Discovery and Antiviral Activity of the Novel 3CL Protease Inhibitor ALG-097558 in the SARS-CoV-2 Hamster Infection Model

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Introduction: There is an urgent need for novel antiviral drugs for the treatment of Covid-19, especially in unvaccinated individuals and at-risk patients. In 2021, the FDA granted emergency use authorization for the SARS-CoV-2 3CL protease (3CLpro) inhibitor Paxlovid (nirmatrelvir/ritonavir). Here, we describe ALG-097558, a novel 3CLpro inhibitor with a favorable resistance and ADME profile without the need of a pharmacoenhancer such as ritonavir.

Table 1: Biochemical Potency and Selectivity

Compound	SARS-CoV-2 3CLpro			HRV 3C Protease IC_{50} (nM)	Cathepsin L IC_{50} (nM)
	IC_{50} (nM)	Hill Slope	K_i (nM)		
ALG-097558	**0.26**	**1.99**	**0.074**	**> 10000**	**> 10000**
Nirmatrelvir	2.92	0.91	2.03	> 10000	> 10000
PBI-0451	3.6	1.74	3.4	> 10000	1493
Ensitrelvir	4.0	1.31	2.6	> 500	> 10000

- Low 3CLpro enzyme concentration (0.3 nM) was used to accurately determine the K_i of highly active 3CLpro inhibitors in a mass spectrometry-based assay
- ALG-097558 is a selective SARS-CoV-2 3CLpro inhibitor without off-target activity against human Cathepsin L and the Human Rhinovirus protease
- ALG-097558 is a reversible 3CLpro binder based on guanidine denaturation experiments (not shown)

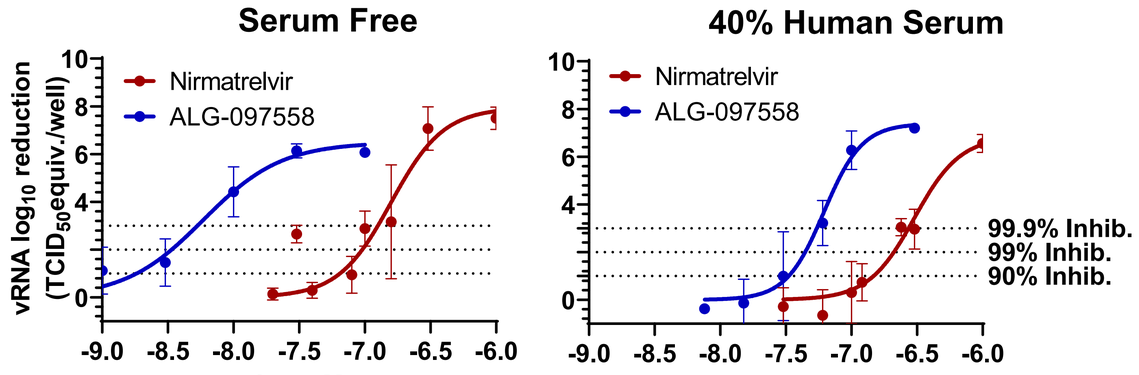
Table 2: Pan-Coronavirus Activity of ALG-097558 in Cellular Assays

Virus	Variant	EC_{50} (µM)			
		ALG-097558	Nirmatrelvir	PBI-0451	Ensitrelvir
SARS-CoV-2	03021/2020 [1]	**0.010**	0.116	n.d.	n.d.
	B.1.1.7 (alpha) [2]	**0.012**	0.099	0.038	0.023
	B.1.617.2 (delta) [2]	**0.013**	0.217	0.126	0.141
	B.1.1.529 (omicron) [1]	**0.008**	0.059	0.136	0.095
	BA.2 [1]	**0.003**	0.027	0.087	0.028
SARS-CoV-1	Isolate Vietnam [1]	**0.022**	0.173	0.323	0.154
β-hCoV	OC43 [3]	**0.009**	0.047	0.168	n.d.
α-hCoV	229E [4]	**0.017**	0.476	0.281	6.30

Cell lines used: (1) VeroE6 (in presence of 2 µM of P-glycoprotein inhibitor CP-100356), (2) A549-ACE2-TMPRSS2, (3) HeLa, (4) Huh-7; No cytotoxicity was detected for ALG-097558 at concentrations up to 100 µM.

