P 526

DHODH inhibitors based on the vidofludimus scaffold containing carboxylic acid bioisosters exert a superior broad-spectrum antiviral activity

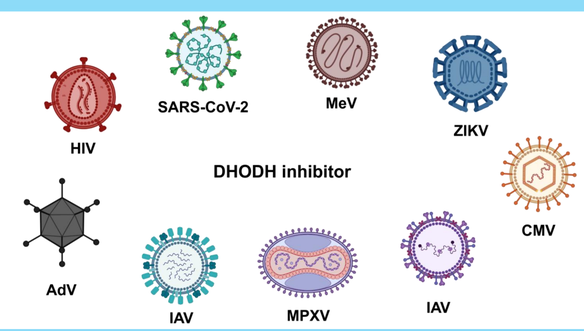
Alexandra Herrmann[1,4], Friedrich Hahn[2,3], Christina Wangen[2], Sabrina Wagner[2], Arne Cordsmeier[2], Pascal Irrgang[2], Tatjana Weil[4], Victoria Hunszinger[4], Rüdiger Groß[4], Natalie Heinen[6], Stephanie Pfaender[7,8], Sebastian Reuter[9], Nadja Uhlig[10], Valentina Eberlein[10], Leila Issmail[10], Thomas Grunwald[10], Robert Klopfleisch[11], Armin Ensser[2], Matthias Tenbusch[2], Jan Münch[4], Konstantin M.J. Sparrer[4,5], Manfred Marschall[2], Hella Kohlhof[1], Daniel Vitt[1], Christian Gege[1]

[1]Immunic AG, Gräfelfing, Germany; [2]Harald zur Hausen Institute of Virology, Uniklinikum Erlangen, Friedrich-Alexander-Universität Erlangen-Nürnberg (FAU), Erlangen, Germany; [3]Institute of Virology, Ulm University Hospital, Ulm, Germany; [4]Institute of Molecular Virology, Ulm University Medical Center, Germany; [5]German Center for Neurodegenerative Diseases (DZNE), Neurovirology and Neuroinflammation, Ulm, Germany; [6]Ruhr University Bochum, Department of Molecular and Medical Virology, Bochum, Germany; [7]University of Luebeck, Institute of Virology and Cell Biology, Lübeck, Germany; [8]Leibniz Institute of Virology, Hamburg, Germany; [9]University Hospital Essen – Ruhrlandklinik, Department of Pulmonary Medicine, Experimental Pneumology, Essen, Germany; [10]Fraunhofer-Institute for Cell Therapy and Immunology (IZI), Department of Infection Research and Diagnostics, Unit Preclinical Validation, Leipzig, Germany; [11]Freie Universität Berlin, Institute of Veterinary Pathology, Berlin, Germany

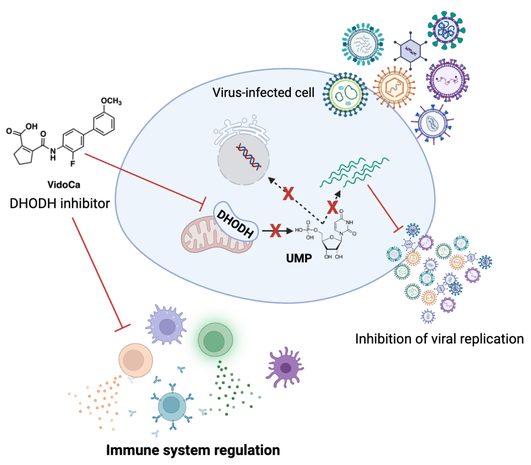
Research highlights

Design, screening, and characterization of optimized DHODH inhibitors[1]

- with improved drug-like properties and target engagement
- with potent antiviral activity against DNA and RNA viruses as well as a retrovirus
- synergistic effects in combination with nucleoside analogs
- that reduce SARS-CoV-2 viral load in mice and influenza-virus replication in human lung tissue

