Development of a Pharmacokinetic-Pharmacodynamic (PK-PD) Model of Fitusiran, an Investigational RNAi Therapeutic Targeting Antithrombin for the Treatment of Hemophilia in Patients With and Without Inhibitors

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Background

Hemophilia A and B are bleeding disorders characterized by ineffective clot formation due to insufficient TG.

Fitusiran is a subcutaneously (SC) administered investigational RNA interference (RNAi) therapeutic targeting antithrombin (AT) with goal of promoting sufficient thrombin generation (TG) to restore hemostasis and prevent bleeding in patients with hemophilia.

**Fitusiran Therapeutic Hypothesis**

- Hemophilia A and B are bleeding disorders characterized by ineffective clot formation due to insufficient TG.
- Fitusiran is a subcutaneously (SC) administered investigational RNA interference (RNAi) therapeutic targeting antithrombin (AT) with goal of promoting sufficient thrombin generation (TG) to restore hemostasis and prevent bleeding in patients with hemophilia.

**Hepatocyte Targeted siRNA Delivery and RNAi Mechanism**

- Fitusiran is targeted to hepatocytes – where AT is produced – through trivalent N-acetyl galactosamine (GalNAc) ligand via the asialoglycoprotein receptor (ASGPR).
- After internalization, fitusiran binds to RNA Induced Silencing Complex (RISC), and mediates cleavage of AT mRNA and consequently lowers AT production.
Objectives

To quantify the relationship between fitusiran dose, predicted fitusiran liver concentrations and serum anti-thrombin (AT) lowering in hemophilia patients

To quantify the relationship between serum AT levels and thrombin generation (TG) in hemophilia patients

To quantify the effects of patient covariates that describe inter-individual variability on AT lowering and TG
Background
PK-PD of Fitusiran in Mice after a Single SC Dose of 2.5 mg/kg

Following fitusiran administration, plasma concentrations decline rapidly and are below lower limit of quantitation within 1 day; fitusiran concentrations in liver and RISC are sustained for several weeks after a single dose

- Lag time is observed between peak liver concentration and maximum AT lowering
- RISC concentrations of fitusiran correlate well with onset, peak and durability of AT lowering
Fitusiran Phase 1 and Phase 2 Open-Label Extension Study Design

**Phase 1, Part A (N=4 healthy volunteers)**

<table>
<thead>
<tr>
<th>Dose</th>
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<tbody>
<tr>
<td>0.03 mg/kg or placebo x 1 SC</td>
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</table>

**Phase 1, Part B (N=12 patients with HA or HB)**

<table>
<thead>
<tr>
<th>Dose</th>
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<tr>
<td>15, 45, 75 mcg/kg weekly x 3 SC</td>
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**Phase 1, Part C (N=18 patients with HA or HB)**

<table>
<thead>
<tr>
<th>Dose</th>
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<tr>
<td>225, 450, 900, 1800 mcg/kg, or 80 mg monthly x 3 SC</td>
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**Phase 1, Part D (N=16 patients with HA or HB with inhibitors)**

<table>
<thead>
<tr>
<th>Dose</th>
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<tr>
<td>50, 80 mg monthly x 3 SC</td>
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**Phase 2 OLE‡ (n= 33)**

<table>
<thead>
<tr>
<th>Dose</th>
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<tbody>
<tr>
<td>50 mg monthly SC</td>
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<tr>
<td>80 mg monthly SC</td>
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- Individual patient dose adjustment may be allowed (per SRC)

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OLE, open-label extension; SC, subcutaneous


^ClinicalTrials.gov Identifier: NCT02554773; EudraCT: 2015-001395-21

†5 patients participating in Part C previously participated in Part B

‡3 patients started Phase 2 OLE at their original Phase 1 dose; later they were converted to 50 mg or 80 mg
Patient Demographics and Disease Status

<table>
<thead>
<tr>
<th>Covariate</th>
<th>Result (N=41)</th>
</tr>
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<tbody>
<tr>
<td>Age, median (range), years</td>
<td>36 (19-65)</td>
</tr>
<tr>
<td>Weight, median (range), kg</td>
<td>75.5 (52-116)</td>
</tr>
<tr>
<td>Baseline peak thrombin, median (range), nM</td>
<td>16 (5-47)</td>
</tr>
<tr>
<td>Hemophilia sub-type</td>
<td>N (%)</td>
</tr>
<tr>
<td>Hemophilia A</td>
<td>34 (83)</td>
</tr>
<tr>
<td>Hemophilia B</td>
<td>7 (17)</td>
</tr>
<tr>
<td>Inhibitor status</td>
<td>N (%)</td>
</tr>
<tr>
<td>Positive</td>
<td>16 (39)</td>
</tr>
<tr>
<td>Negative</td>
<td>25 (61)</td>
</tr>
</tbody>
</table>

Observed Time Course of AT Lowering after Monthly Dosing (Study ALN-AT3SC-001)

