Enabling the development of serum [TTR] as a biomarker for treatment of ATTR amyloidosis

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Disclosures

- Adviser for Alnylam, AstraZeneca, Attralus, BridgeBio, Ionis, Pfizer, and Intellia
Treatment outcomes in systemic amyloidosis associate with the residual concentration of the amyloid precursor protein\textsuperscript{1,2}

AA Amyloidosis\textsuperscript{1}

- More amyloid = worse outcomes
- Less amyloid = better outcomes
- “Natural” clearance of \textit{in vivo} amyloid deposits occurs slowly
- Clearance of amyloid occurs at different rates in different organs
  - \textit{Liver vs heart}
- The rate of amyloid clearance varies between individuals
  - A 75\% reduction in fibril precursor protein concentrations may be sufficient to permit amyloid regression in one patient but may result in amyloid accumulation in another

AL Amyloidosis\textsuperscript{a}


AA, amyloid A; AL, amyloid light chain; SAA, serum amyloid A.
Our community has incorporated this information to advance therapy in AA and AL amyloidosis.

Specific concentration thresholds inform outcomes.

**AA amyloidosis**

Unadjusted relative risk of death associated with the most recent median annual SAA concentration during follow-up.

- SAA Octile (mg/L)
  - <4
  - ≥4 to <9
  - ≥9 to <16.7
  - ≥16.7 to <28
  - ≥28 to <45.6
  - ≥45.6 to <87
  - ≥87 to <155
  - ≥155

- Relative risk (95% CI)
  - <4
  - ≥4 to <9
  - ≥9 to <16.7
  - ≥16.7 to <28
  - ≥28 to <45.6
  - ≥45.6 to <87
  - ≥87 to <155
  - ≥155

- P value
  - <0.001

- The SAA value is the median concentration within each 12-month period and was incorporated into the Cox regression model as a time-dependent covariate.

**AL amyloidosis**

Survival (proportion) vs. Time (months).

- SAA Octile (mg/L)
  - <4
  - ≥4 to <9
  - ≥9 to <16.7
  - ≥16.7 to <28
  - ≥28 to <45.6
  - ≥45.6 to <87
  - ≥87 to <155
  - ≥155

- Relative risk (95% CI)
  - <4
  - ≥4 to <9
  - ≥9 to <16.7
  - ≥16.7 to <28
  - ≥28 to <45.6
  - ≥45.6 to <87
  - ≥87 to <155
  - ≥155

- P value
  - <0.001


There is an equilibrium between circulating amyloid fibril precursor protein concentration and change in amyloid burden.

- Reduce supply of amyloid precursor protein
- Stabilise amyloid-forming proteins
  - β sheet breakers
- Immunotherapy
- SAP depletion

Therapeutic strategies in amyloidosis:
- Enhance removal of existing amyloid
  - Immunotherapy
  - SAP depletion
- Reduce supply of amyloid precursor protein
- Stabilise amyloid-forming proteins
  - β sheet breakers
- Reversion to normally folded protein

AA, amyloid A; AL, amyloid light chain; ATTR, transthyretin amyloidosis; SAP, serum amyloid P component.
Percent reduction in serum [TTR] is associated with clinical benefit in ATTR amyloidosis

Pharmacodynamics:
% change in serum TTR over 18 months

- Median reduction in serum TTR in the patisiran group was 81% (range, -38% to 95%) and was similar across age, sex, or genotype

- In a post hoc analysis of the cardiac subpopulation (n=126), there was an approximate 45% reduction in the composite rate of cardiac hospitalization and all-cause mortality

Primary endpoint:
mNIS+7 neuropathy score

- 56% vs 4% of patisiran- and placebo-treated patients, respectively, experienced a halting or reversal of disease progression (change <0 point mNIS+7)

Serum [TTR] is typically expressed as % reduction from baseline – should we be using residual absolute serum [TTR] instead?

% serum TTR reduction vs ΔmNIS+7 by non ile in APOLLO A

At the population level, ≈80% TTR lowering is associated with improved score

Same % serum TTR reduction can mean different risk for ongoing fibril formation

<table>
<thead>
<tr>
<th></th>
<th>Patient 1</th>
<th>Patient 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Predose [TTR] (µg/mL)</td>
<td>350</td>
<td>150</td>
</tr>
<tr>
<td>Postdose [TTR] (µg/mL)</td>
<td>70</td>
<td>30</td>
</tr>
</tbody>
</table>

Both represent 80% knockdown, but >2× available substrate for ongoing amyloid formation in Patient 1 post-treatment

Should we be using residual absolute serum [TTR] instead?

