Discovery of ALG-097558, a Novel 3CL Protease Inhibitor with Activity 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 (data not shown)

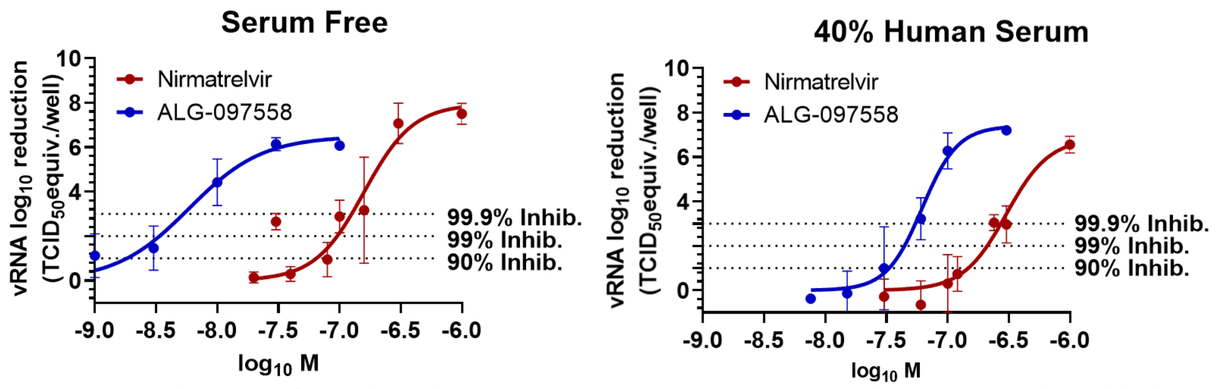
Table 2: Pan-Coronavirus Activity of ALG-097558 in Cellular Assays

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 0.5 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
- 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 (data not shown)

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



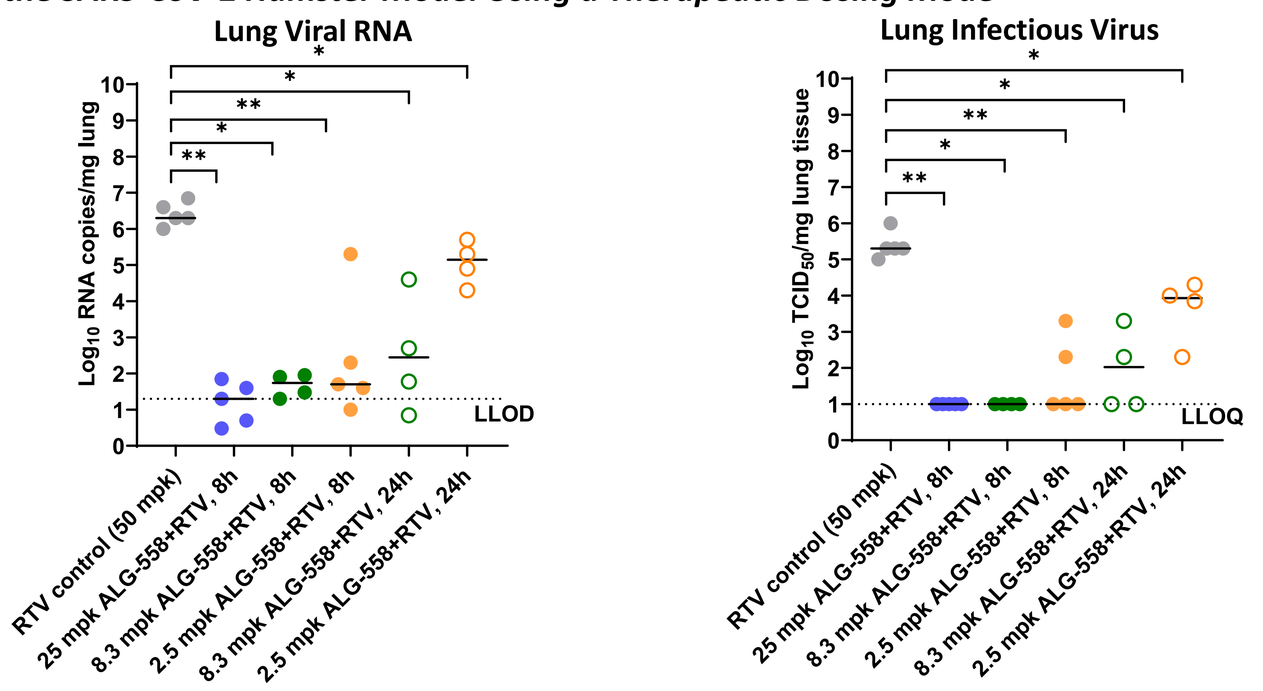
- HAE-ALI cultures infected with SARS-CoV-2 B.1.1.7 on apical, compounds added on basolateral side. Viral replication assessed on day 4 post-infection via RT-qPCR
- ALG-097558 $EC_{99.9}$ of 5.3 and 54 nM, in the absence or presence of 40% human serum, respectively

Table 3: 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

- 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. Therapeutic dosing regimen with dosing start 8h or 24h post-infection (p.i.), BID, for 3 d
- ALG-097558 is metabolically unstable in hamsters and requires boosting with ritonavir (RTV)
- 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)
- ALG-097558 plasma concentrations at all dose levels were above the hamster fu-adjusted EC_{90} (not shown)

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**
- **Projected human oral efficacious dose of 350-600 mg BID without the need of ritonavir boosting**
- **First-in-human clinical trials expected to start in first half of 2023**

Financial disclosure: AJ, SC, RS, KG, KL, CL, AS, SKS, SR, RJ, VS, JD, SC, LMB, LB and JAS are current or former employees of Aligos Therapeutics, Inc. KV, PR and TL are current or former employees of Aligos Belgium BV."