### Preclinical Development of Optimized DHODH Inhibitors as Broad-Spectrum Antivirals for the Treatment of Respiratory Virus Infection

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# Background Imidine nthesis Antiviral Effects

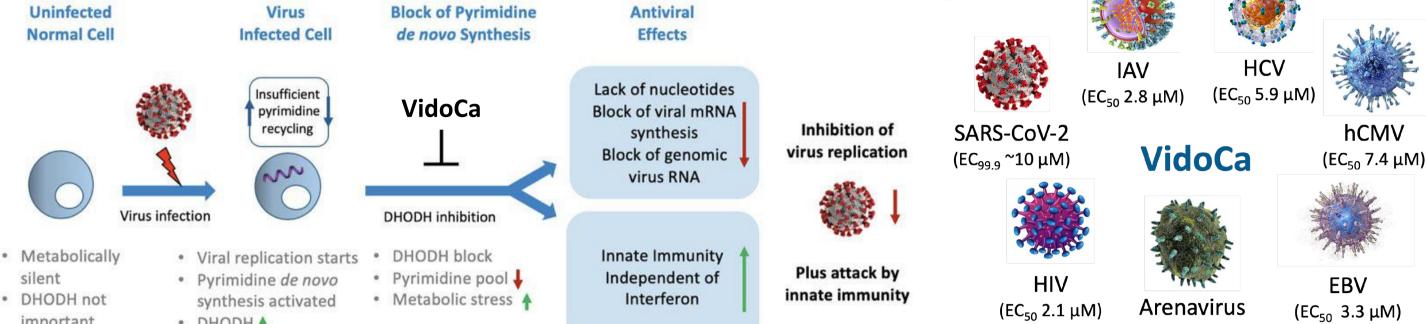


Fig. 1: Broad-spectrum antiviral and immunomodulatory activity of the DHODH inhibitor vidofludimus calcium (VidoCa). (A) Dihydroorotate dehydrogenase (DHODH) that catalyzes the rate-limiting step of *de novo* pyrimidine synthesis represents a promising target for development of host-directed antivirals (HDAs). VidoCa specifically targets DHODH, thereby inhibiting viral replication and reactivation. VidoCa displays a unique dual antiviral mode of action. First, it directly affects viral replication by depletion of the cellular pyrimidine pool. Second, VidoCa indirectly induces an antiviral state by inducing interferon-independent innate immune responses. (B) VidoCa inhibits replication of DNA, RNA, and retroviruses in the lower micromolar range *in vitro*.

