
# Preclinical Antiviral Profile of ALG-097558, a Novel Pan-Coronavirus 3CL Protease Inhibitor

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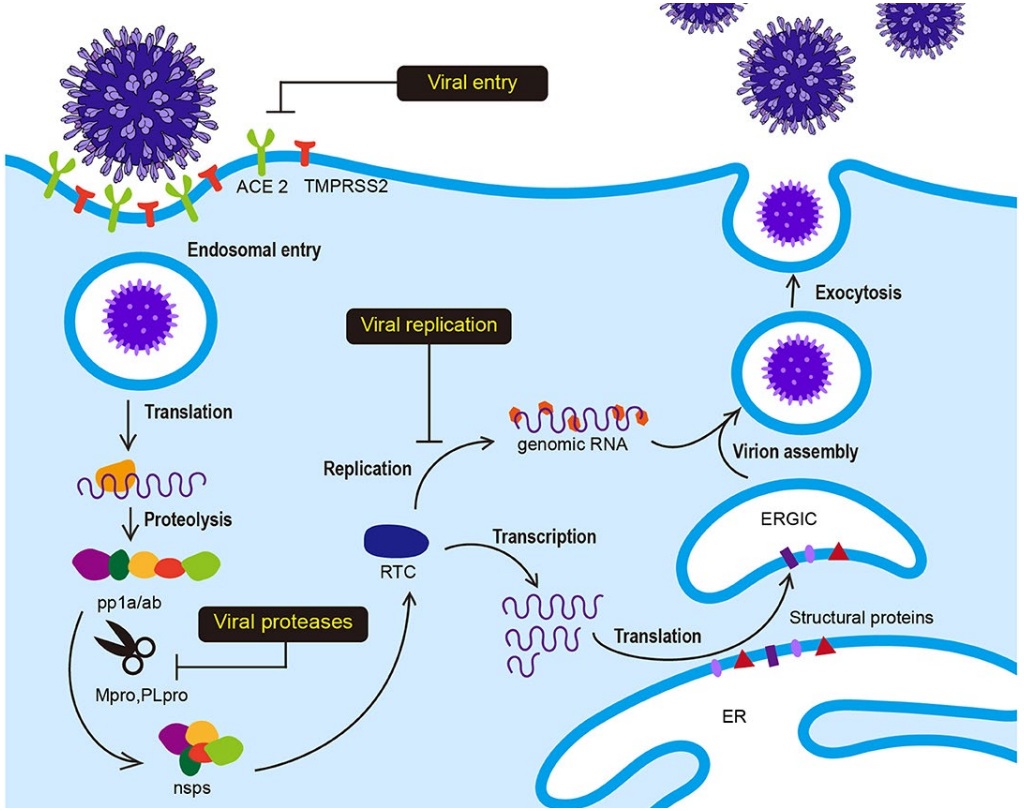
# SARS-CoV-2 3CL Protease Inhibitor Program Goals

- Collaboration established in 2020 between Aligos Therapeutics, CD3, Cistim and the Rega Institute at the KU Leuven
- Viral protease inhibitors clinically validated
  - HIV, HCV
- Key criteria
  - Orally bioavailable
  - Pan-coronavirus antiviral activity
  - Favorable resistance profile
  - No need for a pharmaco-enhancer such as ritonavir




https://www.frontiersin.org/articles/10.3389/fmicb.2020.01723/full.

