Preclinical Evaluation of ALG-097558: A Novel, Orally Bioavailable Pan-Coronavirus 3CLpro Inhibitor for the Treatment of COVID-19

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# Overview of SARS-CoV-2 Antivirals

<table>
<thead>
<tr>
<th>Name</th>
<th>MoA</th>
<th>Approval</th>
<th>Route of Admin.</th>
<th>Pros/cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Veklury (Remdesivir)</td>
<td>RdRp Inhibitor</td>
<td>FDA full</td>
<td>IV</td>
<td>IV; low risk of resistance</td>
</tr>
<tr>
<td>Bebtelovimab</td>
<td>Mab</td>
<td>FDA EUA</td>
<td>IV</td>
<td>IV, susceptible to mutations in spike protein</td>
</tr>
<tr>
<td>Evusheld (tixagevimab + cilgavimab)</td>
<td>Mab</td>
<td>FDA EUA</td>
<td>IV</td>
<td></td>
</tr>
<tr>
<td>Paxlovid (nirmaltrelvir + ritonavir)</td>
<td>Protease Inhibitor</td>
<td>FDA EUA</td>
<td>oral</td>
<td>Strong clinical efficacy; low risk of resistance; drug-drug interactions</td>
</tr>
<tr>
<td>Lagevrio (molnupiravir)</td>
<td>RdRp inhibitor</td>
<td>FDA EUA</td>
<td>oral</td>
<td>Low risk of resistance</td>
</tr>
</tbody>
</table>

- Activity of tixagevimab + cilgavimab reduced > 600-fold against most Omicron subvariants including BQ1, BQ1.1 ¹, ²
- Paxlovid reduced risk of death or hospitalization due to Covid-19 by 89% compared to placebo ³
- Protease and polymerase inhibitors provide high variant coverage
  - Very limited clinical resistance observed

Antivirals with 3 different MoAs available

1: Evusheld fact sheet: [https://www.fda.gov/media/154701/download](https://www.fda.gov/media/154701/download)
2: Bebtelovimab fact sheet: [https://www.fda.gov/media/156152/download](https://www.fda.gov/media/156152/download)
3: Hammond J NEJM 2022; DOI: 10.1056/NEJMoa2118542
Paxlovid Retains Clinical Efficacy Against Omicron Variants

3CLpro Inhibitors such as Nirmatrelvir Retain Activity Against Omicron Variants

EPIC-HR Phase 2/3 trial
- July-Dec 2021
- Largely Delta
- No/minimal preexisting immunity

Clalit health system (Israel), retrospective study
- Jan-March 2022
- >90% Omicron
- >78% with preexisting immunity (vaccine or infection)
Need for 2nd Generation Protease Inhibitors

- Ritonavir Drug-Drug Interactions are a major limitation of Paxlovid

Do not take PAXLOVID if:
- You are allergic to nirmatrelvir, ritonavir, or any of the ingredients in PAXLOVID.
- You are taking any of the following medicines:
  - alfuzosin
  - amiodarone
  - apalutamide
  - carbamazepine
  - colchicine
  - dihydroergotamine
  - dronedarone
  - eletriptan
  - eplerenone
  - ergotamine
  - finerenone
  - flecaïnide
  - flibanserin
  - ivabradine
  - Iomitapide
  - lovastatin
  - lumacaftor/ivacaftor
  - methylergonovine
  - midazolam (oral)
  - naloxegol
  - phenobarbital
  - phenytoin
  - pimozide
  - primidone
  - propafenone
  - quinidine
  - ranolazine
  - rifampin
  - St. John's Wort (hypericum perforatum)
  - sildenafil (Revatio®) for pulmonary arterial hypertension
  - simvastatin
  - tolvaptan
  - triazolam
  - ubrogepant
  - voclosporin

- University of Liverpool Drug Interaction website https://www.covid19-druginteractions.org/