## XAVI-101 inhibits SARS-CoV-2 in vitro and acts synergistically with nucleoside analogs

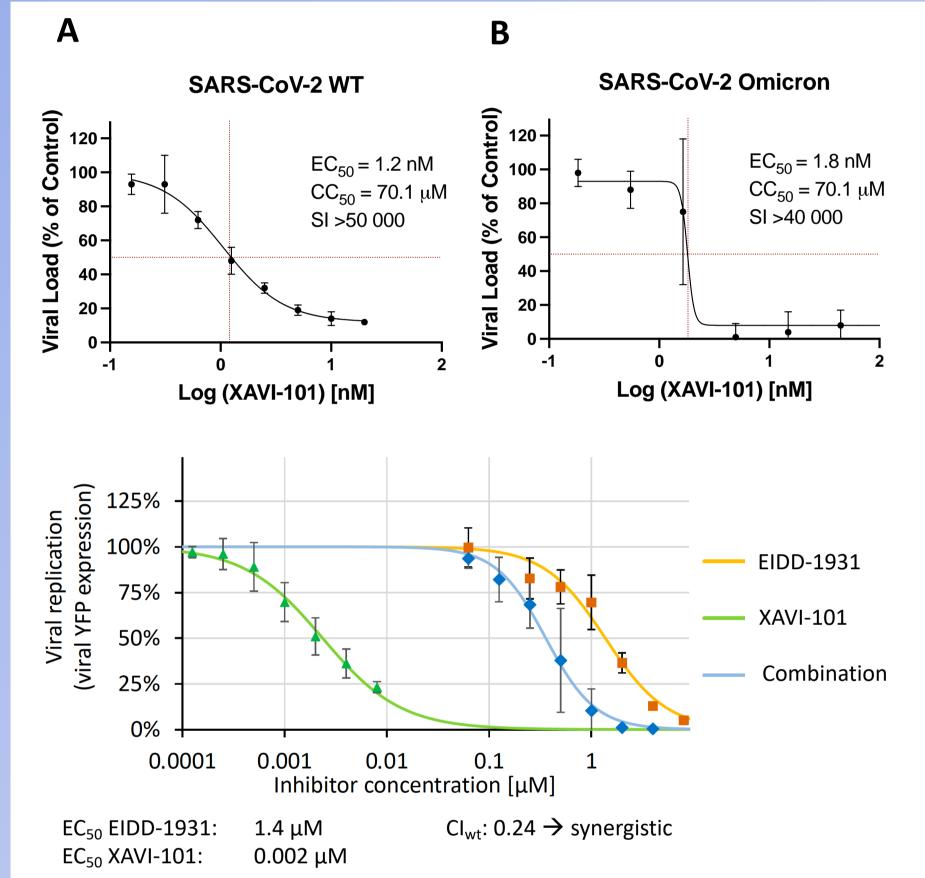
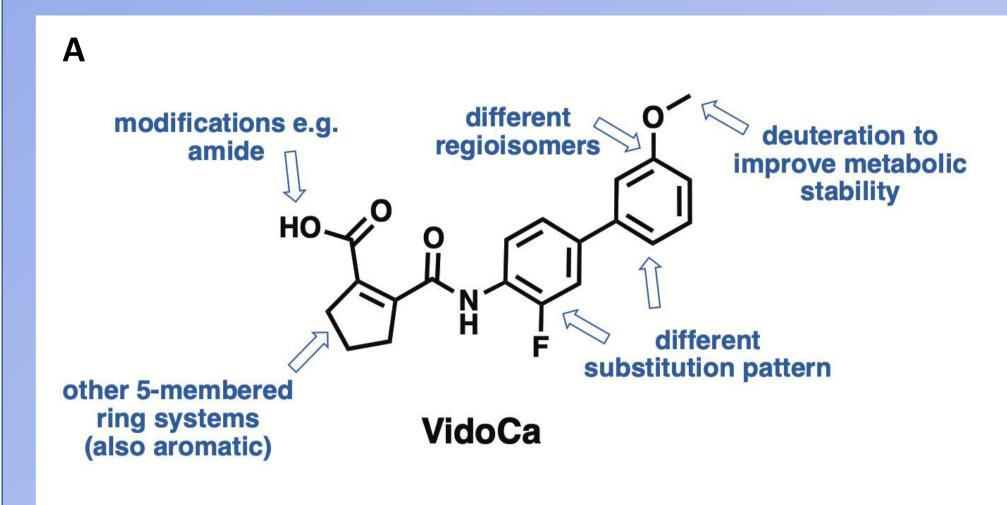
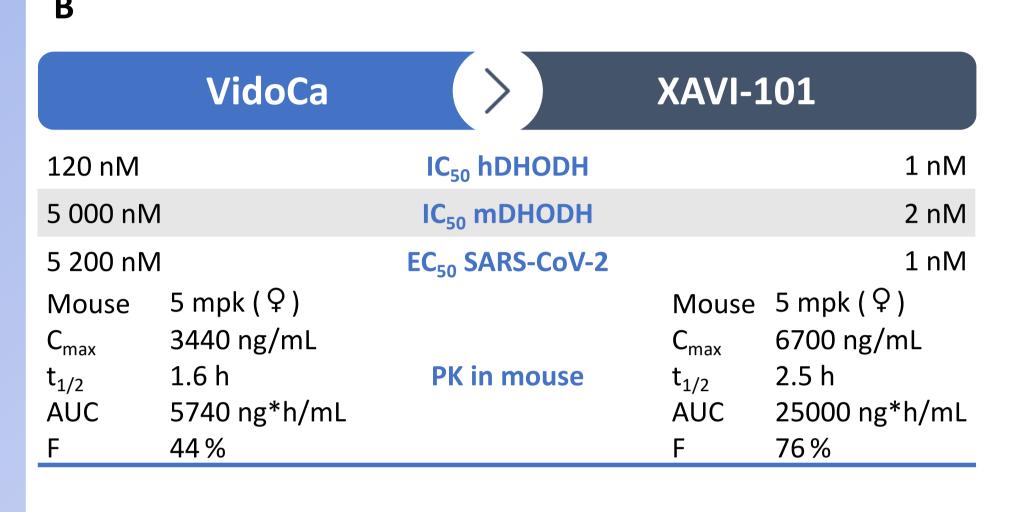


