Potent, Pan-Coronavirus Antiviral Activity of the Novel 3CLpro Inhibitor ALG-097558

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BACKGROUND

Despite widespread immunity from SARS-CoV-2 vaccination and/or past infection, safe, effective, and convenient treatments for acute infection are still needed for individuals who remain at high risk for disease progression. The Infectious Disease Society of America (IDSA) recommends Paxlovid for the treatment of ambulatory patients with mild-to-moderate COVID-19, who are at risk for progression to severe disease. Paxlovid is a combination of the 3CLpro inhibitor nirmatrelvir and the CYP3A4 inhibitor ritonavir (RTV). Due to significant drug-drug-interactions (DDI) caused primarily by RTV, Paxlovid is contra-indicated in a large group of patients with the greatest need for antiviral treatment, for example immunocompromised subjects, or patients with renal or hepatic impairment.

The risk of another coronavirus pandemic is an ongoing threat to global health. Over the last 20 years, three beta coronaviruses (SARS-CoV-1, MERS-CoV and SARS-CoV-2) caused major outbreaks or pandemics. In addition, other human alpha CoVs (229E, NL63) and beta CoVs (OC43, HKU-1) cause in general mild disease. In this context, the development of novel orally available therapeutics with a reduced risk of DDI and pan-coronavirus activity is a critical component of pandemic preparedness to avoid a repeat of the immense public health and societal costs caused by COVID-19.

METHODS

The 3-chymotrypsin like protease (3CLpro) from SARS-CoV-2 and other human and bat coronaviruses was used in enzymatic assays to assess the inhibitory activity of ALG-097558. The antiviral activity was tested in cell-based assays with SARS-CoV-2, SARS-CoV-1, MERS-CoV and other α- and β-coronaviruses using cytopathic effect (CPE) or N-protein immunofluorescence as readout. Bioinformatics analysis was performed to assess sequence conservation in currently circulating SARS-CoV-2 strains and predict susceptibility to ALG-097558.

MAIN FINDINGS

- ALG-097558 is a novel inhibitor of the SARS-CoV-2 3CLpro with **pan-coronavirus activity.**
- In cellular assays, ALG-097558 is **consistently more active than nirmatrelvir, ensitrelvir and ibuzatrelvir** against current (Omicron) and older SARS-CoV-2 isolates and other CoVs including MERS-CoV and SARS-CoV-1.
- ALG-097558 demonstrates **potent, broad-spectrum inhibition of 3CLpro** proteins from various SARS-CoV-2 strains, other human CoVs as well as bat, cat, bird and whale CoVs in biochemical experiments.
- ALG-097558 demonstrated **favorable safety and PK properties in Phase I clinical testing**, including ritonavir-free dosing.
- Through the AGILE clinical trial platform, ALG-097558 is currently being evaluated in a **Phase 2 clinical trial for safety, efficacy and virological response** in monotherapy and as combination with remdesivir in a high-risk COVID-19 population (NCT04746183).

RESULTS: ANTIVIRAL ACTIVITY AGAINST SARS-COV-2

Table 1. Antiviral activity of ALG-097558 and other 3CLpro inhibitors in clinical development against SARS-CoV-2 variants in cell-based assays using cytopathic effect as the readout.

Variant	Cell line	EC_{50} (µM) mean ± SD (n≥3)			
		ALG-097558	Nirmatrelvir	Ibuzatrelvir	Ensitrelvir
GHB-03021/2020 (Wuhan)	VeroE6-eGFP [a]	0.012±0.004	0.114±0.053	0.167±0.067	ND
B.1.1.7 (Alpha)	A549-hACE2-hTMPRSS2	0.011±0.008	0.106±0.069	0.090±0.034	0.022±0.010
B.1.617.2 (Delta)		0.013±0.005	0.217±0.119	ND	0.141±0.066 [d]
B.1.1.529 (Omicron)	VeroE6-eGFP [a]	0.008±0.003	0.069±0.027	ND	0.123±0.060
BA.2 (Omicron)		0.007±0.004	0.045±0.026	ND	0.035±0.011
BA.5 (Omicron)		0.013±0.007	0.089±0.018	0.262 [c]	0.119±0.042
BQ.1 (Omicron)	VeroE6-TMPRSS2 [b]	0.030±0.010	0.097±0.021	ND	0.237±0.086
XBB1.5 (Omicron)		0.040±0.020	0.110±0.026	ND	0.243±0.110
JN.1 (Omicron)		0.006±0.001	0.047±0.004	ND	0.087±0.010
KP.1.1.1 (Omicron)		0.006±0.002	0.022±0.003	ND	0.016±0.002

ND=not determined; in presence of 2 µM of the P-gp inhibitor CP-100356 [(a)], or elacridar [(b)]. [(c)]: n=1; [(d)]: n=2.