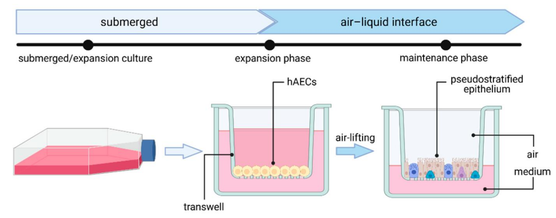


Background

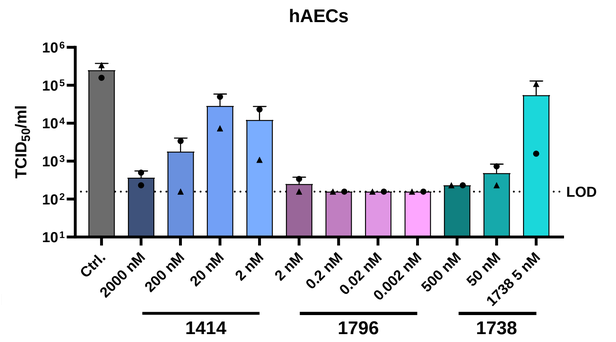


- Dihydroorotate dehydrogenase (DHODH) catalyzes the rate-limiting step of the *de novo* pyrimidine synthesis
- Direct effect on virus replication[2]
- Direct inhibition of hyperactive immune cells[2]
- Secondary activation of interferon-stimulated genes[3]
- Vidofludimus calcium (VidoCa):
  - orally bioavailable small molecule
  - Currently investigated in phase 2 and 3 trials for multiple sclerosis[4,5] and post-COVID syndrome[6]

SARS-CoV-2 inhibition in airway epithelial cells

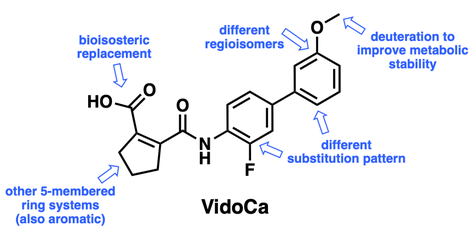


Human airway epithelial cells (hAECs) treated with DHODH inhibitor from the basolateral site, 3x10⁴ PFU SARS-CoV-2 from the apical site; 48 hpi



→ **Optimized DHODH inhibitors potently restrict SARS-CoV-2 replication at nanomolar to sub-nanomolar concentrations**

Compound optimization of vidofludimus calcium



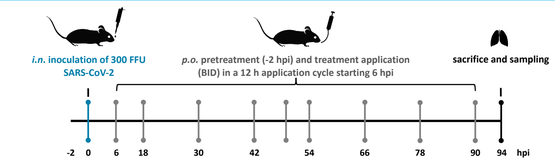
VidoCa		1796
120 nM	IC_{50} hDHODH	1 nM
5,000 nM	IC_{50} mDHODH	2 nM
5,200 nM	EC_{50} SARS-CoV-2	1 nM
Mouse 5 mpk (♀)	PK in mouse	Mouse 5 mpk (♀)
C_{max} 3440 ng/mL		C_{max} 6700 ng/mL
$t_{1/2}$ 1.6 h		$t_{1/2}$ 2.5 h
AUC 5740 ng*h/mL		AUC 25000 ng*h/mL
F 44 %		F 76 %

C_{max}: maximum serum concentration; AUC: area under the curve; exposure over time
$t_{1/2}$: half-life; F: bioavailability

