dependent effect assays[6]

- OT-I and OT-III murine transgenic CD8 T cells having high affinity or low affinity to $OVA_{257-264}$, respectively, were stimulated in presence or absence of 10 μM VidoCa, 10 μM teriflunomide or vehicle for 3 days:
  - Antigen specific: Splenocytes loaded with 50 ng/ml $OVA_{257-264}$
  - Polyclonaly:αCD3/αCD28
  - Supernatant: harvested and stored → cytokine detection (ELISA)
  - Cells: flow cytometry for CD8, viability, proliferation

Metabolic measurements[6]

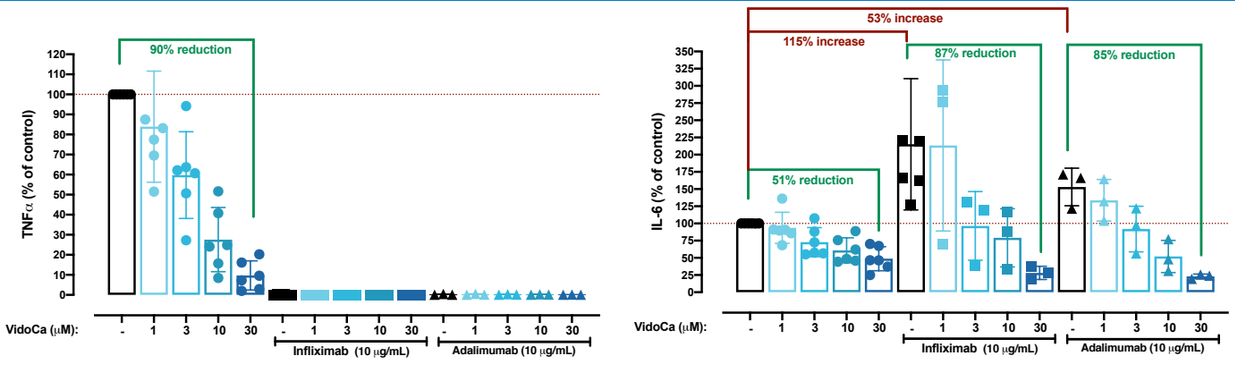
Mitochondrial respiration and glycolysis were measured by Seahorse XFp or XFe96 Extracellular Flux Analyzer (Agilent Technologies) in human and murine CD4 and CD8 T cells.

VidoCa Shows a Minor Induction of Regulatory Macrophages, Which is Strongly Enhanced in Combination With Anti-TNF



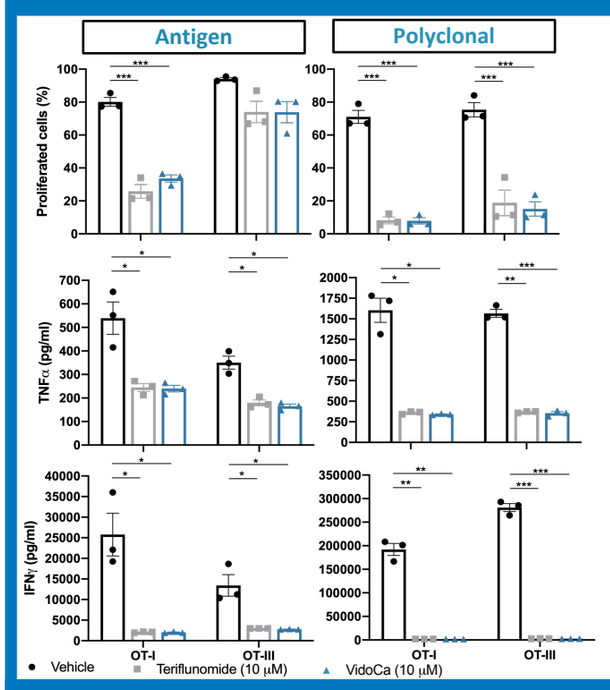
VidoCa induced Mregs in an MLR assay by 20%. Both anti-TNF antibodies were more potent in inducing Mregs alone. However, combining anti-TNF with VidoCa even further augmented Mregs between 58-83% for the higher VidoCa concentrations.

VidoCa Strongly and Dose-Dependently Inhibits TNFα and IL-6 Secretion in an MLR Assay



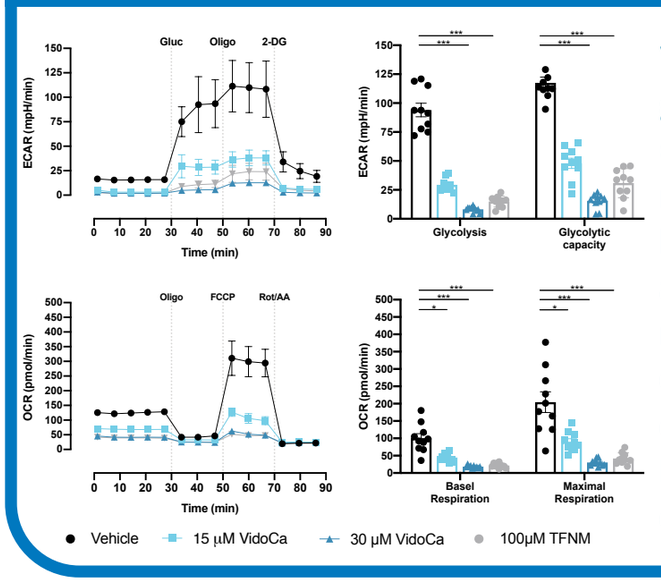
VidoCa strongly reduced TNFα secretion. Also, IL1β secretion was reduced up to 60% and up to 70-75% when combined with anti-TNF. Interestingly, anti-TNF strongly induced the secretion of IL-6. Nonetheless, VidoCa potently inhibited the secretion of IL-6 even when combined with anti-TNF.

VidoCa Shows Activity on T Cell Proliferation, Dependent on TCR Activation Strength



VidoCa suppresses the proliferation of antigen stimulated T cells containing high affinity TCR. Upon polyclonal stimulation, VidoCa is able to suppress proliferation in high and low affinity cells to a similar extent, indicating that the effect seen with VidoCa upon antigen stimulation is not dependent on the T cell, but on the stimulation strength. VidoCa also strongly inhibited the secretion of IFNγ and TNFα. The size of the effect also seems to be due to the stimulation strength.

VidoCa Inhibits Murine and Human T Cell Metabolism



VidoCa potently suppresses glycolysis and glycolytic capacity (upper panel) as well as basal and maximal respiration (lower panel) in human CD4 T cells. Similar results are seen for human CD8 T cells and murine CD4 and CD8 T cells. In all cell types, VidoCa exerts a similar effect as teriflunomide (another DHODH inhibitor, used at a higher concentration).

Conclusions

- VidoCa reduces proinflammatory immune cell responses by inducing Mregs, reducing pro-inflammatory cytokine secretion and reducing T cell proliferation.
- VidoCa shows an additive to synergistic effect with anti-TNF in the MLR assay suggesting a new and promising combination treatment opportunity for UC.
- DHODH is important in cells that receive a strong stimulus and are highly metabolically active. Therefore, VidoCa was observed to only target these cells and, thus, allows for a normal immune response, representing a huge safety advantage for treatment of IBD patients.

References

1. Tan *et al.* 2016, *J. Molcel.*
2. Vos *et al.* 2012. *Inflamm. Bowel Dis.*
3. King *et al.* 2012. *Immunity*
4. Amrani *et al.* 2000. *Nature*
5. Vos *et* al. 2011. *J.Gastro*
6. *Klotz et al.* 2019 *Sci.Transl.Med.*

Contact: info@imux.com