- ALG-097558 demonstrates pan-coronavirus activity in cell-based assays
- BA.5 testing pending; bioinformatics predicts retained activity against BA.2.12.1, BA.3, BA.4 and BA.5
- Pan-coronavirus activity confirmed in FRET assay using 3CLpro derived from SARS-CoV-2, MERS, human α-CoV 229E and NL63, and human β-CoV HKU-1 (not shown)

Figure 1: Inhibition of SARS-CoV-2 Replication in 3D Human Airway Epithelium (HAE) Air-Liquid-Interphase (ALI) Cultures



- HAE-ALI cultures infected with SARS-CoV-2 B.1.1.7 on apical, compounds added on basolateral side
- Viral replication is assessed on day 4 post-infection via RT-qPCR
- ALG-097558 $EC_{99.9}$ of 5.3 and 54 nM, in the absence or presence of 40% human serum, respectively

Table 3: ADME and In vitro Toxicology Profile of ALG-097558

Parameter	Data	Profile
Stability in simulated gastric and intestinal fluids, and in plasma (R, D, H)	$t_{1/2}$ > 480 min	Stable
Stability in liver microsomes $t_{1/2}$ min Ha/M/C/R/D/H	<15/ <15/ <15/ >60/ >60/ 50	Good stability in R,D and H
Systemic clearance across species	4.33 -50.7 mL/min/kg	Low to moderate in non-rodents; high in rodents
Steady state volume of distribution	0.434-1.23 L/kg	Low to moderate
Bioavailability using solution formulations	5 to > 50% in R, D, and C	Orally bioavailable
Plasma $t_{1/2}$ (PO)	2-3 h in R, D, and C	Short, suggestive of BID regimen
hERG/CaV/NaV	10 µM	IC_{50} >10 µM
Ames assay (+/-S9)	Up to 250 µg/well	Negative
In Vitro Micronucleus (+/-S9)	Up to 500 µg/mL	Negative

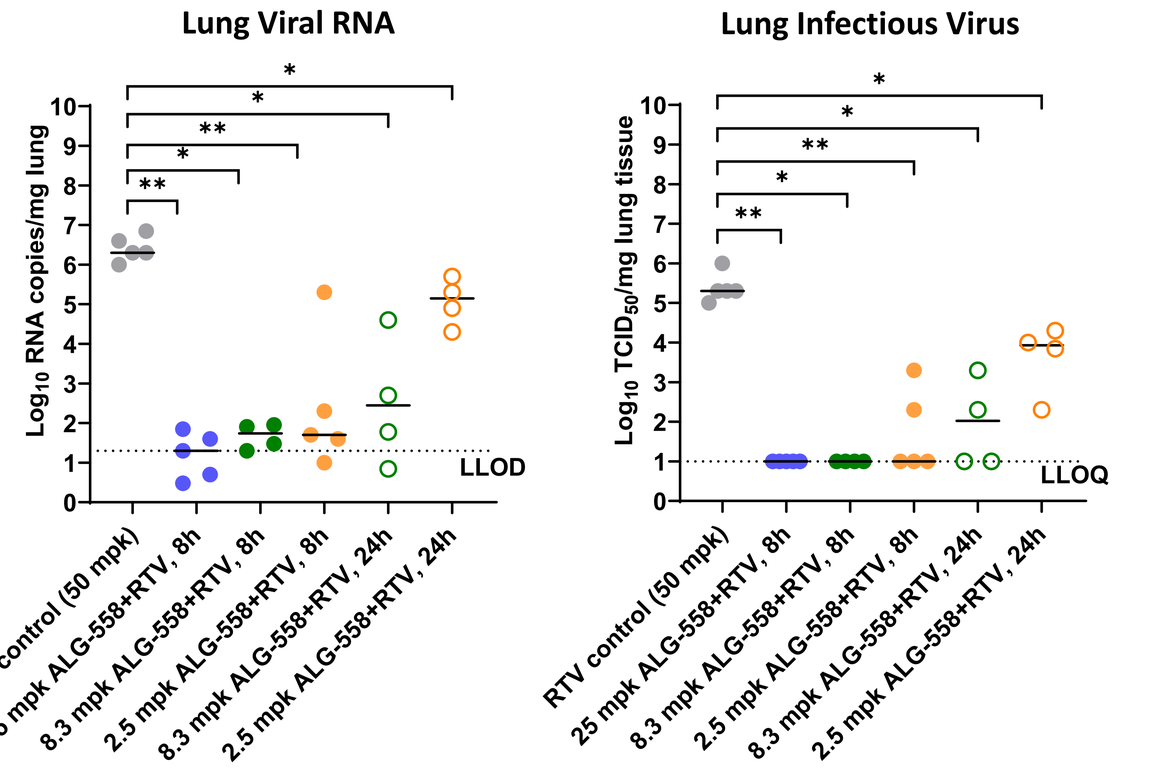
Ha/M/R/D/C/H: hamster/mouse/rat/dog/monkey/human

