Oral 3CL-Protease Inhibitors for Treating Coronaviral Infections

Discovery on Target
October 20th 2022, Boston
From the Start of the SARS-CoV-2 Pandemic, SARS-CoV-2 3CLpro Represented an Attractive Target…

- Oral PIs are clinically validated (HIV/HCV)
- 3CLpro is conserved across CoV’s,
  - Pan-CoV coverage for future variants and pandemics
- Amenable to structure-based drug design
- No human homolog of 3CLpro
- Collaboration on Aligos and CD3/Rega at KU Leuven established in 2020

*SARS-CoV-2 life cycle*

*https://www.frontiersin.org/articles/10.3389/fmicb.2020.01723/full*
…Triggering Intensive 3CLpro Targeting R&D Activities

As with the unprecedented monumental effort to combat Covid-19 with Vaccines/antivirals/treatments…

Subselection of oral 3CLpro inhibitors in (pre)clinical development:

- Repurposed/purpose build
- (Non)Selective
- IV/nasal/oral administration
- (non)Covalent
- (Ir)Reversible
- QD/BID dosing
- +/- ritonavir

… a broad array of 3CLpro inhibitors were investigated and are currently in active (pre)clinical development

SARS-CoV-2 Protease Substrate and Inhibitor

SARS-CoV-2 Polyprotein Substrate

SARS-CoV-2 Protease Inhibitor

Glutamine Surrogate

Warhead
Lessons from Pre-Covid-19 3C(L) Protease Inhibitors

• Orally available 3C-inhibitors for Human Rhinovirus are described, indicating feasibility for oral administration of the P1-glutamine surrogate peptidomimetic chemotype

![Chemical Structures]

- **Rupintrivir**
  - 1999
  - Clinical stage non-PO

- **AG-7404**
  - 2005
  - Clinical stage PO

- **PF-00835231**
  - 2004-2005
  - PO F(%): <0.1-1.4% (monkey/rat)

- **PO F(%) ~20% (rat)**
  - Kansas State University

- **PF-07304814**
  - 2020
  - IV-dosing

- **PF-07321332**
  - 2021
  - PO-dosing

• P1-glutamine peptidomimetic inhibitors were described for SARS-CoV-1 (and were a steppingstone to the first clinical 3CLpro SARS-CoV-2 inhibitors)

- **SARS-CoV-1**
  - 2004-2005
  - PF-00835231

- **SARS-CoV-2**
  - 2020
  - IV-dosing
  - PF-07304814

  - 2021
  - PO-dosing
  - PF-07321332

*http://dx.doi.org/10.1016/j.antiviral.2015.11.010*
Our Effort Towards 3CLpro Inhibitors…

• Preference for designing a 3CLpro inhibitor selective over host targets, aiming for a highly efficacious pan-corona antiviral with a high tolerability.
  › Not targeting host proteases (cysteine/serine) involved in SARS-CoV-2 entry (Cathepsin/TMPRSS2)
  › Observed discrepancy between activity in enzymatic 3CL-protease activity and cellular activity on SARS-CoV-2, singling out cathepsin L inhibition as a possible culprit

• Target product profile evolved with emerging new information on the SARS-CoV-2 pandemic and 3CLpro inhibitor landscape

<table>
<thead>
<tr>
<th>ID</th>
<th>SARS-CoV-2</th>
<th>Cathepsin L</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>3CLpro IC₅₀ (µM)</td>
<td>Vero E6 + CP EC₅₀ (µM)</td>
</tr>
<tr>
<td>Cpd A1</td>
<td>0.58</td>
<td>0.094</td>
</tr>
<tr>
<td></td>
<td>SI = 481</td>
<td>SI = 2380</td>
</tr>
<tr>
<td>Cpd 11r</td>
<td>1.2</td>
<td>0.074</td>
</tr>
<tr>
<td></td>
<td>SI = 451</td>
<td>SI &gt; 1000</td>
</tr>
</tbody>
</table>

10 human proteases tested: inhibiting Cathepsin L/B and calpain
SARS-CoV-2 Protease Inhibitor Screening Cascade

In vitro antiviral potency and selectivity

Structure based drug design

3CL Protease IC\textsubscript{50}
Cathepsin L selectivity
SARS-CoV-2/OC43 cell-based EC\textsubscript{50}
Cell Toxicity CC\textsubscript{50}
In vitro ADME

SARS-CoV-1/229E/SARS-CoV-2 variants
In vivo rodent IV/PO PK, SARS-CoV-2 ALI assay

Biochemical Cathepsin L assay early in the screening cascade

Hamster efficacy study Tox Profiling

Candidate

ADME Tox and DMPK Animal efficacy

Medicinal Chemistry LLO
Early Reported SARS-CoV-2 3CLpro Inhibitors are also Cathepsin L Inhibitors

