Dual Inhibition of SARS-CoV-2 and Human Rhinovirus with Protease Inhibitors in Clinical Development

Jerome Deval, March 24th, 2022
Disclosures

This presentation contains forward-looking statements within the meaning of the Private Securities Litigation Reform Act of 1995. All statements other than statements of historical facts contained in this presentation, including without limitation statements regarding our future plans and goals in the research and development of our current or future drug candidates, are forward-looking statements. These statements involve known and unknown risks, uncertainties and other important factors that may cause our actual results, performance or achievements to be materially different from any future results, performance or achievements expressed or implied by the forward-looking statements. Because forward-looking statements are inherently subject to risks and uncertainties, some of which cannot be predicted or quantified and some of which are beyond our control, you should not rely on these forward-looking statements as predictions of future events. The events and circumstances reflected in our forward-looking statements may not be achieved or occur and actual results could differ materially from those projected in the forward-looking statements. Except as required by applicable law, we do not plan to publicly update or revise any forward-looking statements contained herein, whether as a result of any new information, future events, changed circumstances or otherwise. For a further description of the risks and uncertainties that could cause actual results to differ from those anticipated in these forward-looking statements, as well as risks relating to the business of Aligos Therapeutics in general, see Aligos’ Annual Report on Form 10-K filed with the Securities and Exchange Commission on March 10, 2022, and its future periodic reports to be filed with the Securities and Exchange Commission. Except as required by law, Aligos Therapeutics undertakes no obligation to update any forward-looking statements to reflect new information, events or circumstances, or to reflect the occurrence of unanticipated events.

Except where otherwise indicated, the information contained in this presentation speaks as of the date hereof or as of the date at which such information is expressed to be stated, as applicable, and such information may express preliminary estimated, unaudited results which shall be subject to audit or other year-end adjustments and such audited or adjusted results may materially differ from those contained in this presentation.

This presentation concerns drug candidates, some of which are undergoing nonclinical studies and others of which are under clinical investigation, and all of which have not yet been approved for marketing by the U.S. Food and Drug Administration. These drug candidates are currently limited by federal law to investigational use, and no representation is made as to their safety or effectiveness for the purposes for which they are being investigated.
SARS-CoV-2 3CLpro Represents an Attractive Target

- Oral PIs are clinically validated (HIV/HCV)
- Main protease (3CLpro) is conserved across CoVs and picornaviruses
  - Pan-CoV coverage makes target attractive for possible future pandemics
- Emerging SARS-CoV-2 variants (alpha, beta, gamma, delta, lambda, omicron)
  - Are likely susceptible to 3CLpro inhibitors due to very few relevant mutations/polymorphism
- No human homolog of 3CLpro
Oral SARS-CoV-2 3CLpro Inhibitors in Development

Preclinical:
- **Nirmatrelvir**
  - Pfizer
  - (Nirma. / PF-07321332)
  - PAXLOVID™
  - (Nirma. + ritonavir)
  - Oral BID dosing

Clinical:
- **EDP-235**
  - Enanta
  - QD

- **PBI-0451**
  - Pardes Bio
  - QD/BID

Preclinical:
- **STI-1558**
  - Sorrento
  - No ritonavir

- **SH-879**
  - Sosei Heptares
  - Grant funding from Welcome trust

- **EDDC-2214**
  - Everest medicines
  - Singapore EDDC

- **ASC-11**
  - Asceltis
  - With ritonavir

Approved in the US and Europe
- **Nirmatrelvir**
  - Pfizer
  - (Nirma. / PF-07321332)
  - PAXLOVID™
  - (Nirma. + ritonavir)
  - Oral BID dosing

Filed for approval in Japan
- **Ensitrelvir**
  - Shionogi
  - Xocova™
  - (S-217622)
  - Oral QD dosing

Desired profile for a best-in-class:
1) QD/BID drug orally bioavailable without ritonavir
2) Broad pan-CoV spectrum, including resistance mutations and circulating variants
Coronavirus PI Collaboration: CD3/Rega at KU Leuven

Centre for Drug Design and Discovery ("CD3")