Dose Dependent Maximum AT Lowering is Observed that Appears to be Sigmoidal
PK-PD Model for AT Lowering: In Liver, Fitusiran is Loaded into RISC Complex and Inhibits AT Synthesis

Modeling Strategy

- Rat liver PK concentrations were described by a 2-compartment PK model
- Human liver PK was predicted based on allometric scaling of liver PK parameters from rats
- Within liver, fitusiran gets loaded onto RISC complex
- Fitusiran loaded RISC concentrations inhibit the synthesis rate of AT thus leading to lowering of AT activity
- Lowering of AT activity led to increase in thrombin generation and this relationship was best described using a quadratic function

\[ Q = \text{Rate of uptake into RISC compartment}, \quad RV = \text{Volume of RISC compartment}, \quad CL_{\text{RISC}} = \text{Clearance from RISC compartment}, \quad K_{\text{syn}} = \text{Synthesis rate of AT Protein}, \quad K_{\text{deg}} = \text{Degradation rate of AT Protein}, \quad IC_{50} = \text{Concentration producing 50\% of the maximal effect}, \quad I_{\text{max}} = \text{Maximum inhibition of } K_{\text{syn}} \]

Covariate effects were tested on IC\text{50} parameter
Human Liver PK was Predicted Based on Allometric Scaling of Rat Liver PK

Human liver PK was predicted based on allometric scaling of PK parameters in rats with exponents of 1 for volume and 0.75 for clearance parameters

- Predicted half-life of fitusiran in human liver is 12 days
Model Adequately Describes the Kinetics and Interpatient Variability of AT Activity

PK-PD model adequately describes AT response during onset, steady-state and recovery phases

• The estimated degradation half-life of AT was similar to that reported in literature\(^1\), at ~2 days
• Predicted half-life of RISC loaded fitusiran in human liver is 20.5 days

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AT, antithrombin; RISC, RNA induced silencing complex
Predicted Dose-Response for Steady State AT Lowering

Dose response curve shows an asymptote at doses >80 mg
80 mg QM Results in Larger Proportion of Patients Maintaining Greater AT Lowering During Dosing Interval

<table>
<thead>
<tr>
<th>% of Patients with &gt;80% AT Lowering at Steady-State</th>
<th>50 mg QM</th>
<th>80 mg QM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peak</td>
<td>72</td>
<td>92</td>
</tr>
<tr>
<td>Trough</td>
<td>66</td>
<td>88</td>
</tr>
</tbody>
</table>

AT, antithrombin
Simulation of Dosing Regimen

Greater AT lowering with minimal difference between peaks and troughs is expected with monthly regimen compared to bimonthly regimen.
Impact of Covariates on AT Lowering

Similar AT lowering is expected in hemophilia A or B patients, with or without inhibitors, across range of observed baseline weights and age in the trial.
More than Proportional (Non-Linear) Increase in TG is Observed with Increase in AT Lowering

There is generally good agreement between the observed TG values and model predictions, thus, confirming the predictability of the final model and its utility in predicting TG across different doses

- AT-TG relationship is described by the equation

\[ TG = 16.1 + 0.0065 \times (AT \text{ Lowering})^2 \]

Observed and Model Predicted AT-TG Relationship across 41 Patients

AT, antithrombin; TG, thrombin generation
Predicted Fitusiran Dose-Response of Thrombin Generation

Dose response curve shows greater TG at 80 mg compared to lower doses

- Doses greater than 80 mg are not anticipated to cause substantial additional increase in TG, suggesting that near maximal PD effect is anticipated at 80 mg QM dose

Solid blue line: Median TG at steady state
Shaded blue area: 90% Prediction Interval steady state TG response
Symbols = Observed mean TG after >1 monthly doses
Continued monthly dosing of fitusiran at 80 mg results in an increased TG with minimal difference between peaks and troughs throughout the dosing interval.

Simulation performed at baseline TG of 16 nM
Solid blue line = Median; Shaded blue area = 90% Prediction Interval;
Symbols = Observations (ALN-AT3SC-001)
Impact of Covariates on Thrombin Generation

TG is similar across patients with hemophilia A or B, with or without inhibitors, across range of observed baseline weights, age, baseline TG in the trial

- Patients with higher baseline TG have greater absolute increase in thrombin generation from baseline
Summary

PK-PD model was developed that adequately described the time course and inter-individual variability in observed AT lowering and TG in hemophilia patients.

There was no significant covariate effect of age, weight, hemophilia subtype, or inhibitor status on AT lowering and thrombin generation, suggesting fitusiran is similarly effective in these sub-populations.

Based on population PK-PD modeling, recommended Phase 3 dose is 80 mg administered monthly:

- Data suggest that 80 mg is adequate for maximizing PD effect.
- Higher doses are not anticipated to yield substantial additional increase in AT lowering and TG.
- 80 mg results in larger proportion of patients maintaining >80% AT lowering during dosing interval compared to 50 mg.
- Monthly regimen results in minimal variation between peak and trough compared to bimonthly regimen.