<table>
<thead>
<tr>
<th>compound</th>
<th>Cpd 11a</th>
<th>6j</th>
<th>6e</th>
<th>Cpd 13b</th>
<th>Cpd 11r</th>
<th>A9</th>
<th>PF-00835231</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cathepsin L IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td>0.21</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>290</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>168</td>
</tr>
<tr>
<td>SARS-CoV-2 3CLpro IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td>9</td>
<td>8</td>
<td>10</td>
<td>472</td>
<td>1154</td>
<td>4891</td>
<td>5</td>
</tr>
<tr>
<td>HRV 3Cpro IC&lt;sub&gt;50&lt;/sub&gt; (nM)</td>
<td>9</td>
<td>170</td>
<td>90</td>
<td>2590</td>
<td>58</td>
<td>294</td>
<td>1675</td>
</tr>
<tr>
<td>SARS-CoV-2 Huh-7 EC&lt;sub&gt;50&lt;/sub&gt; (nM)*</td>
<td>54</td>
<td>53</td>
<td>242</td>
<td>3676</td>
<td>1</td>
<td>&lt;0.5</td>
<td>952</td>
</tr>
</tbody>
</table>

*EC<sub>50</sub> E64D: 80 nM; Z-FA-FMK <50 nM
Impact of Cathepsin L and 3CLpro Activity on SARS-CoV-2 Inhibition in Huh-7 Cells

High cellular SARS-CoV-2 potency was observed for two types of compounds:

1) **Desired profile**: Highly selective and potent 3CLpro compounds

2) **Undesired profile**: Cathepsin L inhibitors show high potency in Huh-7, independent of their 3CLpro activity → results in overestimation of cellular potency

Unselective inhibitors all have:
1) P2:

   ![Chemical structure](image)

2) Aldehyde or ketoamide warhead

Cathepsin L inhibitors show high potency against SARS-CoV-2 in Huh-7, independent of 3CLpro activity

Highly selective and potent 3CLpro compounds also show good potency in Huh-7
Biochemical Activity ALG-097111 and ALG-097070 vs. PF-00835231 and GC-376

<table>
<thead>
<tr>
<th></th>
<th>PF-00835231</th>
<th>GC-376</th>
<th>ALG-097111</th>
<th>ALG-097070</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC₅₀ (µM)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3CLpro</td>
<td>0.005</td>
<td>0.025</td>
<td>0.007</td>
<td>0.010</td>
</tr>
<tr>
<td>HRV3C</td>
<td>1.82</td>
<td>0.042</td>
<td>&gt;2</td>
<td>0.049</td>
</tr>
<tr>
<td>Cathepsin L</td>
<td>0.155</td>
<td>&lt;0.0005</td>
<td>&gt;10</td>
<td>1.63</td>
</tr>
</tbody>
</table>

Higher SARS-CoV-2 3CLpro selectivity over CatL observed for ALG-097111 and ALG-097070
SARS-CoV-2 Hamster Model with ALG-097111 and ALG-097200

ALG-097111
SARS-CoV-2 (A549+ACE2): EC₅₀ = 200 nM

ALG-097200
Prodrug of ALG-097070
SARS-CoV-2 (A549+ACE2): EC₅₀ = 757-1122 nM

ALG-097111 and ALG-097200 were dosed SC in a SARS-CoV-2 Hamster model in a prophylactic setting with molnupiravir as control.

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Treatment</th>
<th>mg/kg/dose</th>
<th>Dosing Frequency</th>
<th>Dose route</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>5</td>
<td>Vehicle/RTV</td>
<td>50</td>
<td>BID</td>
<td>SC/PO</td>
</tr>
<tr>
<td>Group 2</td>
<td>5</td>
<td>Molnupiravir</td>
<td>200</td>
<td>BID</td>
<td>PO</td>
</tr>
<tr>
<td>Group 3</td>
<td>5</td>
<td>ALG-097111/RTV</td>
<td>200/50</td>
<td>BID/BID</td>
<td>SC/PO</td>
</tr>
<tr>
<td>Group 4</td>
<td>5</td>
<td>ALG-097200/RTV</td>
<td>200/50</td>
<td>BID/BID</td>
<td>SC/PO</td>
</tr>
</tbody>
</table>
SARS-CoV-2 Hamster Model with ALG-097111 and ALG-097200

• ALG-097111 and ALG-097200 significantly reduced infectious virus titers in the lungs of treated hamsters

• Plaque assay confirmed with reduction in viral RNA loads (not shown)

• Hamster plasma and lung $C_{\text{trough}}$ (based on PK in uninfected hamster):
  
  ALG-097111: 5.5 and 3-fold SARS-CoV-2 A549-ACE2 EC$_{50}$
  ALG-097070: 7-10 and 11-16 fold SARS-CoV-2 A549-ACE2 EC$_{50}$

• Molnupiravir reduced the infectious virus titers by 3.3 log with some variations among treated hamsters.

• ALG-097111 and ALG-097200 were tool-compounds and optimization continued to improve oral availability and potency, resulting in the selection of **ALG-097558**

First report of SARS-CoV-2 inhibition in SARS-CoV-2 hamster model for a selective protease inhibitor
ALG-097558, a reversible covalent binder, $K_i$ is 27-46 fold more potent vs. competitors in the 3CLpro biochemical assay.