Polydefkis M, et al. Presented at ISA, Mar 26-29, 2018; Kumamoto, Japan. Graph used with permissions from first author.
Two-part, open-label, multicenter study in adults with hereditary ATTR amyloidosis with polyneuropathy (ATTRv-PN) or ATTR amyloidosis with cardiomyopathy (ATTR-CM)

PART I: Single-Ascending Dose Escalation

**ATTRv-PN patients**
(n=36)

- 0.1 mg/kg (n=3)
- 0.3 mg/kg (n=3)
- 0.7 mg/kg (n=3)
- 1.0 mg/kg (n=6)

**ATTR-CM patients**
(n=36)

- 0.7 mg/kg NYHA Class I/II (n=3)
- 0.7 mg/kg NYHA Class III (n=6)
- 1.0 mg/kg NYHA Class I/II (n=3)

PART II: Dose Expansion

**n=15**
- 55 mg NYHA Class I/II (n=12)
- 80 mg (n=5)

**n=21**
- 55 mg NYHA Class III (n=12)
- 55 mg NYHA Class I/II (n=12)

**n=24**
- 55 mg NYHA Class III (n=12)

**n=24**
- 55 mg NYHA Class I/II (n=12)
- 80 mg (n=5)

**n=72**

Primary Objectives
Evaluate safety, tolerability, PK, and PD
- Measure serum TTR levels

Secondary Objectives
Evaluate efficacy on clinical measures of:
- Neurologic function in patients with ATTRv-PN
- Cardiac disease in patients with ATTR-CM

The NTLA-2001 phase 1 study in ATTR amyloidosis has completed enrollment (N=72)

ATTR, transthyretin amyloidosis; Cas, CRISPR-associated protein; CRISPR, clustered regularly interspaced short palindromic repeats; NYHA, New York Heart Association; PD, pharmacodynamics; PK, pharmacokinetics.

Clinicaltrials.gov ID: NCT0460105.

ATTR, transthyretin amyloidosis; Cas, CRISPR-associated protein; CRISPR, clustered regularly interspaced short palindromic repeats; NYHA, New York Heart Association; PD, pharmacodynamics; PK, pharmacokinetics.
## Patient demographics and characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>PN Patients (N=36)</th>
<th>CM Patients (N=29)</th>
<th>All Patients (N=65)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, years</strong></td>
<td>Median (min, max)</td>
<td>61 (19, 75)</td>
<td>78 (46, 86)</td>
</tr>
<tr>
<td><strong>Sex, n (%)</strong></td>
<td></td>
<td>Male</td>
<td>26 (72)</td>
</tr>
<tr>
<td><strong>Weight, kg</strong></td>
<td>Median (min, max)</td>
<td>77 (55, 117)</td>
<td>82 (63, 115)</td>
</tr>
<tr>
<td><strong>TTR genotype, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>p.V50M</td>
<td>11 (31)</td>
<td>0</td>
<td>11 (17)</td>
</tr>
<tr>
<td>p.V142I</td>
<td>1 (3)</td>
<td>6 (21)</td>
<td>7 (11)</td>
</tr>
<tr>
<td>p.T80A</td>
<td>7 (19)</td>
<td>1 (3)</td>
<td>8 (12)</td>
</tr>
<tr>
<td>p.S97Y</td>
<td>7 (19)</td>
<td>0</td>
<td>7 (11)</td>
</tr>
<tr>
<td>p.E62D</td>
<td>4 (11)</td>
<td>0</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Other</td>
<td>6 (17)</td>
<td>2 (7)</td>
<td>8 (12)</td>
</tr>
<tr>
<td>WT</td>
<td>0</td>
<td>20 (69)</td>
<td>20 (31)</td>
</tr>
<tr>
<td><strong>NYHA Class, n (%)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No diagnosis of heart failure</td>
<td>12 (33)</td>
<td>0</td>
<td>12 (18)</td>
</tr>
<tr>
<td>I</td>
<td>19 (53)</td>
<td>3 (10)</td>
<td>22 (34)</td>
</tr>
<tr>
<td>II</td>
<td>5 (14)</td>
<td>14 (48)</td>
<td>19 (29)</td>
</tr>
<tr>
<td>III</td>
<td>0</td>
<td>12 (41)</td>
<td>12 (18)</td>
</tr>
<tr>
<td><strong>NT-proBNP, ng/L</strong></td>
<td>Median (min, max)</td>
<td>127 (&lt;50, 1878)</td>
<td>1845 (851, 19,624)</td>
</tr>
</tbody>
</table>