Can NOT be co-administered with many immuno-suppressants, heart-failure/hypertension medications, anti-convulsants, lipid-lowering agents and others
Program Goals

- Collaboration established in 2020 between Aligos Therapeutics, CD3, Cistim and the Rega Institute at the KU Leuven

- 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

ALG-097558
Antiviral Activity and Selectivity in Biochemical Assays

<table>
<thead>
<tr>
<th>Compound</th>
<th>SARS-CoV-2 3CLpro IC₅₀ (nM)</th>
<th>SARS-CoV-2 3CLpro Hill Slope</th>
<th>Cathepsin L IC₅₀ (nM)</th>
<th>HRV 3C Protease IC₅₀ (nM)</th>
<th>Cathepsin L IC₅₀ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALG-097558</td>
<td>0.26</td>
<td>1.99</td>
<td>0.074</td>
<td>&gt; 10000</td>
<td>&gt; 10000</td>
</tr>
<tr>
<td>Nirmatrelvir</td>
<td>2.92</td>
<td>0.91</td>
<td>2.03</td>
<td>&gt; 10000</td>
<td>&gt; 10000</td>
</tr>
<tr>
<td>PBI-0451</td>
<td>3.6</td>
<td>1.74</td>
<td>3.4</td>
<td>&gt; 10000</td>
<td>1493</td>
</tr>
</tbody>
</table>

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
**ALG-097558**

Pan-Coronavirus Activity in Cellular Assays

<table>
<thead>
<tr>
<th>Virus</th>
<th>Variant</th>
<th>EC₅₀ (µM)</th>
<th>ALG-097558</th>
<th>Nirmatrelvir</th>
<th>PBI-0451</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>ALG-097558</td>
<td>Nirmatrelvir</td>
<td>PBI-0451</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>03021/2020¹</td>
<td>0.010</td>
<td>0.116</td>
<td>n.d.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B.1.1.7 (alpha)²</td>
<td>0.012</td>
<td>0.099</td>
<td>0.038</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B.1.617.2 (delta)²</td>
<td>0.013</td>
<td>0.217</td>
<td>0.126</td>
<td></td>
</tr>
<tr>
<td></td>
<td>B.1.1.529 (omicron)¹</td>
<td>0.008</td>
<td>0.059</td>
<td>0.136</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA.2¹</td>
<td>0.003</td>
<td>0.027</td>
<td>0.087</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BA.5¹</td>
<td>0.012</td>
<td>0.075</td>
<td>0.215</td>
<td></td>
</tr>
<tr>
<td>SARS-CoV-1</td>
<td>Isolate Vietnam¹</td>
<td>0.022</td>
<td>0.173</td>
<td>0.323</td>
<td></td>
</tr>
<tr>
<td>β-hCoV</td>
<td>OC43³</td>
<td>0.009</td>
<td>0.047</td>
<td>0.168</td>
<td></td>
</tr>
<tr>
<td>α-hCoV</td>
<td>229E⁴</td>
<td>0.017</td>
<td>0.476</td>
<td>0.281</td>
<td></td>
</tr>
</tbody>
</table>

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.

- MERS testing pending; bioinformatics predicts retained activity BA.2.12.1, BA.3, BA.4, BQ.1, BQ1.1, BF.1
- 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)

ALG-097558 demonstrates pan-coronavirus activity in cell-based assays
Inhibition of SARS-CoV-2 Replication in 3D Human Airway Epithelium (HAE) Air-Liquid-Interphase (ALI) Cultures

- Human airway epithelial cells are cultured in a 3-dimensional cell culture model at the air-liquid interphase
- Infection with SARS-CoV-2 B.1.1.7 on apical side
- Test compounds are added on basolateral side
- Viral replication is assessed on day 4 post-infection via RT-qPCR
  - Allows determination of EC$_{90}$, EC$_{99}$ and EC$_{99.9}$, in presence/absence of 40% human serum
Inhibition of SARS-CoV-2 Replication in 3D Human Airway Epithelium (HAE) Air-Liquid-Interphase (ALI) Cultures