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# ALG-097558
## Antiviral Activity and Selectivity in Biochemical Assays

| Compound | SARS-CoV-2 3CLpro [1] | | HRV 3C Protease $IC_{50}$ (nM) | Cathepsin L $IC_{50}$ (nM) |
|---|---|---|---|---|
| | $IC_{50}$ (nM) | $K_i$ (nM) | | |
| **ALG-097558** | **0.27** | **0.074** | **> 10,000** | **> 10,000** |
| Nirmatrelvir | 4.8 | 3.3 | > 10,000 | > 10,000 |
| Ensitrelvir | 4.0 | 2.6 | > 500 | > 10,000 |
| Pomotrelvir | 4.1 | 3.2 | > 10,000 | 1493 |

[1] Low 3CLpro enzyme concentration (0.3 nM) was used to accurately determine the Ki 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 exhibits reversible 3CLpro binding based on guanidine denaturation experiments (not shown)

ALG-097558 is a highly potent and selective inhibitor of the SARS-CoV-2 3CLpro

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# ALG-097558
## Pan-Coronavirus Activity in Cellular Assays

| Virus | Variant | $EC_{50}$ (µM) | | | |
|---|---|---|---|---|---|
| | | ALG-097558 | Nirmatrelvir | Ensitrelvir | Pomotrelvir |
| SARS-CoV-2 | 03021/2020 (Wuhan) [1] | **0.012** | 0.114 | n.d. | n.d. |
| | B.1.1.7 (alpha) [2] | **0.011** | 0.106 | 0.022 | 0.038 |
| | B.1.617.2 (delta) [2] | **0.013** | 0.217 | 0.141 | 0.126 |
| | B.1.1.529 (omicron) [1] | **0.008** | 0.069 | 0.123 | 0.152 |
| | BA.2 [1] | **0.007** | 0.045 | 0.035 | 0.137 |
| | BA.5 [1] | **0.013** | 0.089 | 0.119 | 0.215 |
| SARS-CoV-1 | Isolate Vietnam [1] | **0.022** | 0.148 | 0.150 | 0.323 |
| MERS | EMC [1] | **0.005** | 0.025 | 0.1 | >0.1 |
| β-hCoV | OC43 [3] | **0.008** | 0.047 | 0.135 | 0.168 |
| α-hCoV | 229E [4] | **0.017** | 0.502 | 6.30 | 0.281 |

Cell lines used: (1) VeroE6-eGFP or Vero76 (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.

- Bioinformatics analysis predicts retained activity against currently circulating subvariants BA.4.6, BQ1.1, BN.1, XBB1.5 and XBB.1.9.1
- ALG-097558 inhibits viral replication in a 3D human airway epithelial cell culture model with $EC_{99.9}$ values of 5.3 and 54 nM, in the absence or presence of 40% human serum, respectively

**ALG-097558 demonstrates pan-coronavirus activity in cell-based assays**

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# ALG-097558
## Preliminary Resistance Characterization

- T21I is the only mutation selected during in vitro passaging with SARS-CoV-2 B.1.1.7
  - T21I causes minor 5-fold loss in antiviral activity of ALG-097558 in cell-based assay
  - Further resistance selection experiments with B.1.1.7 and B.1.1.529 ongoing
- E166V identified as only major resistance mutation in enzymatic assay
  - Smaller fold loss compared to nirmatrelvir
  - E166V exhibits low enzymatic fitness and prevalence (0.00004%)[1,2]
  - ALG-097558 retains activity against other nirmatrelvir resistance mutations such as F140A, E166A, L167F, and H172Y (next slide)
- Antiviral testing using additional known 3CLpro resistance mutations is ongoing
- In cell-based assays, T21I, T21I+S144A, E166A/L157F and L50F/E166A/L167F cause smaller fold loss of activity for ALG-097558 than nirmatrelvir

**ALG-097558 has a favorable activity profile against selected resistance mutants**

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[1] Iketani, S Nature 2022; [2] covdb.Stanford.edu