Data cutoff May 11, 2023.
Interim data presented are for the first 65 (dosed and reached at least 28 days post-infusion by the data cutoff) of 72 patients dosed. Results from the final 7 patients will be reported at a future date. CM, cardiomyopathy; NT-proBNP, N-terminal pro-B-type natriuretic peptide; NYHA, New York Heart Association; PN, polyneuropathy; TTR, transthyretin.
### Most frequent treatment emergent adverse events

#### TEAEs by Maximum Toxicity Grade and Preferred Term Reported in >5% of All ATTRv-PN and ATTR-CM Patients (N=65)

<table>
<thead>
<tr>
<th>AE, Preferred Term</th>
<th>Any Grade</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade ≥3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Infusion-related reaction</td>
<td>25 (38)</td>
<td>10 (15)</td>
<td>14 (22)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Headache</td>
<td>12 (18)</td>
<td>12 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea</td>
<td>11 (17)</td>
<td>10 (15)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Back pain</td>
<td>7 (11)</td>
<td>7 (11)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>COVID-19 infection</td>
<td>6 (9)</td>
<td>5 (8)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Cardiac failure</td>
<td>6 (9)</td>
<td>2 (3)</td>
<td>2 (3)</td>
<td>2 (3)</td>
</tr>
<tr>
<td>Upper respiratory tract infection</td>
<td>6 (9)</td>
<td>6 (9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AST increased</td>
<td>5 (8)</td>
<td>3 (5)</td>
<td>1 (2)</td>
<td>1 (2)</td>
</tr>
<tr>
<td>Dizziness</td>
<td>5 (8)</td>
<td>5 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatigue</td>
<td>5 (8)</td>
<td>5 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Muscle spasms</td>
<td>5 (8)</td>
<td>4 (6)</td>
<td>1 (2)</td>
<td></td>
</tr>
<tr>
<td>Vision blurred</td>
<td>5 (8)</td>
<td>5 (8)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Atrial flutter</td>
<td>4 (6)</td>
<td>1 (2)</td>
<td>3 (5)</td>
<td></td>
</tr>
<tr>
<td>Constipation</td>
<td>4 (6)</td>
<td>2 (3)</td>
<td>2 (3)</td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>4 (6)</td>
<td>4 (6)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

- **This includes all reported events, including those unrelated to NTLA-2001 (e.g., atrial flutter and cardiac failure hospitalizations)**
- **Infusion-related reactions were most common; nearly all were considered mild and resolved without sequelae, and all patients received the complete, planned dose**
- **Any liver enzyme elevations resolved spontaneously, were asymptomatic, and required no intervention (e.g., steroids) or hospitalization**

Figure notes: Results for each dose level are shown out to the last time point with complete follow-up for the entire cohort. Interim data presented excludes the 0.1 mg/kg cohort from the dose-escalation of the polyneuropathy arm. The three patients in the 0.1 mg/kg cohort have been re-dosed at 55 mg and results will be shared in a future presentation. The 55 mg and 80 mg doses are the fixed doses corresponding to 0.7 mg/kg and 1.0 mg/kg, respectively.

ATTR-CM
- NYHA I-II 0.7 mg/kg (N=3)
- NYHA III 0.7 mg/kg (N=6)
- NYHA I-II 1.0 mg/kg (N=3)
- NYHA I-II 55 mg (N=11)
- NYHA III 55 mg (N=6)

Baseline IQR: 155 to 234 µg/mL

Median (IQR) Serum [TTR] at Day 28 (n=62)
- Residual absolute TTR concentration at day 28: 17 µg/mL (11 to 24)
- % Change from baseline in serum TTR at day 28: -91% (-88 to -94)

ATTRv-PN
- 0.3 mg/kg (N=3)
- 0.7 mg/kg (N=3)
- 1.0 mg/kg (N=6)
- 55 mg (N=16)
- 80 mg (N=5)

Baseline IQR: 160 to 300 µg/mL
Regardless of baseline TTR levels, NTLA-2001 led to consistently low and sustained absolute serum [TTR] in all patients Data cutoff May 11, 2023.