Fig. 3. XAVI-101 inhibits replication of different SARS-CoV-2 variants and acts synergistically with the active metabolite of molnupiravir EIDD-1931 in vitro. Caco-2 cells were treated with serial dilutions of XAVI-101 and infected with the SARS-CoV-2 WT reporter virus d6-YFP that expresses YFP instead of the viral ORF6 protein (A) or a clinical isolate of the omicron variant (B). Infected cells were quantified by either analyzing the percentage of YFPpositive cells (A) or using an nps3specific antibody and a fluorescently labeled secondary antibody (B) using a Victor X4 multiplate reader. In parallel, cytotoxicity was assessed by neutral red 50% half-maximal staining. The effective concentration ( $EC_{50}$ ) and the 50% cytotoxic concentration ( $CC_{50}$ ) were calculated using the nonlinear regression analysis in GraphPad Prism.

(C) Drug interactions of the DHODH inhibitor XAVI-101 and the active metabolite of the nucleoside analog molnupiravir (EIDD-1931) were assessed in Caco-2 cells infected with SARS-CoV-2 d6-YFP by Loewe additivity fixed dose ratio assay. Therefore, cells were treated either with XAVI-101 alone, EIDD-1931 alone, or a combination of both compounds starting at the respective 4x EC<sub>50</sub> concentration. Calculation of the weighted combination index ( $Cl_{wt}$ ) revealed a synergistic interaction of XAVI-101 and EIDD-1931.

#### Compound optimization of VidoCa to XAVI-101





Compound optimization **XAVI-101** improved antiviral activity and pharmaceutical properties its progenitor compared to a lead-VidoCa. (A) During optimization process, VidoCa was optimized regarding pharmacokinetic properties (e.g., deuteration, different substitution patterns ring) central phenyl target engagement, and antiviral activity (via modification of the central phenyl ring and variations in the 5-membered ring system. These modifications resulted in a large chemical space with new full (i.e., protection patent WO2022/214691). (B) In vitro DHODH enzyme assays revealed an enhanced target inhibition for human and animal protein. Analysis of the antiviral activity in culture experiments confirmed an improved inhibition of viral replication for a variety of different viruses in vitro.