• Viral replication is assessed on day 4 post-infection via RT-qPCR, in the absence or presence of 40% human serum

ALG-097558 EC\textsubscript{99.9} of 5.3 and 54 nM, in the absence or presence of 40% human serum, respectively
Confidential

ALG-097558
Preliminary Resistance Characterization

- Resistance selection using SARS-CoV-2 B.1.1.7 ongoing; preliminary findings, to be confirmed:
  - Only slow increases in drug pressure possible, suggesting high barrier to resistance
  - Increase in drug pressure at slower pace than other 3CLpro inhibitors (e.g., nirmatrelvir)
  - Further increase in ALG-097558 concentration resulted in suppression of viral replication

- ALG-097558 retains antiviral activity against known resistance mutations in enzymatic assay
  - L50F/E166A/L167F mutant demonstrates high replication fitness and transmissibility in hamster model
  - Assessment of additional mutants ongoing
  - Confirmed with recombinant mutant viruses in cell-based experiments

<table>
<thead>
<tr>
<th>Compound</th>
<th>Biochemical Potency Fold IC$_{50}$ Compared to Wild-type (Resistance)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L50F/E166A/L167F</td>
</tr>
<tr>
<td>ALG-097558</td>
<td>3 (n=3)</td>
</tr>
<tr>
<td>Nirmatrelvir</td>
<td>66 (n=6)</td>
</tr>
<tr>
<td>PBI-0451</td>
<td>&gt; 65 (n=2)</td>
</tr>
</tbody>
</table>

- L50F/E166A/L167F mutant demonstrates high replication fitness and transmissibility in hamster model
- Assessment of additional mutants ongoing
- Confirmed with recombinant mutant viruses in cell-based experiments

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

1 Abdelnabi R 2022 doi: https://doi.org/10.1101/2022.09.28.509903
Favorable ADME Profile Allowing Advancement to Clinical Development

Low Stability in Hamster and Mouse (but NOT Other Species) Requires RTV-Boosting in Efficacy Studies
ALG-097558
SARS-CoV-2 Hamster Model: Efficacy After Prophylactic Dosing

- Intranasal infection with SARS-CoV-2 B.1.617.2 delta (1x 10^4 TCID_{50})
- Compounds dosed orally, BID for 3 days
  - Treatment start immediately before infection
  - Nirmatrelvir as positive control
- Five animals/group
- Lungs tested on day 4 for viral RNA and infectious virus
- Significant decrease in lung infectious titer
  - At LLOQ in nearly all animals
  - Confirmed with lung viral RNA read-out (not shown)

Significant reduction in lung viral RNA loads and infectious virus titers after oral treatment with ALG-097558

RTV administered 1 h before ALG-097431, RTV and ALG-097431 formulated as a solution in 43% Ethanol + 27% PG in water.
In a therapeutic setting, low doses of ALG-097558 significantly reduced lung viral RNA and infectious titer

- ~5 log\(_{10}\) vRNA reduction with 8h p.i. dosing start
- At limit of quantification for vRNA and infectious titer with 8h p.i. dosing start
- Significant reductions in vRNA and infectious titer at 24 h p.i.
- Dosing initiation as late as 24h post-infection improves body weight loss due to infection (not shown)

**Significant reduction in lung vRNA and infectious virus titers after therapeutic treatment with ALG-097558**
Projecting Efficacious Human Dose Projection

- $C_{\text{min}}$ targeted as 5x of serum-shifted EC$_{90}$ or serum-shifted EC$_{99.9}$ in the *in vitro* HAE ALI assays
- Methods validated based on comparable clearance and volume of distribution predicted by these methods vs. the parameters derived from reported human PK for nirmatrelvir

Projected efficacious human dose 370 to 600 mg BID without need for ritonavir

1 Owen DR et al., Science 374:6575:1586-93
Summary and Outlook

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

Thank you!