Table 2. Antiviral activity of ALG-097558 and other 3CLpro inhibitors in clinical development against selected SARS-CoV-2 variants in cell-based assays using N-protein immunofluorescence as readout.

Variant	Cell line	EC_{50} / EC_{90} (µM) (n≥3)			
		ALG-097558	Nirmatrelvir	Ibuzatrelvir	Ensitrelvir
BQ.1 (Omicron)	VeroE6-TMPRSS2 [a]	0.016/0.040	0.081/0.191	0.118/0.157 [b]	0.154/0.402
XBB1.5 (Omicron)		0.016/0.030	0.067/0.139	0.082/0.138 [b]	0.176/0.366
JN.1 (Omicron)		0.014/0.016	0.124/0.137	ND	0.236/0.260
KP.1.1.1 (Omicron)		0.009/0.014	0.070/0.131	ND	0.044/0.169

[(a)] :in presence of 2 µM of the P-gp elacridar; [(b)]: n=2.

RESULTS - ANTIVIRAL ACTIVITY AGAINST SARS-COV-2

ALG-097558 inhibits 10 different SARS-CoV-2 isolates with a narrow EC_{50} range from 0.006 to 0.040 µM in cellular assays with cytopathic effect (CPE) as the readout (Table 1). No cytotoxicity was observed at concentrations up to 50 µM. ALG-097558 was consistently more active than other 3CLpro inhibitors, such as nirmatrelvir (the 3CLpro inhibitor in Paxlovid), ibuzatrelvir and ensitrelvir. The results of the CPE read-out was confirmed with a subset of viruses using an antibody against the viral N protein and immunofluorescence as the end point (Table 2).

RESULTS - ANTIVIRAL ACTIVITY AGAINST OTHER CORONAVIRUSES

Table 3. Antiviral activity of ALG-097558 and other 3CLpro inhibitors in clinical development against other coronaviruses in cell-based assays with a cytopathic effect-based read-out.

Virus	Genus	Cell line	EC_{50} (µM) mean ± SD (n≥3)			
			ALG-097558	Nirmatrelvir	Ibuzatrelvir	Ensitrelvir
SARS-CoV-1	Beta	VeroE6-eGFP [a]	0.022±0.013	0.148±0.124	0.120±0.004 [d]	0.150±0.018
MERS-CoV		Vero76 [a]	0.005 [c]	0.025 [c]	ND	0.1 [c]
MERS-CoV		VeroE6-TMPRSS2 [b]	0.037±0.025	0.080±0.036	0.142±0.009 [d]	0.443±0.240
hCoV OC43		HeLa	0.008±0.003	0.047±0.020	0.060±0.012	0.135±0.015
hCoV 229E	Alpha	Huh-7	0.017±0.012 [d]	0.502±0.525	ND	6.30±2.49
hCoV 229E		VeroE6-TMPRSS2 [b, e]	ND	0.605±0.031 [d]	2.00±1.09 [d]	ND
hCoV NL63		A549-Ace2-Dpp4-Tmprss2 [e]	0.213±0.004	2.17±0.142	>6 [d]	>6 [d]
fCoV FIPV		CRFK	0.015±0.001	0.096±0.010	ND	>2 [d]

hCoV: human coronavirus; fCov: feline ronavirus; ND=not determined; experiments performed in presence of 2 µM of the P-gp inhibitor CP-100356 [(a)], or elacridar [(b)]. [(c)]: n=1; [(d)]: n=2. [(e)]: N protein immunofluorescence instead of CPE-based readout

The potent antiviral activity of ALG-097558 extended to other human coronaviruses such as the pathogenic SARS-CoV-1 and MERS-CoV, seasonal hCoVs such as OC43, 229E and NL63 and the feline infectious peritonitis virus (FPIV). These include viruses from the alpha (229E, NL63, FPIV) and beta genus (SARS-CoV-1, MERS-CoV, OC43) of *Coronaviridae*. With the exception of NL63, ALG-097558 is highly active against these viruses with EC_{50} values of < 0.04 µM (Table 3). In contrast, a much more pronounced loss of antiviral activity was observed for other approved 3CLpro inhibitors (nirmatrelvir, ensitrelvir) or ibuzatrelvir, which is in Phase 3 clinical testing, when tested against alpha hCoVs, resulting in EC_{50} values of > 0.5 µM.