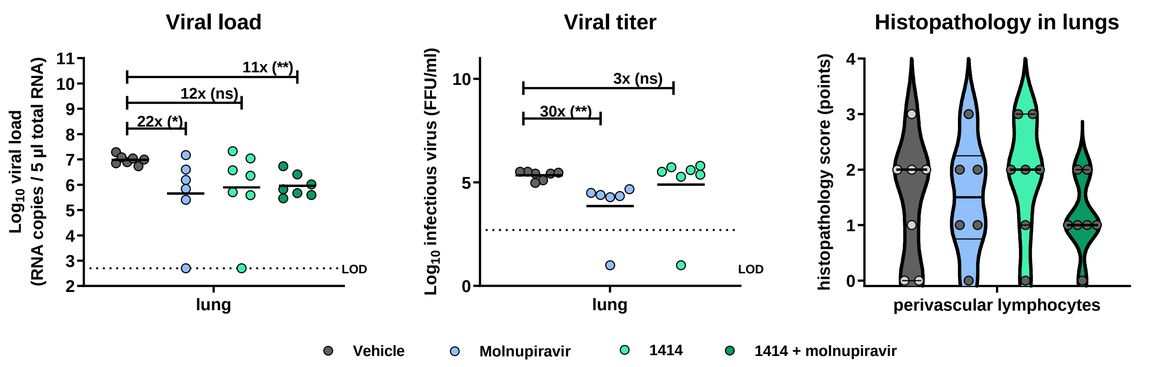
- Introduction of bioisosters, modifications at the central phenyl ring, and variations of the 5-membered ring system lead to improved potency
- Modifications at the central phenyl ring resulted in the elimination of species specificity
- Deuteration and modifications at the central phenyl ring improvement of pharmacokinetic properties

→ **Optimization resulted in a large chemical space with >300 novel DHODH inhibitors**

SARS-CoV-2 inhibition in a mouse model

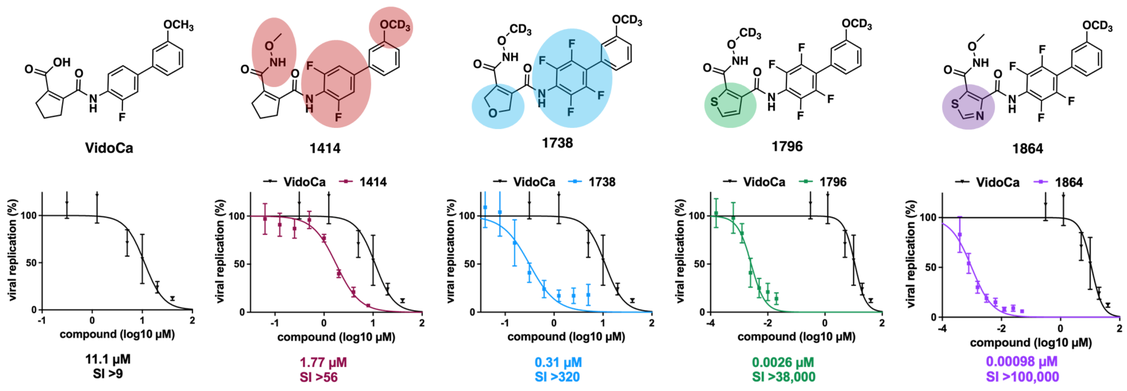


Female K18-hACE mice, 25 mg/kg/bid molnupiravir, 50 mg/kg/bid DHODH inhibitor 1414, or combination of both; analysis 4 dpi. Histopathological analysis using H&E staining. Quantification of infectious virus via plaque assay applying an anti-spike antibody.



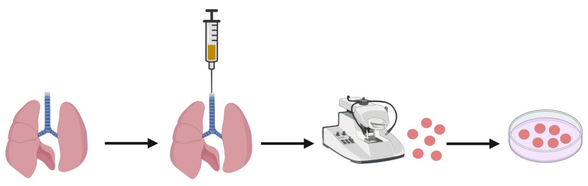
→ **Combination of the DHODH inhibitor 1414 with a nucleoside analog has a beneficial effect on reduction of tissue damage compared to single treatment *in vivo***

