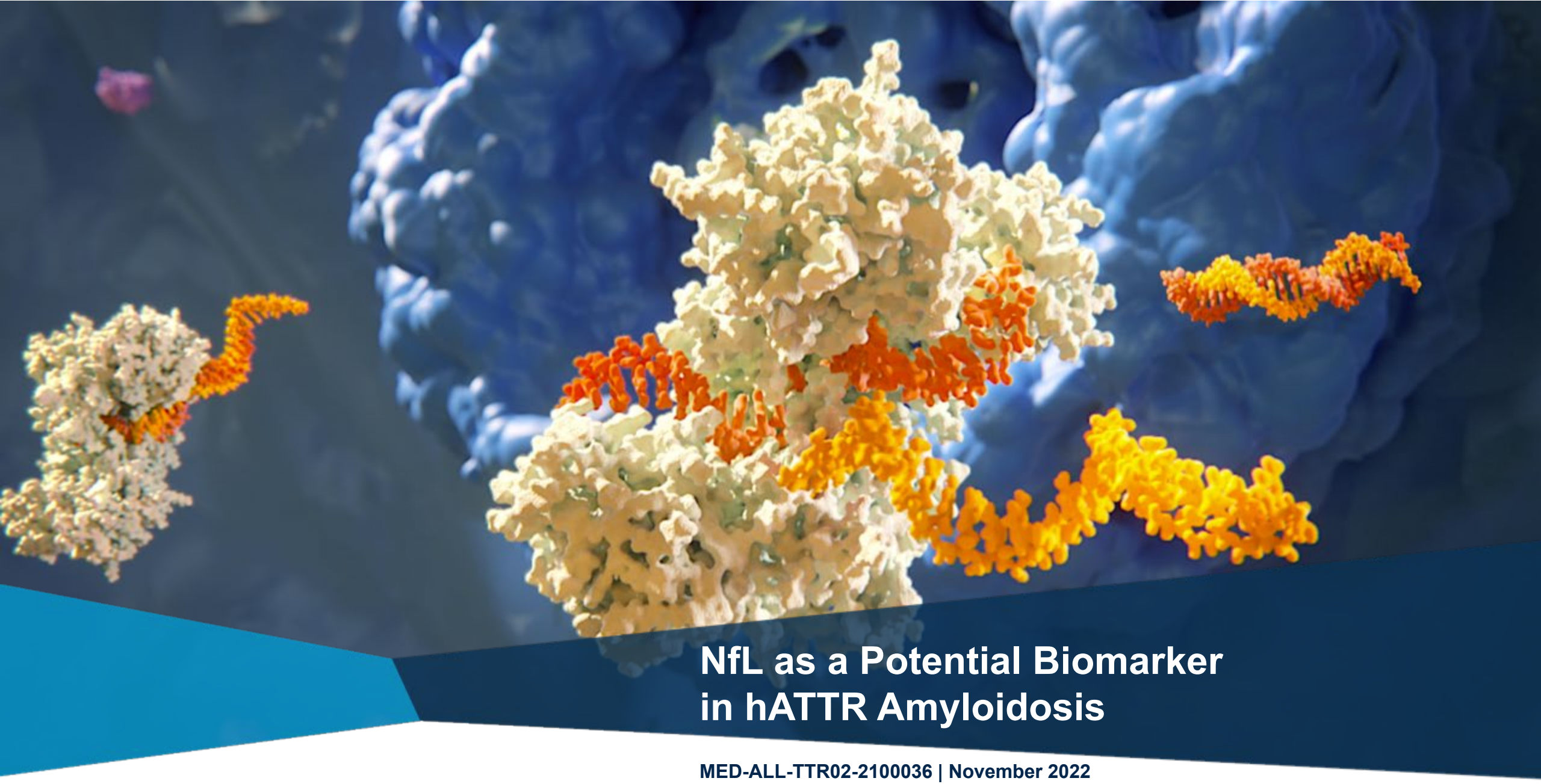


NfL as a Potential Biomarker in hATTR Amyloidosis

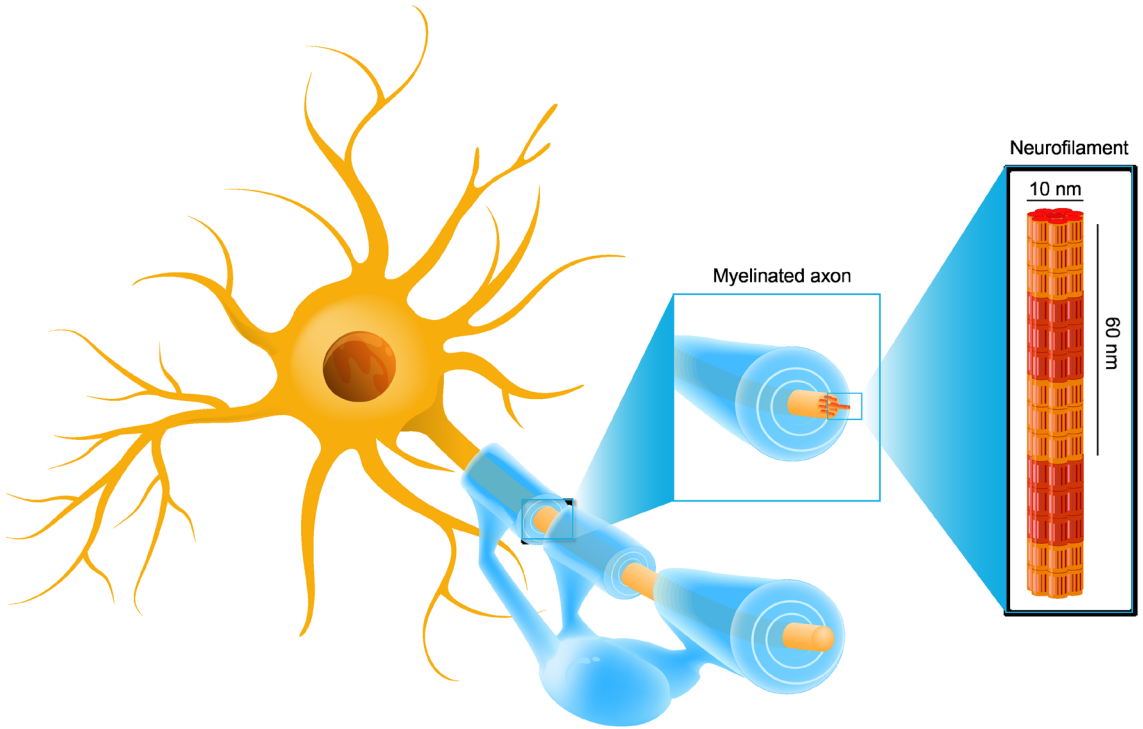
MED-ALL-TTR02-2100036 | November 2022

NfL as a Potential Biomarker in hATTR Amyloidosis

- This resource is intended to support scientific exchange and may contain information that is not in the approved Prescribing Information for ONPATTRO® (patisiran) and AMVUTTRA® (vutrisiran). The information provided is not intended to serve as recommendations for clinical practice.
- Alnylam does not recommend or suggest the use of its products in any manner that is inconsistent with the approved Prescribing Information.
- Please see the ONPATTRO and AMVUTTRA full Prescribing Information for the FDA-approved product labeling.
- This resource may contain hyperlinks that are not functional in this format.
- For further information, please see RNAiScience.com to connect with a Medical Science Liaison, submit a medical information request, or access other Alnylam medical education resources.

Neurofilament Light Chain (NfL)

Neurofilament Structure[1]



- Neurofilaments are the most abundant group of proteins in mature myelinated axons[2]
- Neurofilaments perform key roles in neuronal structure and function[1,2]
  - Resistance against external pressure
  - Radial growth of axons
  - Indirect moderation of the conduction velocity
  - Attachment for organelles and other proteins

NfL, neurofilament light chain.

1. Gaetani L et al. *J Neurol Neurosurg Psychiatry* 2019;90:870–881; 2. Thebault S et al. *Biomedicines* 2020;8:523.

NfL Pathophysiology

- Under normal conditions, low levels of NfL are constantly released from axons, with higher levels observed at older ages[1,2]

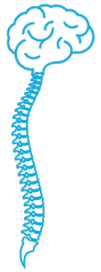
- In response to central or peripheral axonal damage, the release and levels of NfL in CSF and blood sharply increase[2]
  - Concentration in blood is roughly 40-fold lower than in CSF[2]
  - Strong correlations between blood and CSF NfL levels have been demonstrated[1]

CNS, central nervous system; CSF, cerebrospinal fluid; NfL, neurofilament light chain.