RESULTS – INHIBITION OF 3CLPRO ENZYMATIC ACTIVITY

In a high-sensitivity mass-spectrometry-based biochemical assay using 0.3 nM of the SARS-CoV-2 3CLpro protein derived from the ancestral Wuhan strain, ALG-097558 was at least 30-fold more active than nirmatrelvir, ensitrelvir and ibuzatrelvir (based on K_i) (Table 4).

Table 4: Biochemical potency of ALG-097558 and other 3CLpro inhibitors in a high-sensitivity SARS-CoV-2 3CLpro assay

Compound	IC_{50} (nM) (n≥2)	K_i (nM) (n≥2)
ALG-097558	0.22±0.04	0.071±0.045
Nirmatrelvir	4.3±1.6	3.1±1.2
Ensitrelvir	4.0±0.7	2.6±0.5
Ibuzatrelvir	3.5 (n=1)	2.3 (n=2)

The pan-coronavirus activity of ALG-097558 observed in cellular assays was confirmed in biochemical experiments, where ALG-097558 inhibited the enzymatic activity of 3CLpro proteins derived from various human alpha and beta CoVs (Table 5). Furthermore, the pan-coronavirus activity was also observed against a diverse panel of 3CLpros from bat, cat, bird and whale CoVs, which included the alpha, beta and gamma genus (Table 6). The slightly elevated IC_{50} for ALG-097558 and other 3CLpro inhibitors against MERS-CoV and bat NL63 may be due to the higher enzyme concentrations needed for sufficient assay sensitivity. ALG-097558 was consistently more active than nirmatrelvir, ensitrelvir and ibuzatrelvir. Substantially lower inhibition of the enzymatic activity was noted for ibuzatrelvir and ensitrelvir against 3CLpros from alpha-CoVs such as NL63, 229E, and FIPV and gamma-CoVs from bird (AIVB) and beluga whale CoV.

Table 5: Biochemical potency of ALG-097558 and other 3CLpro inhibitors against 3CLpro proteins (5nM) derived from various human coronaviruses.

Human Coronavirus 3CLpro	Genus	Enzyme Conc. (nM)	IC_{50} (nM) (n≥3)			
			ALG-097558	Nirmatrelvir	Ibuzatrelvir	Ensitrelvir
SARS-CoV-2	Beta	5	1.34±0.35	3.28±0.72	2.34±0.97	3.30±0.70
SARS-CoV-2 (Omicron)		5	2.30±0.50	5.13±0.31	3.33±0.55	6.1±1.1
MERS-CoV		50	59.1±30.8	185±119	97.4±69.7	1741±449
hCoV OC43		5	0.61±0.20	2.18±0.81	2.54±0.94	9.18±1.96
hCoV HKU1		5	1.57±0.53	5.09±2.53	3.63±3.62	3.24±1.30
hCoV NL63	Alpha	5	5.77±1.31	112±62	332±224	>2000
hCoV 229E		5	2.83±0.25	52.9±18.4	178±73	1484±568

Table 6: Biochemical potency of ALG-097558 and other 3CLpro inhibitors against 3CLpro proteins (5nM) derived from bat, cat, whale and bird coronaviruses.

Bat Coronavirus 3CLpro	Genus	Enzyme Conc. (nM)	IC_{50} (nM) (n≥3)			
			ALG-097558	Nirmatrelvir	Ibuzatrelvir	Ensitrelvir
Bat HKU4	Beta	5	1.3±0.1	2.2±0.2	1.5±0.2	16.5±0.9
Bat PDF-2180		25	7.2±1.1	18.7±2.4	20.1±4.2	160±76
Bat RsYN04		10	2.15±0.95	9.97±2.6	4.2±1.9	14.0±2.5
Bat 229E	Alpha	5	3.2±0.7	19.6±6.7	99.0±24.8	1241±658
Bat NL63		25	64.4±6.2	660±44	>2000	>2000
Cat CoV FIPV		5	2.43±0.71	25.7±8.5	41.1±1.1	>2000
Beluga whale CoV	Gamma	5	1.33±0.21	6.25±3.40	9.96±5.81	>2000
Avian Infectious Bronchitis Virus		5	2.01±0.50	50.0±27.2	38.9±10.4	1610±675

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