Human Translation of GalNAc-siRNA Conjugates with Improved Specificity

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Multiple Human POCs Demonstrate Reproducible and Modular Nature of ESC Conjugate Platform

1. Fitusiran
2. Inclisiran
3. Givosiran
4. Lumasiran
5. Vutrisiran
6. Cemdisiran
7. ALN-AAT
8. ALN-AAT02
9. ALN-AGT
10. ALN-HBV02 (VIR-2218)
Inclisiran: Investigational RNAi Therapeutics Targeting PCSK9

Study Conducted by The Medicines Company; Acquired by Novartis International AG

ORION-11: Efficacy
Potent, consistent response to inclisiran

Individual patient responses contributing to primary endpoint – 17 months
Inclisiran: Investigational RNAi Therapeutics Targeting PCSK9

Study Conducted by The Medicines Company: Acquired by Novartis International AG

ORION-11: Safety and tolerability

No evidence of liver, kidney, muscle or platelet toxicity

<table>
<thead>
<tr>
<th>Laboratory tests</th>
<th>Placebo</th>
<th>Inclisiran</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N = 804</td>
<td>N = 811</td>
</tr>
<tr>
<td>Liver function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ALT &gt;3x ULN</td>
<td>4 (0.5%)</td>
<td>4 (0.5%)</td>
</tr>
<tr>
<td>AST &gt;3x ULN</td>
<td>4 (0.5%)</td>
<td>2 (0.2%)</td>
</tr>
<tr>
<td>ALP &gt;2x ULN</td>
<td>2 (0.5%)</td>
<td>1 (0.1%)</td>
</tr>
<tr>
<td>Bilirubin &gt;2x ULN(^3)</td>
<td>8 (1.0%)</td>
<td>6 (0.7%)</td>
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<tr>
<td>Kidney function</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Creatinine &gt;2 mg/dL</td>
<td>11 (1.4%)</td>
<td>5 (0.6%)</td>
</tr>
<tr>
<td>Muscle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CK &gt;5x ULN</td>
<td>9 (1.1%)</td>
<td>10 (1.2%)</td>
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<tr>
<td>Hematology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Platelet count &lt;75x10^9/L</td>
<td>1 (0.1%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

1. Safety population includes all patients who received at least 1 dose of study medication  
2. Patients may be counted in more than one category  
3. No cases met H Ny’s Law

- Inclisiran safety profile similar to placebo, with no adverse changes in laboratory markers
- Injection site events 4.2% - predominantly mild and none persistent
- Numerically fewer CV events reported for inclisiran than placebo (exploratory endpoint)
Characteristics of the Human LFT Signal with Subset of ESC Conjugates: Does Not Occur Across All Programs, Suggesting Sequence Specificity

ALN-AAT01 Phase 1/2 Interim Results

- **Anecdotal evidence for RISC-mediated mechanism:**
  - Onset of LFT elevations coincides with onset of maximum RNAi activity
  - High knockdown appears necessary (but not sufficient) for LFT elevations
- **Similar profile seen in other programs with sporadic LFT elevations**
Subset of ESC Conjugates Show Rat Hepatotoxicity at Exaggerated Doses

In silico prediction & In vitro efficacy

In vitro screen for predicted off-targets

Rodent Knockdown

Rat Tox @ >100x PD dose

NHP Knockdown

DC

No hepatotoxicity (60%)

Show hepatotoxicity (40%)

Single cell necrosis and/or hepatocellular degeneration with ↑LFT 2x upper limit of normal

These compounds drop out of DC selection process
Seed-Based Off-Target Effects Are Important Drivers of Rodent Hepatotoxicity for Subset of ESC Conjugates

1. **Non-RNAi effects**
e.g. siRNA chemistry, metabolites, protein binding, drug accumulation

2. **Competition for RISC loading with miRNAs**

3. **Undesired seed-based off-target activity**

   - Partial sequence match
   - Off-target binding

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**ARTICLE**

**Selection of GalNAc-conjugated siRNAs with limited off-target-driven rat hepatotoxicity**


Janas, Schlegel et al. Nat Commun. 2018
ESC+ Seed Pairing Destabilization Strategy Improved Specificity and Therapeutic Index in Rats

Important Considerations
1. On-target potency must be maintained in vivo
2. Off-target activity should be minimized

Rat Liver Histopath for 30 mg/kg dose

How would ESC+ design translate in humans?
- Evaluated ESC+ versions of ALN-HBV and ALN-AAT01

Bramslen et. al. Nucleic Acids Res. 2010
Vaish et. al. Nucleic Acids Res. 2011
Lee et. al. Nat. Comm. 2015
Janas, Schlegel et al. Nat. Comm. 2018
Improved Specificity of RNAi Activity by VIR-2218

RNA-Seq analysis in HepG2.2.15 cells showed fewer differentially expressed genes and a lower magnitude of gene dysregulation, supporting reduced off-target effects with VIR-2218 compared with ALN-HBV.
VIR-2218-1001: Phase 1/2 study design

