Familial amyloidotic polyneuropathy (FAP) is a fatal disease caused by transthyretin (TTR) aggregation. Patisiran is an RNAi therapeutic targeting hepatic TTR production. Results of an ongoing Phase 2 open-label extension (OLE) study of patisiran in FAP (n=27; 0.3 mg/kg IV every 3 weeks for up to 2 years) demonstrated >80% sustained knockdown of serum TTR and stable neuropathy scores over 12 months. As non-native conformations of TTR (NNTTR), including misfolded monomeric and oligomeric forms, have been implicated in the pathogenesis of the polyneuropathy in FAP, we used an immunobassay (ELISA) that specifically detects NNTTR in FAP patient plasma samples to evaluate the effect of patisiran on NNTTR levels in patients treated on the OLE study. Serial NNTTR measurements were performed on 25 patients, including 19 concurrently receiving a TTR tetramer stabilizer. Rapid and sustained lowering of NNTTR was observed in patients on or off tetramer stabilizers, with ~90% reduction maintained out to Day 248. These results demonstrate that the TTR knockdown with patisiran is associated with marked reductions in NNTTR, providing direct mechanistic evidence supporting the therapeutic hypothesis that TTR knockdown could result in clinical benefit in FAP.

### FAP NNTTR Assay

#### Table 1. Plasma samples from patisiran Phase 2 OLE study were analyzed using the FAP NNTTR assay

<table>
<thead>
<tr>
<th>Study</th>
<th>ALN-TTR02-003</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>25</td>
</tr>
<tr>
<td>Sample Collection Time</td>
<td>Days 0 and 231: Pre-dose and Days 1, 3, 7, and 17 Post-dose Days 84 and 182: Pre-dose</td>
</tr>
<tr>
<td>Total number of Samples</td>
<td>432</td>
</tr>
<tr>
<td>Concurrent Diffusals</td>
<td>6</td>
</tr>
<tr>
<td>Concurrent Tafamidis</td>
<td>13</td>
</tr>
<tr>
<td>Patisiran Alone</td>
<td>6</td>
</tr>
<tr>
<td>Genotypes</td>
<td>V30M(18), R454C(1), F464L(1)<em>, S77F(2), S77Y(2), Y116S(1)</em></td>
</tr>
</tbody>
</table>

Misfolding Diagnostics’ proprietary FAP NNTTR ELISA assay has been previously shown to:
- Sensitive and specific towards NNTTR
- Microtainer plasma or serum sample needed
- 100% accuracy for V30M FAP patient identification
- Detects >90% TTR genotypes associated with ATTR amyloid neuropathy phenotype

#### Results

Figures 1 and 2 demonstrate the high sensitivity and specificity of the ELISA assay in detecting misfolded TTR protein. Consistent with previous results, levels of native TTR were maintained in patients on both the TTR stabilizer and patisiran. The assay was able to effectively detect a broad range of TTR genotypes associated with FAP, including those with a low detection sensitivity due to amino acids close to post-translational modifications (PTMs) or genetic variations such as V30M, R54TC, and F64L.

### Discussion

#### Figure 3. Native TTR and NNTTR reduction by individual patients, split by concurrent stabilizer usage.

- NNTTR appears to be a suitable disease relevant efficacy biomarker to complement Alnylam’s current total TTR PO marker.
- Misfolding Diagnostics serves as disease relevant relevant mechanistic biomarker bridging total TTR knockdown and clinical outcome.
- Schematic drawing of total TTR and NNTTR levels in relationship to clinical outcome.

#### Summary

- Patisiran effectively and sustainably lowers NNTTR level in FAP patients over the treatment period
  - Correlates with total TTR reduction
  - Time dependent reduction; nadir within several weeks after start of patisiran treatment
  - Sustained ~90% reduction of NNTTR (compared to pre-treatment value) throughout the treatment duration
  - NNTTR level should provide a critical mechanistic link between TTR knockdown and clinical benefit

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