#### **XAVI-101** inhibits diverse RSV strains in vitro

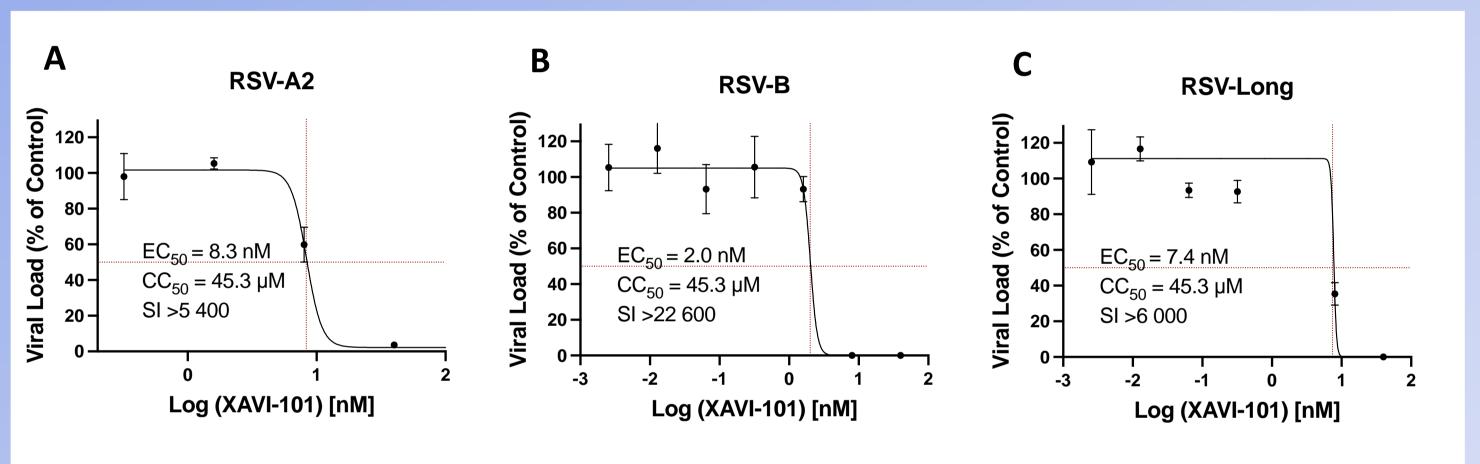


Fig. 5. XAVI-101 inhibits infection of different RSV strains in the lower nanomolar range. Hep-2 cells were treated with serial dilutions of XAVI-101 and infected with respiratory syncytial virus (RSV) A2 expressing eGFP (A), RSV-B (B), or RSV-Long (C) (MOI = 0.001). RSV infection was quantified 2 dpi either by counting eGFP-positive cells using a CTL Immunospot reader or by IC staining with a RSV-targeting antibody. In parallel, cytotoxicity was assessed by quantification of ATP levels using CellTiter-Glo Luminescent Cell Viability Assay (Promega) 48 h post-treatment. The  $EC_{50}$  and  $CC_{50}$  concentrations were calculated using the nonlinear regression analysis in GraphPad Prism.

#### XAVI-101 analog diminishes SARS-CoV-2 viral load in vivo

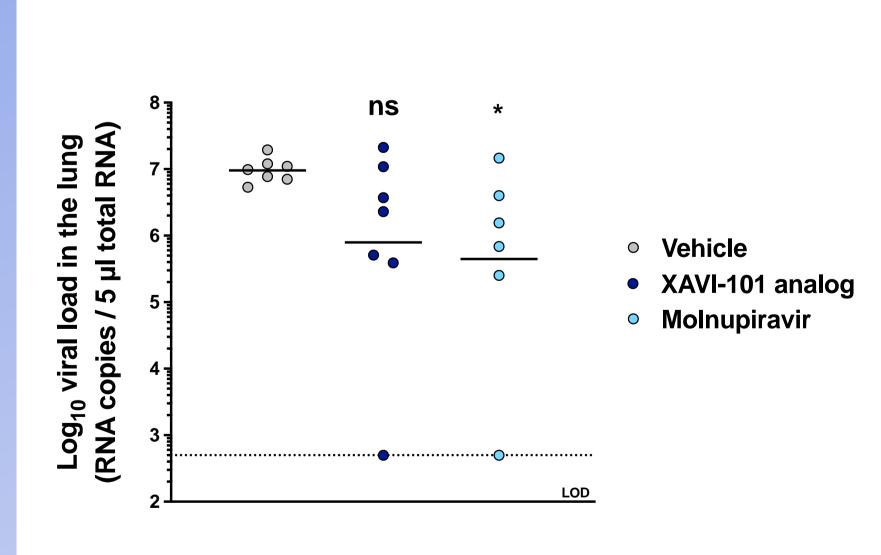


Fig. 4. XAVI-101 reduces SARS-CoV-2 viral load in lungs of K18 hACE2 mice. Female K18-hACE2 mice (n = 7) received either 100 mg/kg/bid XAVI-101, 50 mg/kg/bid molnupiravir, or 10% DMSO in PEG400 as vehicle control via oral gavage. Mice were infected intranasally with 300 FFU SARS-CoV-2 (Wuhan strain) and euthanized 4 dpi. Viral load in lung homogenates was quantified by RT-qPCR. Reduction of viral load is shown as fold reduction compared to vehicle control. Statistical analysis was performed by Mann-Whitney U test relative to vehicle control in GraphPad Prism (ns: not significant; \*: p ≤ 0.05).