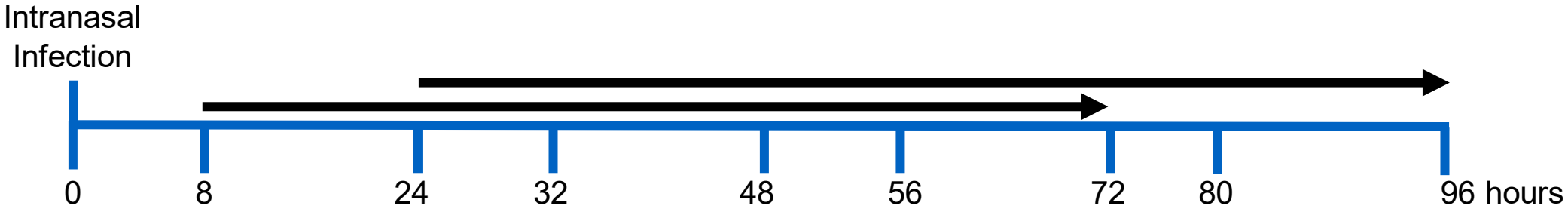
# ALG-097558
# Resistance Profile - Enzymatic Assay

| Mutation | Prevalence (%)[1] | Fold Change Compared with Wildtype (based on $IC_{50}$) | | | |
|---|---|---|---|---|---|
| | | ALG-097558 | Nirmatrelvir | Pomotrelvir | Ensitrelvir |
| T21I | 0.02 | 1 | 1 | 1 | 1 |
| T21I/S144A | <0.00008 | 2 | 5 | 3 | 5 |
| T21I/S144A/T304I | <0.00008 | 1 | 4 | 3 | 3 |
| T21I/C160F/A173V/V186A/T304I | <0.001 | 1 | 3 | 1 | 1 |
| T21I/A173V/T304I | <0.001 | 1 | 3 | 1 | 1 |
| L50F/F140L/L167F/T304I | <0.00002 | 1 | 4 | 5 | 2 |
| L50F/E166A/L167F | <0.00004 | 4 | 68 | >89 | >90 |
| P132H (Omicron) | | 2 | 1 | 1 | ND |
| F140A | <0.00001 | 1 | 3 | 5 | 4 |
| F140L/A173V | <0.00006 | 1 | 5 | 2 | 1 |
| S144A | 0.00008 | 2 | 3 | 4 | 5 |
| E166A | 0.00004 | 2 | 13 | ND | ND |
| E166V | 0.00004 | 43 | >472 | 19 | 18 |
| T21I/E166V | < 0.00004 | 32 | >472 | 11 | 8 |
| L50F/E166V | < 0.00004 | 19 | >472 | 9 | 9 |
| L167F | 0.00002 | 1 | 5 | ND | ND |
| H172Y | 0.0001 | 2 | 8 | 41 | 8 |
| A173V/T304I | <0.002 | 1 | 3 | 1 | 1 |

No shift for any of the 4 inhibitors tested: T21I,T21I/D263G, T21I/T304I, M49I/T169I, P252L, P252L/T304I

ALG-097558 retains activity against all mutations except E166V and E166V double-mutations
Smaller fold shift compared to nirmatrelvir

# ALG-097558
## SARS-CoV-2 Hamster Model: Efficacy After Therapeutic Dosing




- Hamsters infected intra-nasally with $10^4$ $TCID_{50}$ of SARS-CoV-2 B.1.617.2 (Delta)
- ALG-097558 administered PO, BID
  - Low doses of 25, 8.3 and 2.5 mg/kg to find minimal efficacious dose
  - Co-dosed with ritonavir to overcome hamster-specific metabolic instability
- Dosing start 8 or 24h post-infection
- Read-out 72 or 96h post-infection
  - Lung vRNA and infectious titer
  - Body weight
  - Lung histological assessment