Improved inhibition of SARS-CoV-2 replication

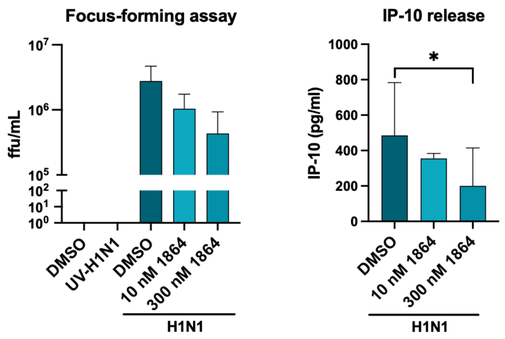


→ **Compound optimization resulted in a more than 10,000-fold improvement of SARS-CoV-2 inhibition *in vitro***

Inhibition of influenza A virus in lung tissue

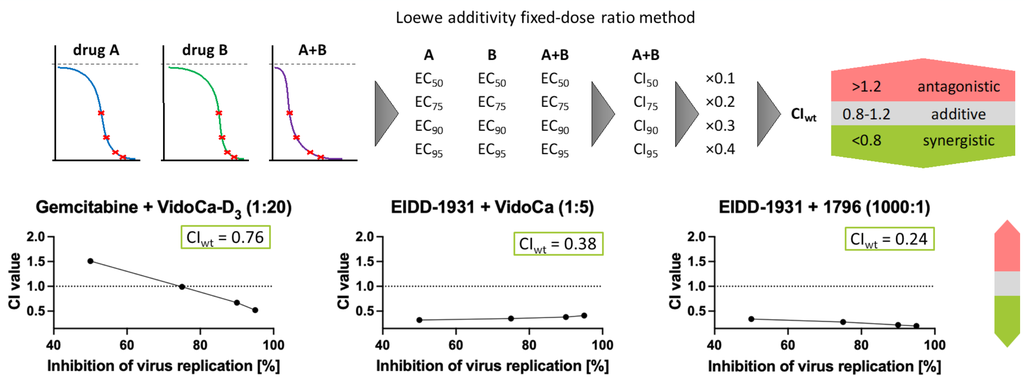


Human precision cut lung slices (PCLs), A/H1N1/California/4/2009pandemic, 72 hpi; in collaboration with Fraunhofer ITEM, Hannover.



→ **The optimized DHODH inhibitor 1864 inhibits influenza virus replication in human lung tissue and reduces the release of pro-inflammatory cytokines**

Drug interaction with nucleoside analogs



→ **Optimized DHODH inhibitors act synergistically with nucleoside analogs *in vitro***

Broad-spectrum antiviral activity

	Baltimore	VidoCa (nM)	1738 (nM)	1864 (nM)
		non-enveloped		
HAdV	I	6400	350	0.4
		enveloped		
CMV	I	7000	670	-
MPXV	I	700	58.2	0.5
SARS-CoV-2	IV	5200	330	1.0
CoV-229E	IV	-	172	3.4
CoV-OC43	IV	-	172	4.1
ZIKV	IV	-	470	44.2
IAV	V	2200	460	3.0
MeV	V	-	295	3.1
RSV	V	-	19.9	-
HIV-1	VI	2100	8.7	0.001

→ **Inhibition of non-enveloped and enveloped viruses**

→ **Inhibition of DNA and RNA viruses**

→ **Inhibition of a retrovirus**

References

[1] Herrmann *et al.*, *ACS*, in revision.
[2] Luganini *et al.*, 2025, *Antiviral Res.*
[3] Luthra *et al.*, 2018, *Antiviral Res.*
[4] Muehler *et al.*, 2020, *Mult. Scler. Relat. Disord.*
[5] Fox *et al.*., 2022, *Ann. Clin. Transl. Neurol.*
[6] Weipert *et al.*, 2025, *Trials*.
[7] Heinen *et al.*, 2021, *Viruses.*
[8] Kollareth and Scharma, 2024, *Front. Immunol.*

Acknowledgements

Immunic THERAPEUTICS
Fraunhofer ITEM
Bayerische Forschungsstiftung

Contact

Dr. Alexandra Herrmann
Institute of Molecular Virology
Ulm University Medical Center
Meyerhofstr. 1, N27

alexandra-1.herrmann@uni-ulm.de