Goal: to identify potent, selective coronavirus and rhinovirus PIs

Professor Johan Neyts
Rega Institute
KU Leuven
Comparison Between Standard FRET and SAMDI-MS Assay

Advantages of MS assay: 1) higher sensitivity, 2) lower propensity for false positive, and 3) can be multiplexed
Next Step: Multiplex the Assay at Low Enzyme Concentration

- Monitoring the activity of SARS-CoV-2 3CLpro and human rhinovirus (HRV) 3C simultaneously
- Standard assay condition: 3 nM 3CLpro and 6 nM HRV3C (384-well plate)

Advantage: dual readout on SARS-CoV-2 and human rhinovirus proteases for all tested compounds
Compound Selectivity Profile

- **Rupintrivir**
  - SELECTIVE (rhinovirus)
  - cathepsin L -

- **GC376**
  - NON-SELECTIVE
  - cathepsin L +

- **PF-00835231**
  - SELECTIVE (SARS-CoV-2)
  - Weak cathepsin L inhibitor

---

**GC376 is non-selective: it inhibits both viral proteases as well as human cathepsin L**

Optimization for Protease Selectivity

Compounds were optimized to maximize ligand occupancy beyond covalent binding of warhead to cysteines

Rupintrivir

PF-00835231

HRV3C protease (PDB 1CQQ)

SARS-CoV-2 3CLpro (PDB 6XMH)

Enzyme Titration with Tight Binding Inhibitors

At 3 nM 3CLpro: risk of under-predicting compound potency with tight binding inhibitors

<table>
<thead>
<tr>
<th></th>
<th>SARS-CoV-2 3CLpro IC_{50} (nM)</th>
<th>SARS-CoV-2 3CLpro Hillslope</th>
<th>HRV 3C Protease IC_{50} (nM)</th>
<th>Cathepsin L IC_{50} (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nirma.</td>
<td>17.9 ± 6.6</td>
<td>1.25*</td>
<td>&gt; 10000</td>
<td>&gt; 10000</td>
</tr>
<tr>
<td>PF-00835231</td>
<td>5.48 ± 1.78</td>
<td>1.70*</td>
<td>1675 ± 1000</td>
<td>172 ± 134</td>
</tr>
</tbody>
</table>

* High hillslope (>1) is indicative of enzyme titration.
Further Reducing Enzyme Concentration

- Lower 3CLpro enzyme (from 3 to 0.3 nM) provides a more sensitive assay that was developed to address the issue of enzyme titration with very potent compounds (IC₅₀ < 10 nM)

**Nirmatrelvir**
(Nirma. / PF-07321332)

\[
\text{IC₅₀ (nM) = 18 nM, } \text{Hillslope = 1.25} \\
\text{IC₅₀ (nM) = 2.9 nM, } K_i = 2.03 \text{ nM, Hillslope = 0.91}
\]

Using low protease concentration is needed to accurately assess the potency of tight binding inhibitors
A Few Words About Resistance to 3CLpro Inhibitors

- Resistance selection performed at Rega/KU Leuven with Compound-1 (Early Lead)
- Passaging of SARS-CoV-2 in VeroE6 cells in the presence of increasing concentrations of Compound-1 resulted in the selection of mutations at amino acids 50, 166 and 167

More details shown in poster

Liu C. et al., Abstract 214

Passaging of SARS-CoV-2 with compound-1 resulted in selection of 3 amino acid mutations
Effect of Mutations on Resistance to 3CLpro Inhibitors

The triple mutant conferred resistance to compound-1 and nirmatrelvir, but not to ‘231
Summary and Conclusion

• The need to develop improved 2nd generation inhibitors of 3CLpro remains high

• Robust and sensitive biochemical assays are key to differentiate inhibitors during drug discovery

• Here, we described a novel biochemical assay with dual readout for SARS-CoV-2 and rhinovirus proteases
  – Validated with known selective inhibitors of each enzyme
  – More sensitive and less prone to false positives than the FRET format

• Picomolar concentration of 3CLpro was required to avoid enzyme titration and underprediction of tight binding inhibitor potency

Our next goal: to understand the activity profile of 3CLpro inhibitors against all major resistance mutations and circulating variants
Thank you!