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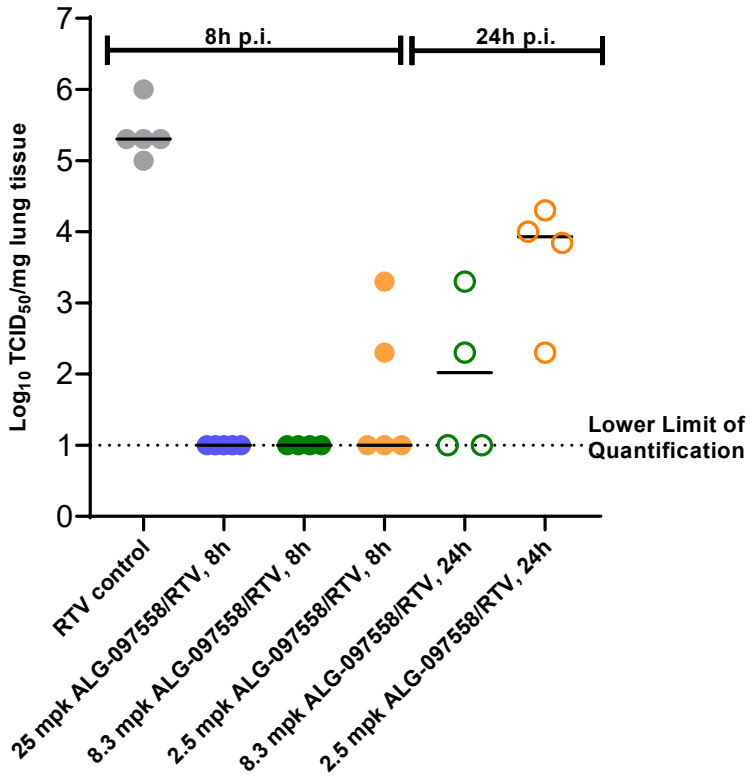
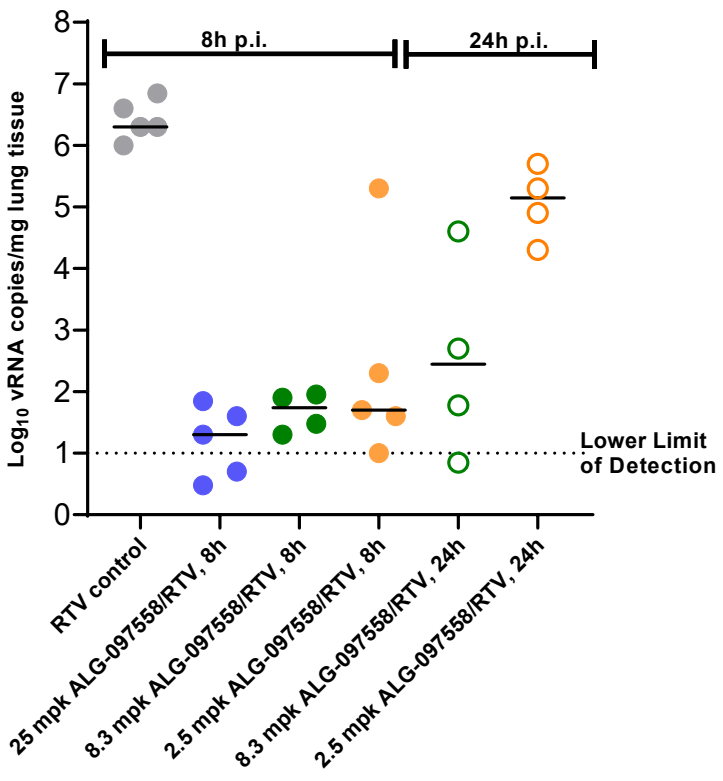
RTV administered 1 h before ALG-097558, RTV and ALG-09558 formulated as a solution in 43% Ethanol + 27% PG in water.

# ALG-097558
## SARS-CoV-2 Hamster Model: Efficacy After Therapeutic Dosing

**Lung Infectious Virus**




**Lung Viral RNA**




Significant reduction in lung vRNA and infectious virus titers after therapeutic treatment with ALG-097558, and improvements in lung histology

