Patisiran, an RNAi therapeutic for the treatment of FAP, lowers non-native TTR species implicated in disease pathology

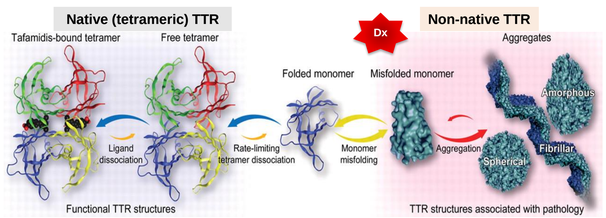
Xin Jiang[1], Justin Chapman[1], Jeffery Kelly[1,2], David Adams[3], Ole Suhr[4], Teresa Coelho[5], Isabel Conceicao[6], Marcia Waddington-Cruz[7], Hartmut Schmidt[8], Juan Buades[9], Josep Campistol[10], Jean Pouget[11], John Berk[12], Brian Bettencourt[13], Renta Hutabarat[13], Jared Gollob[13]

(1) Misfolding Diagnostics, Inc., La Jolla, CA, USA; (2) Department of Molecular and Experimental Medicine, The Scripps Research Institute, La Jolla, CA, USA; (3) Centre Paris-Sud, APHP, Hopital de Bicetre, INSERM U788, Service de Neurologie, and Centre de Reference des Neuropathies Amyloides Familiales, Le Kremlin-Bicetre, France; (4) Department of Public Health and Clinical Medicine, Umea University, Umea, Sweden; (5) Unidade Clinica de Paramiloidose, Hospital de Santo Antonio, Porto, Portugal; (6) Centro Hospitalar Lisboa Norte-Hospital de Santa Maria, Lisbon, Portugal; (7) Hospital Universitario Clementino Fraga Filho, Rio de Janeiro, Brazil; (8) The University Hospital of Muenster, Muenster, Germany; (9) Hospital Son Llatzer, Palma de Mallorca, Spain; (10) Hospital Clinic, Barcelona, Spain; (11) Centre de Reference des Maladies Neuromusculaires et de la SLA, Hopital de la Timone, Marseille, France; (12) Amyloid Treatment and Research Program, Boston University, Boston, MA, USA; (13) Alnylam Pharmaceuticals, Cambridge, MA, USA

Abstract

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 for 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 immunoassay (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.

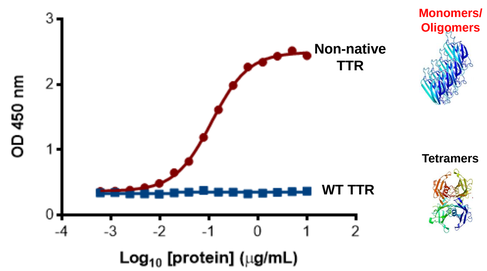
Background



- Tetramer dissociation is the 1st step in TTR amyloidogenesis
- Amyloid hypothesis – protein aggregation causes post-mitotic tissue degeneration
- Detection of misfolded non-native TTR conformations:
  - Correlates with the pathophysiological **root cause** of TTR amyloidosis
  - Reduction of non-native conformations is a **necessary** step in achieving therapeutic efficacy; likely to predict clinical benefit
- NNTTR level should provide a critical mechanistic link between TTR knockdown and clinical benefit

FAP NNTTR Assay

Figure 1. FAP NNTTR sandwich ELISA assay specifically recognizes misfolded TTR protein.



Misfolding Diagnostics' proprietary FAP NNTTR ELISA assay has been previously shown to:

- Sensitive and specific towards NNTTR
- Microliter plasma or serum sample needed
- 100% accuracy for V30M FAP patient identification
- Identifies >90% TTR genotypes associated with ATTR polyneuropathy phenotype
- Detects significant reduction of NNTTR in FAP patients treated with TTR kinetic stabilizer (tafamidis or diflunisal)

Clinical Samples

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

Study	ALN-TTR02-003
N	25
Sample Collection Time	Days 0 and 231: Pre-dose and Days 1, 3, 7, and 17 Post-dose Days 84 and 182: Pre-dose
Total number of Samples	432
Concurrent Diflunisal	6
Concurrent Tafamidis	13
Patisiran Alone	6
Genotypes	V30M(18), R54TC(1), F64L(1)*, S77F(2), S77Y(2), Y116S(1)* * Low detection sensitivity due to aa close to antibody epitopes

Results

Figure 2. Rapid and sustained lowering of NNTTR in FAP patients treated with patisiran on Phase 2 OLE study. Blinded plasma samples were tested for NNTTR level using the FAP NNTTR sandwich ELISA assay.

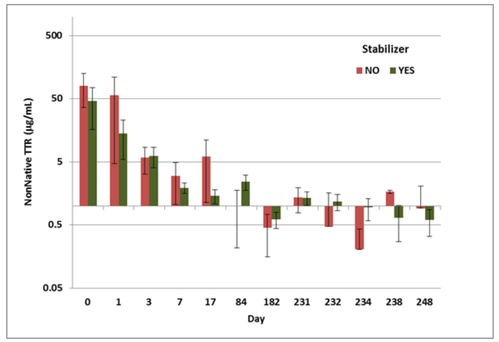
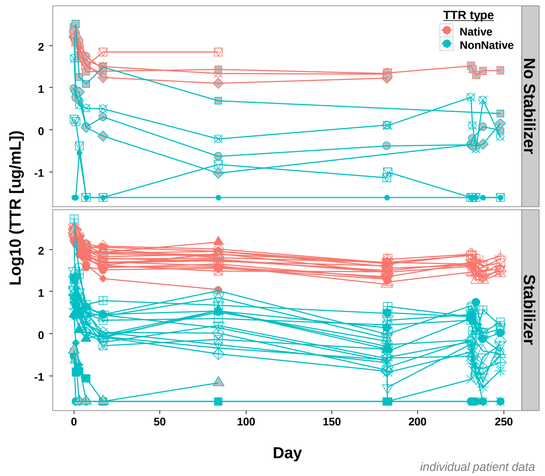


Figure 3. Native TTR and NNTTR reduction by individual patients, split by concurrent stabilizer usage.



individual patient data

Disclosure:
The current study is funded by Alnylam Pharmaceuticals

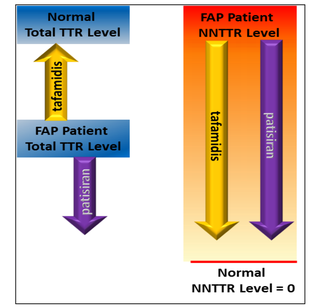
misFolding Diagnostics

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
- NNTTR appears to be a suitable disease relevant efficacy biomarker to compliment Alnylam's current total TTR PD marker

Discussion

Figure 4. NNTTR level serves as disease relevant mechanistic biomarker bridging total TTR knockdown and clinical outcome. Schematic drawing of total TTR and NNTTR levels in relationship to clinical outcome.



- Improvement in neurological impairment and quality of life associated with therapies that both increase or decrease total TTR
- NNTTR is disease associated (unique to patients, not detectable in healthy controls)
- Reduction of NNTTR is likely necessary for drug effect
- Reductions in total TTR and NNTTR levels post-patisiran therapy coincide with the slowing of neurologic deterioration and maintenance in quality of life

Misfolding Diagnostics, Inc., 3210 Merryfield Row, San Diego, CA 92121 Contact: info@misfoldDx.com