<table>
<thead>
<tr>
<th>SARS-CoV-2 3CLpro</th>
<th>$IC_{50}$ (nM)$^1$</th>
<th>Hillslope</th>
<th>$K_i$ (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nirmatrelvir</td>
<td>2.92</td>
<td>0.91</td>
<td>2.03</td>
</tr>
<tr>
<td>PBI-0451</td>
<td>3.6</td>
<td>1.74</td>
<td>3.4</td>
</tr>
<tr>
<td>Ensitrelvir</td>
<td>4.0</td>
<td>1.31</td>
<td>2.6</td>
</tr>
<tr>
<td>ALG-097558</td>
<td>0.26</td>
<td>1.99$^2$</td>
<td>0.074</td>
</tr>
</tbody>
</table>

$^1$The assays were performed with 0.3 nM 3CLpro.
$^2$High hillslope (>1.5) is indicative of enzyme titration.
**ALG-097558**
Superior Cell-Based Potency Against SARS-CoV-2 and CoV

<table>
<thead>
<tr>
<th>Virus</th>
<th>Variant/Cell line</th>
<th>EC$_{50}$ (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PBI-0451</td>
</tr>
<tr>
<td>SARS-CoV-2</td>
<td>Wuhan Vero E6+CP</td>
<td>n.d.</td>
</tr>
<tr>
<td></td>
<td>B.1.1.7 (alpha) A549-ACE2-TMPRSS2</td>
<td>0.038</td>
</tr>
<tr>
<td></td>
<td>B.1.617.2 (delta) A549-ACE2-TMPRSS2</td>
<td>0.126</td>
</tr>
<tr>
<td></td>
<td>B.1.1.529 (omicron) VeroE6-GFP+CP</td>
<td>0.136</td>
</tr>
<tr>
<td>SARS-CoV-1</td>
<td>Vero-E6+CP</td>
<td>0.323</td>
</tr>
<tr>
<td>OC43 (β-hCoV)</td>
<td>HeLa</td>
<td>0.168</td>
</tr>
<tr>
<td>229E (α-hCoV)</td>
<td>Huh-7</td>
<td>0.281</td>
</tr>
</tbody>
</table>

**Beta**

**Alpha**

ALG-097558 demonstrates pan-coronavirus antiviral activity
ALG-097558 is more active than PF-07321332, PBI-0451 and S-217622 across all CoV’s tested
ALG-097558
Oral Prophylactic Treatment in the SARS-CoV-2 Hamster Model

5 Hamsters / group, intranasal infection, B.1.617.2 (1 x 10^4 TCID_{50}). Treatment started before infection, administered by oral gavage BID over 3-days. RTV administered 1 h before the ALG cpd, RTV and ALG-097558 formulated as a solution in 43% Ethanol + 27% PG in water.

Sacrifice and assessment of infectious virus in lung at day 4.

Significant reduction in infectious virus titers after oral prophylactic treatment with ALG-097558. Use of ritonavir is only needed in the hamster model.
ALG-097558
Oral Therapeutic Treatment in the SARS-CoV-2 Hamster Model

Amount of Infectious Virus at Day 4

Significant reduction in infectious virus titers after oral therapeutic treatment with ALG-097558
Use of ritonavir is only needed in the hamster model

Dosing (BID)
- Ritonavir (RTV), 50 mg/kg
- RTV/ALG-097558, 50/75 mg/kg
- RTV/ALG-097558, 50/25 mg/kg
- RTV/ALG-097558, 50/75 mg/kg

Treatment started before or after infection as indicated, administered by oral gavage BID over 3-days.
RTV administered 1 h before the ALG cpd, RTV and ALG-097558 formulated as a solution in 43% Ethanol + 27% PG in water.
Sacrifice and assessment of infectious virus in lung at day 4.
Summary and Next Steps

• Collaboration with KU Leuven/Rega Institute/CD3, resulted in the identification of highly potent and selective 3CL protease inhibitors with broad anti-Coronaviral activity

• **ALG-097111** and **ALG-097200** were used as tool compounds to confirm *in vivo* activity of selective SARS-CoV-2 3CLpro inhibitors in a hamster model.

• **ALG-097558**
  › Shows high potency *in vitro* in biochemical and cell-based assays
  › High potency *in vivo* in a SARS-CoV-2 hamster model (prophylactic and therapeutic oral dosing)
  › Broadly active against a diverse range of coronaviruses
  › PK profile in preclinical species predicts a projected human oral efficacious dose of 350-600 mg BID without ritonavir
  › Phase 1 enabling nonclinical studies ongoing, Phase 1: H1 2023
Thank you!

Professor Johan Neyts
Rega Institute
KU Leuven

Centre for Drug Design and Discovery (“CD3”)

ALIGOS THERAPEUTICS