ALN-COV: An Investigational RNAi Therapeutic for COVID-19

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Disclosures

• Employee of Alnylam Pharmaceuticals
ALN-COV, an Investigational RNAi Therapeutic for COVID-19

Provides a unique and distinct antiviral strategy for the treatment of COVID-19
- RNAi mechanism of action results in degradation of SARS-CoV-2 viral RNA genome

Direct administration of ALN-COV to lungs, the key site of viral replication and disease manifestations
- Early delivery of a potent antiviral agent directly to the site of replication has the potential to prevent progression to severe pulmonary disease and decrease time to clinical recovery

Initial development plan focused on the treatment of patients with mild to moderate COVID-19
- Potential for development in targeted prophylactic setting

IND filing planned for around year-end 2020
Chemistry Advances Enable Robust Tissue Distribution with Potent and Durable Gene Knockdown in Lung

Surrogate siRNA targeting endogenous lung target (Sod1)

![Sod1 mRNA Reduction](image)

Dose-dependent Sod1 mRNA reduction in lung following single-doses of Sod1 siRNA at 1 and 10mg/kg

![Sod1 mRNA Over Time](image)

Sustained Sod1 mRNA reduction in lung following a single 10mg/kg dose

**Sod1 siRNA Distribution in the Mouse Lung Measured by IHC**

(A) PBS-treated animal on Day 10 post dose. (B) 10 mg/kg Sod1 siRNA on Day 10 post dose. siRNA is magenta. Blue is hematoxylin counterstain.

Robust bronchiolar and alveolar uptake of siRNA in lung histological sections by immunohistochemistry.
Development Candidate Selection
Targeting SARS-CoV-2 genome

- 350 candidate sequences fully conserved in SARS-CoV and SARS-CoV-2 identified by Alnylam; Alnylam team synthesized and tested in vitro using a reporter system.
- 100 top candidates transferred to Vir for testing in the in vitro SARS-CoV-2 infectious system using three concentrations and two different readouts.
- EC\textsubscript{50} evaluation of top 24 sequences against infectious SARS-CoV-2.
- Narrowed down to 5 sequences based on potency, specificity, sequence conservation, and manufacturability.

ALN-COV (VIR-2703)
SARS-CoV-2 Genome Coverage and Activity

~100 siRNAs with ≥80% KD at 10 nM in two luciferase reporter vectors tested

- Graph represents the knockdown (KD) values of each individual duplex.
- Top leads (in black) were selected from duplexes that had ≥80% KD in both luciferase reporter vectors tested.
SARS-CoV-2 Live Virus Assay

Vero E6 cells

-24 h

siRNA reverse transfection

0

Infect with SARS-CoV-2 MOI (0.001 or 0.01)

4 h

wash off virus

24h or 48h

RT-qPCR of supernatant
IFA (CoV-2 nucleoprotein)
In-cell ELISA (CoV-2 nucleoprotein)

controls:
- uninfected
- mock transfected + SARS-CoV-2
- siRNA(luciferase) + SARS-CoV-2

uninfected

infected

SARS-CoV-2 nucleoprotein

RT-qPCR

CoV-2 genomes/ml
Top 24 siRNAs from ~100 screened in live virus in vitro assay

- Screening of 100 siRNAs against live SARS-CoV2 infection led to choosing of 24 candidates for further assessment
ALN-COV Comprises Two siRNAs from Top 5 Candidates

Two siRNAs utilized to mitigate risk of viral escape

Top 5 Candidates (qPCR)

<table>
<thead>
<tr>
<th>siRNA</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; EC&lt;sub&gt;95&lt;/sub&gt; (pM; PCR)</th>
<th>EC&lt;sub&gt;50&lt;/sub&gt; EC&lt;sub&gt;95&lt;/sub&gt; (pM; IFA)</th>
<th>Genome Reactivity* (0 mm)</th>
<th>Genome Reactivity* (1 mm)</th>
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<tbody>
<tr>
<td>siRNA 1</td>
<td>42 1183</td>
<td>66 763</td>
<td>99.91%</td>
<td>100.00%</td>
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<tr>
<td>siRNA 2</td>
<td>86 702</td>
<td>118 608</td>
<td>99.89%</td>
<td>99.98%</td>
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*N=4386 genomes analyzed
**Study Design: Efficacy of ALN-COV in SARS-CoV-2 Hamster Model**

**Hamster model supports SARS-CoV-2 infection**

- Extensive homology to human ACE2 receptor; resembles the manifestations of upper and lower respiratory tract infection in humans
- Rapid loss of body weight gain provides key readout in addition viral titers

**ALN-COV Hamster study Design**

**Pre-treatment**

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<thead>
<tr>
<th>Days:</th>
<th>-3</th>
<th>0</th>
<th>2</th>
<th>4</th>
<th>7</th>
<th>14</th>
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<tbody>
<tr>
<td>ALN-COV treatment (low or high dose)</td>
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<td>SARS-CoV-2 infection</td>
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<td>Lung and trachea harvest</td>
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<tr>
<td>Final bodyweight measurement</td>
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**Co-treatment and infection**

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*Clinical Infectious Diseases, ciaa325, https://doi.org/10.1093/cid/ciaa325*
Target Indication for the Treatment of Mild to Moderate COVID-19

Intervention early during viral response phase of disease

Adapted from HK Siddiqi et al., JHLT 2020
Current Clinical Development Plan

Treatment of adult and adolescent patients with mild to moderate COVID-19

**Phase 1 Study**
Healthy Volunteers  
N~40

**Objective**: Establish safety and select dose of ALN-COV

**Study Design**: Randomized, single-blind, placebo-controlled, ascending dose study in healthy volunteers (HVs)

**Dose Regimen**: Once daily x 2 days inhalation administration via nebulizer

**Randomization**: 3:1, ALN-COV : placebo

**Endpoints**: Safety, tolerability, and pharmacokinetics (PK)

**Phase 2/3 Study**
Mild to Moderate COVID-19  
N~600

**Objective**: Evaluate efficacy and safety of ALN-COV

**Study Design**: Randomized, double-blind, placebo-controlled study in patients with mild to moderate COVID-19 with risk factors for severe disease

**Dose Regimen**: Once daily x 2 days inhalation administration via nebulizer

**Randomization**: 1:1, ALN-COV+SOC : placebo+SOC

**Endpoints**: Progression to severe pulmonary disease, hospitalization, mechanical ventilation, symptom resolution, disease severity, mortality, viral parameters, safety, and PK
To those who say “impossible, impractical, unrealistic,” we say:

CHALLENGE ACCEPTED