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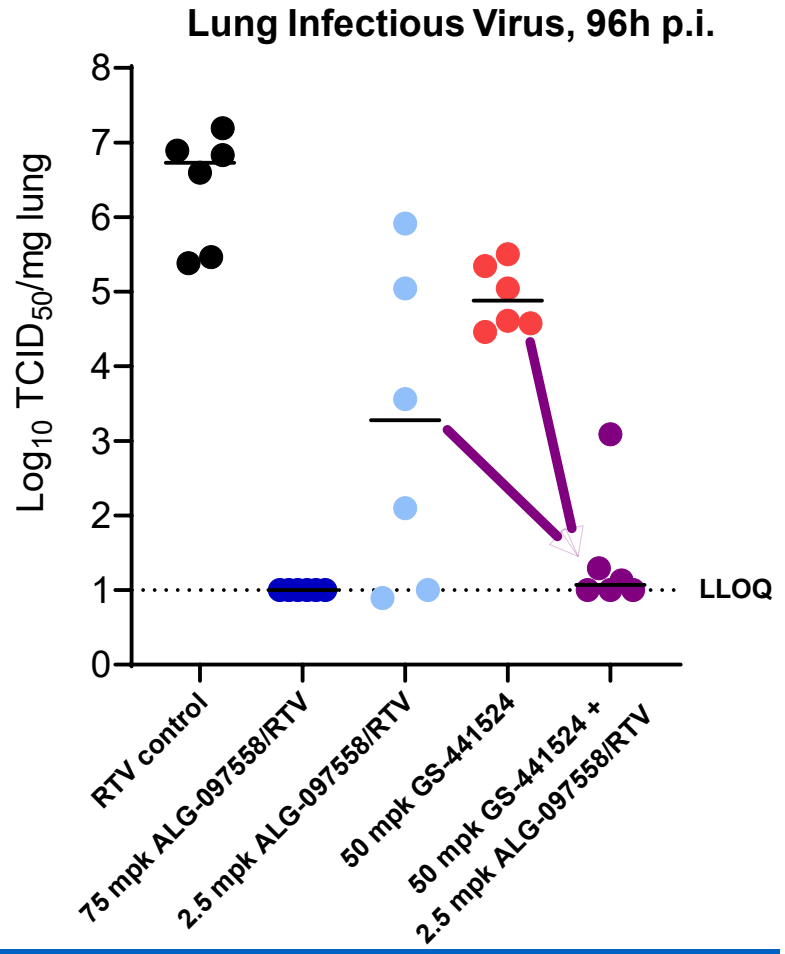
# ALG-097558 SARS-CoV-2 Hamster Model Combination with Remdesivir Parent (GS-441524)

- An intentionally selected suboptimal dose of ALG-097558 (2.5 mg/kg) combined with GS-441524, the parent of remdesivir
  - Therapeutic dosing regimen where dosing was initiated 24h post-infection
- ALG-097558 + GS-441524 resulted in a greater reduction in lung infectious titer than ALG-097558 or GS-441534 monotherapy

| Treatment Group | $Log_{10}$ Reduction of Infectious Virus |
|---|---|
| 75 mg/kg ALG-097558 | 6.7 |
| 2.5 mg/kg ALG-097558 | 3.5 |
| 50 mg/kg GS-441524 | 1.8 |
| 2.5 mg/kg ALG-097558 + GS-441524 | 5.7 |




Additive inhibition of viral replication with a combination of ALG-097558 and GS-441524

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# ALG-097558
## Summary and Outlook

- Pan-coronavirus 3CLpro inhibitor, nanomolar antiviral activity in biochemical and cellular assays
- Favorable, initial resistance profile
  - E166V only mutation causing significant (>4-fold) loss of activity in enzymatic assay
- Efficient reduction of viral replication in the SARS-CoV-2 hamster model using low, oral doses and a therapeutic dosing regimen
  - Combination of suboptimal doses of ALG-097558 and GS-441524 demonstrates additive inhibition of viral replication
- Potential for more convenient, less complex treatment regimen
  - PK profile in preclinical species predicts a projected human efficacious dose of 240-380 mg BID without ritonavir
- Phase 1 enabling activities ongoing, HV dosing to start H1 2023

**Thank you !**

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