<table>
<thead>
<tr>
<th>SAD</th>
<th>Healthy volunteers</th>
<th>50 mg SC</th>
<th>100 mg SC</th>
<th>200 mg SC</th>
<th>400 mg SC</th>
<th>600 mg SC</th>
<th>900 mg SC</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>1 dose</td>
<td>1 dose</td>
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<table>
<thead>
<tr>
<th>MAD</th>
<th>Chronic HBV HBeAg−</th>
<th>20 mg SC</th>
<th>50 mg SC</th>
<th>100 mg SC</th>
<th>200 mg SC</th>
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<tbody>
<tr>
<td></td>
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<td>x 2 doses</td>
<td>x 2 doses</td>
<td>x 2 doses</td>
<td>x 2 doses</td>
</tr>
<tr>
<td></td>
<td></td>
<td>n=4</td>
<td>n=8</td>
<td>n=8</td>
<td>n=4</td>
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<tr>
<th>MAD</th>
<th>Chronic HBV HBeAg+</th>
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<tr>
<td></td>
<td>50 mg SC x 2 doses</td>
</tr>
<tr>
<td></td>
<td>n=4</td>
</tr>
<tr>
<td></td>
<td>200 mg SC x 2 doses</td>
</tr>
<tr>
<td></td>
<td>n=4</td>
</tr>
</tbody>
</table>

Double-blind, randomized, placebo-controlled, MAD study in patients with chronic HBV infection. At each dose level, 4 or 8 patients randomized 3 active:1 placebo

HBeAg, hepatitis B e antigen; MAD, multiple ascending dose; SAD, single ascending dose.
VIR-2218-1001: Phase 1 SAD (Part A, healthy volunteers): ALT

ALT (IU/mL)

Placebo

VIR-2218

50 mg  100 mg  200 mg  400 mg  600 mg  900 mg

Week

D1 4 8 12  D1 4 8 12  D1 4 8 12  D1 4 8 12  D1 4 8 12  D1 4 8 12

0 50 100 150 200

3x ULN

ULN

0 50 100 150 200
Human: Treatment-emergent post-baseline ALT elevations in healthy volunteers with normal ALT at baseline

- No post-baseline ALT elevations to >ULN in the VIR-2218 or ALN-HBV cohorts were associated with increases in bilirubin >ULN.
- No changes in functional status of the liver (e.g., albumin, coagulation parameters) or clinical signs/symptoms of hepatic dysfunction were observed in any ALN-HBV- or VIR-2218-treated patient.

*Approximate mg/kg dose based on an average adult weight of 60 kg; fixed doses ranged from 50–900 mg. ULN, upper limit of normal.
ALN-AAT01 Phase 1/2 Study Design


- 0.1 mg/kg x 1 SC, N=4
- 0.3 mg/kg x 1 SC, N=4
- 1.0 mg/kg x 1 SC, N=4
- 3.0 mg/kg x 1 SC, N=4
- 6.0 mg/kg x 1 SC, N=4

Part B: Multiple-Ascending Dose (MAD) | Randomized 4:2, Single-blind, Placebo-controlled

- 1.0 mg/kg, q28d x4 SC, N=6

Healthy adult volunteers
Outcome evaluations: Safety and Pharmacodynamic Response (reduction in plasma AAT level)

SAD-only Phase 1 in males and females

Part A:
N= 8 per cohort, double blind, randomized 3:1 active:placebo
5th (10mg/kg) cohort optional

- 0.3 mg/kg
- 1.0 mg/kg
- 3.0 mg/kg
- 6.0 mg/kg
- 10.0 mg/kg
Positive ESC+ Human POC
ALN-AAT02 Clinical Activity and Safety

<table>
<thead>
<tr>
<th>Structure*</th>
<th>ALN- AAT01</th>
<th>ALN-AAT02</th>
</tr>
</thead>
<tbody>
<tr>
<td>Efficacy</td>
<td>Up to 89% KD</td>
<td>Up to 89% KD</td>
</tr>
<tr>
<td>Liver Safety</td>
<td>1/15 (up to 6 mg/kg dose)</td>
<td>0/18 (up to 6 mg/kg dose)</td>
</tr>
</tbody>
</table>

* Images are representative
ESC+ Technology: Basis of Alnylam Product Engine
Source of Sustainable Innovation

- Expect 2-4 INDs per year
- Large number of opportunities
- Organic capability & growth
Summary

• ESC+ strategy mitigates seed-mediated off-target effects, improves specificity and further expands therapeutic window of siRNA conjugates in preclinical species

• Achieved encouraging translation of ESC+ design in humans
  ◦ Directly assessed the impact of the new ESC+ design with follow-on compounds in two separate programs (same sequence but new ESC+ design)

• Multiple additional ESC+ conjugates have advanced into clinical development
To those who say “impossible, impractical, unrealistic,” we say:

CHALLENGE ACCEPTED

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