#### XAVI-101 inhibits influenza virus replication in vitro

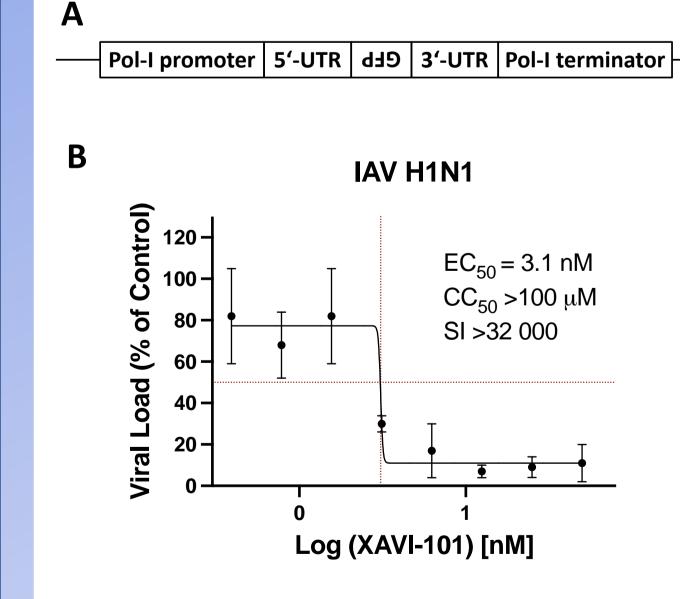


Fig. 6. XAVI-101 efficiently blocks influenza virus infection in vitro. (A) HEK293T cells were transfected with a plasmid-based influenza A virus (IAV) polymerase reporter system that encodes an RNA polymerase I (Pol-I) promoter/terminator cassette that expresses GFP flanked by untranslated regions (UTRs) of the IAV nucleoprotein segment. (B) After one day, cells were treated with serial dilutions of XAVI-101 and infected with the IAV H1N1/PR8 isolate (MOI = 0.1). Infected cells were quantified by analyzing GFP-positive cells using a PicoMD imaging platform. In parallel, cytotoxicity was assessed by neutral red assay. The EC<sub>50</sub> and CC<sub>50</sub> concentrations were calculated using the nonlinear regression analysis in GraphPad Prism.

#### **Summary and Conclusions**

- > XAVI-101 shows improved target engagement and pharmaceutical properties compared to its progenitor vidofludimus calcium (VidoCa)
- > XAVI-101 potently restricts replication of different respiratory viruses in the single-digit nanomolar range *in vitro*
- > XAVI-101 analog reduces the viral load in lungs of SARS-CoV-2 infected mice
- > XAVI-101 represents a promising candidate with broad-spectrum antiviral activity for future clinical development

**Broad Antiviral Specificity** 

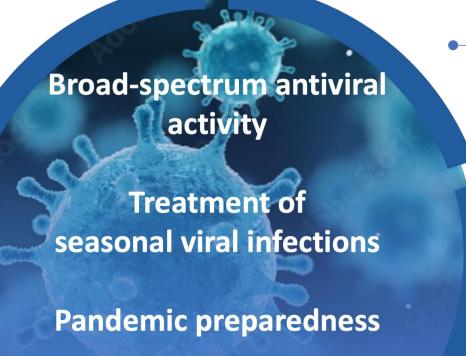
Targeting host pathways essential and universal for viral propagation

High potency against human and animal model species targets

Low Potential for Resistance

Low risk of host targets being affected by viral escape mutations

Potential for prophylactic applications



Potential for prophylactical

treatment

#### Safety / tolerability

Lead candidates derived from mother compounds with proven clinical safety and tolerability

In vitro safety already proven for novel candidate

#### Target selection

Targeting pathways that require induction for viral propagation while host functions are maintained by salvage pathways

Hahn *et al.*, 2020; *Viruses* Dec 5;12(12):1394. doi: 10.3390/v12121394 Vehreschild *et al.*, 2022; *Infect. Dis. Ther.* Dec;11(6):2159-2176. doi: 10.1007/s40121-022-00690-0

**References:**