Table 4: Favorable Activity Profile Against Selected Resistance Mutants

Compound	Biochemical Potency Fold IC_{50} Compared to Wild-type (Resistance)				
	L50F/E166A/L167F	F140A	S144A	H172Y	Q189K
ALG-097558	**3 (n=3)**	**0.8**	**1**	**2**	**1**
Nirmatrelvir	66 (n=6)	4	3	7	1
PBI-0451	> 65 (n=2)	4	2	20	0.6
Ensitrelvir	> 67 (n=2)	3	3	5	0.5

- Resistance selections with ALG-097558 are ongoing
- ALG-097558 shows minor loss of activity against mutations conferring resistance to other SARS-CoV-2 PIs in a FRET-based assay

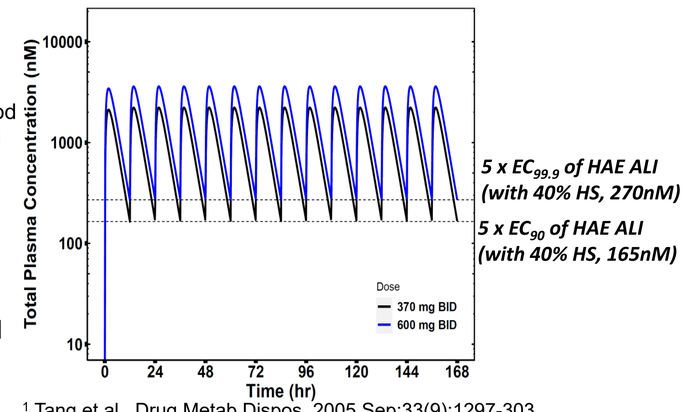
Figure 2: Significant Reduction of Viral Replication By Orally Dosed ALG-097558 in the SARS-CoV-2 Hamster Model Using a Therapeutic Dosing Mode



- Intranasal infection with SARS-CoV-2 B.617.2 delta, ($1x10^4$ $TCID_{50}$/animal); 5 animals per group
- ALG-097558 is metabolically unstable in hamsters and requires boosting with ritonavir (RTV)
- Therapeutic dosing regimen with dosing start 8h or 24h post-infection (p.i.), BID, for 3 days
- ALG-097558 causes ~ 5 log_{10} reduction in vRNA with 8h p.i. dosing start; all animals at or near LLOD, confirmed with infectious titer read-out
- With 24h p.i. dosing start, significant and dose-dependent reduction in vRNA and infectious titer observed
- Improvements in body weight loss (due to viral infection) observed with ALG-097558 dosing start of 8h and 24h p.i. (not shown)
- Plasma conc. of ALG-097558 at all dose levels were above the hamster fu-adjusted EC_{90} (not shown)

Figure 3: Human Efficacious Dose Projection

- Methods and assumptions
  - Clearance predicted using fu intercept correction method (FCIM) [1]
  - Volume of distribution predicted using $OT_{in\text{-}vivo}$ method where V_{ss} is function of the physiological description of Øie–Tozer [2]
  - C_{min} targeted as 5x of serum-shifted EC_{90} or serum-shifted $EC_{99.9}$ in the *in-vitro* HAE ALI assays
  - Oral bioavailability of 25% as an average of bioavailability in nonclinical species
- Methods validated based on comparable clearance and volume of distribution predicted by these methods vs. the parameters derived from reported human PK for nirmatrelvir [3]
- Projected efficacious human dose 370 to 600 mg BID without need for ritonavir



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[2] Obach RS et.al., J Pharmacol Exp Ther. 1997 Oct;283(1):46-58.
[3] Owen Dr et al., Science 374:6575:1586-93

Summary and Outlook for ALG-097558:

- **Pan-coronavirus, nanomolar antiviral activity in biochemical and cellular assays**
- **Efficient inhibition of viral replication in human airway epithelium ALI cultures with $EC_{99.9}$ of 5.3 and 54 nM, respectively, with or without 40% human serum**
- **Efficient reduction of viral replication in SARS-CoV-2 hamster model using low, oral doses and a therapeutic dosing regimen**
- **Favorable ADME profile indicates a 370-600 mg BID dosing regimen in humans without the need of ritonavir boosting**
- **First-in-human clinical trials expected to start in first half of 2023**

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