The median (IQR) maximum change from day 28 onward (measure of fluctuations) = -1.4 μg/mL (-4.7 to 1.7)

**Figure notes:** Mean depicted only when there are two or more data points. Subsequent points connected by a dashed line denotes less than full group follow-up. Interim data presented excludes the 0.1 mg/kg cohort from the dose-escalation of the polyneuropathy arm. The three patients in the 0.1 mg/kg cohort have been redosed at 55 mg and results will be shared in a future presentation. The 55 mg and 80 mg doses are the fixed doses corresponding to 0.7 mg/kg and 1.0 mg/kg, respectively. ATTR-CM, transthyretin amyloidosis with cardiomyopathy; ATTRv-PN, hereditary transthyretin amyloidosis with polyneuropathy; IQR, interquartile range; NYHA, New York Heart Association; SD, standard deviation; TTR, transthyretin.
Summary

• In other systemic amyloidoses, the residual, absolute concentration of the amyloid precursor protein is closely associated with clinical outcomes

• Interim data from 62 patients with ATTR amyloidosis treated with NTLA-2001 continue to show a favorable safety and tolerability profile, with rapid, consistent, and durable reductions of serum [TTR] to low levels in all patients

• For treatments that reduce total serum [TTR], with nonfluctuating steady state measures, the residual absolute serum [TTR] could be a robust biomarker of ATTR amyloidosis therapy outcomes

• With collaboration, this approach to biomarker development has facilitated progress in care and better outcomes in AA and AL amyloidosis
Acknowledgments

We extend our gratitude to the patients who participated in the NTLA-2001 Phase 1 study and their family and caregiver networks.

Staff executing the study on behalf of:

- Richmond Pharmacology, London
- New Zealand Clinical Research, Auckland
- Umea University, Sweden
- CHU Bicetre, University of Paris-Saclay
- Intellia Therapeutics
- Regeneron Pharmaceuticals

United Kingdom
National Amyloidosis Centre

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- Prof. Philip Hawkins
- Prof. Paul Simons
- Guglielmo Verona

Histology
- Janet Gilbertson
- Imaging
- Dorota Rowczenio
- Genetics
- David Hutt

Editorial support was provided by James Banigan, PhD, and Shannon Davis of Apollo Medical Communications, part of Helios Global Group, and funded by Intellia Therapeutics.
Dose-responsive rapid and deep serum TTR reduction sustained across all patients

**Figure notes:** Results for each dose level are shown out to the last time point with complete follow-up for the entire cohort.

**ATTR-CM**
- NYHA I-II 0.7 mg/kg (N=3)
- NYHA III 0.7 mg/kg (N=6)
- NYHA I-II 1.0 mg/kg (N=3)
- NYHA I-II 55 mg (N=11)
- NYHA III 55 mg (N=6)

**ATTRv-PN**
- 0.1 mg/kg (N=3)
- 0.3 mg/kg (N=3)
- 0.7 mg/kg (N=3)
- 1.0 mg/kg (N=6)
- 55 mg (N=16)
- 80 mg (N=5)

**% Change from baseline in serum TTR at Day 28 (n=62, excludes the 0.1 mg/kg cohort)**

<table>
<thead>
<tr>
<th>Description</th>
<th>Mean (SE) serum [TTR]</th>
<th>Median (IQR) serum [TTR]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SE) serum [TTR]</td>
<td>-90% (0.86)</td>
<td>-91% (-88 to -94)</td>
</tr>
</tbody>
</table>

Data cutoff May 11, 2023.

Figure notes: Results for each dose level are shown out to the last time point with complete follow-up for the entire cohort.

ATTR-CM, transthyretin amyloidosis with cardiomyopathy; ATTRv-PN, hereditary transthyretin amyloidosis with polyneuropathy; NYHA, New York Heart Association; SE, standard error; TTR, transthyretin.