1. Thebault S et al. *Biomedicines* 2020;8:523; 2. Gaetani L et al. *J Neurol Neurosurg Psychiatry* 2019;90:870–881.

NfL: A Well–Studied Biomarker in Neurological Disorders

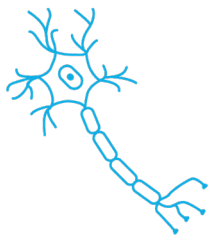
- NfL has been widely described as a biomarker of neuroaxonal injury across:



CNS Diseases

- Multiple sclerosis[1]
- Alzheimer's disease[2,3]
- Huntington's disease[4]

Review
Blood Neurofilament Light Chain: The Neurologist's Troponin?[9]
Thebault S et al. Biomedicines 2020;8:523



PNS Diseases

- Vasculitic neuropathy[5]
- Chronic inflammatory demyelinating polyneuropathy[6]
- Guillain-Barré syndrome[7]
- Charcot-Marie-Tooth disease[8]

Scientific Commentaries
Peripheral Blood Neurofilament Light Chain Levels: The Neurologist's C-reactive Protein?[10]
Giovannoni G et al. Brain 2018;141:2235–2237

CNS, central nervous system; NfL, neurofilament light chain; PNS, peripheral nervous system.

1. Thebault S et al. *Mult Scler* 2022; 28(10): 1491-1497; 2. Lewczuk P et al. *Alzheimers Res Ther* 2018;10:71; 3. Lin YS et al. *Sci Rep* 2018;8:17368;
4. Byrne LM et al. *Lancet Neurol* 2017;16:601–609; 5. Bischof A et al. *Ann Rheum Dis* 2018;77:1093–1094; 6. Van Lieverloo GGA et al. *J Peripher Nerv Syst* 2019;24:187–194; 7. Mariotto S et al. *J Peripher Nerv Syst* 2018;23:174–177; 8. Sandelius A et al. *Neurology* 2018;90:e518–e524; 9. Thebault S et al. *Biomedicines* 2020;8:523; 10. Giovannoni G et al. *Brain* 2018;141:2235–2237.

NfL is Being Evaluated as a Potential Biomarker for PN in hATTR Amyloidosis

Study/analysis/publication	Patient population and analyses
Alnylam Pharmaceuticals analyses*	
APOLLO[1,2]	Analysis of the proteome and NfL levels at baseline and through 18 months of patisiran and placebo treatment in hATTR amyloidosis patients with PN
HELIOS–A[3]	Analysis of NfL levels at baseline in hATTR amyloidosis patients with PN
Patisiran global OLE[4–6]	Analysis of NfL levels through 24 months of patisiran treatment in hATTR amyloidosis patients with PN during their participation in the Global OLE following APOLLO and phase 2 OLE
Patisiran phase 2 OLE[5]	Analysis of NfL levels in hATTR amyloidosis patients with PN through 24 months of patisiran treatment
External analyses	
Kapoor et al. (2019)[7]	Analysis of NfL levels in hATTR amyloidosis patients with PN and asymptomatic carriers of various *TTR* variants
Maia et al. (2020)[8]	Analysis of NfL levels in hATTR amyloidosis patients with PN (*V30M* variant) and asymptomatic carriers of *V30M* variant of *TTR*
Louwsma et al. (2020)[9]	Analysis of NfL levels in hATTR amyloidosis patients with PN and asymptomatic carriers of various variants of *TTR,* and patients with AL amyloidosis with and without PN

* All NfL and other proteomic analyses conducted by Alnylam and presented in this deck are post-hoc

AL, amyloid light–chain; ATTR, transthyretin–mediated; hATTR, hereditary transthyretin–mediated; NfL, neurofilament light chain; OLE, open–label extension; TTR, transthyretin; PN, polyneuropathy.

1. Ticau S et al. *Neurology* 2021;96:e412–e422; 2. Adams D et al. *N Engl J Med* 2018;379:11–21; 3. Alnylam Pharmaceuticals. Data on file; 4. Ticau S et al. *PNS Virtual Meeting* 2020; 5. Polydefkis M et al. *AAN Virtual Meeting 2021*; 6. Ticau S et al. *HFSA Virtual Meeting* 2020; 7. Kapoor M et al. *J Peripher Nerv Syst* 2019;24:314–319; 8. Maia LF et al. *Amyloid* 2020;27:97–102; 9. Louwsma J et al. *Amyloid* 2020;28:50–55.

Analyses from the APOLLO Study[1*]

Neurofilament Light Chain as a Biomarker of Hereditary Transthyretin-Mediated Amyloidosis

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Neurology® 2021;96:e412-e422. doi:10.1212/WNL.0000000000011090

Objective

- To identify changes in the proteome and NfL levels associated with the onset and progression of hATTR amyloidosis, and treatment with patisiran in patients with the polyneuropathy of hATTR amyloidosis who participated in the phase 3 APOLLO study[1]

* All NfL and other proteomic analyses conducted by Alnylam and presented in this deck are post-hoc

hATTR, hereditary transthyretin–mediated; NfL, neurofilament light chain.

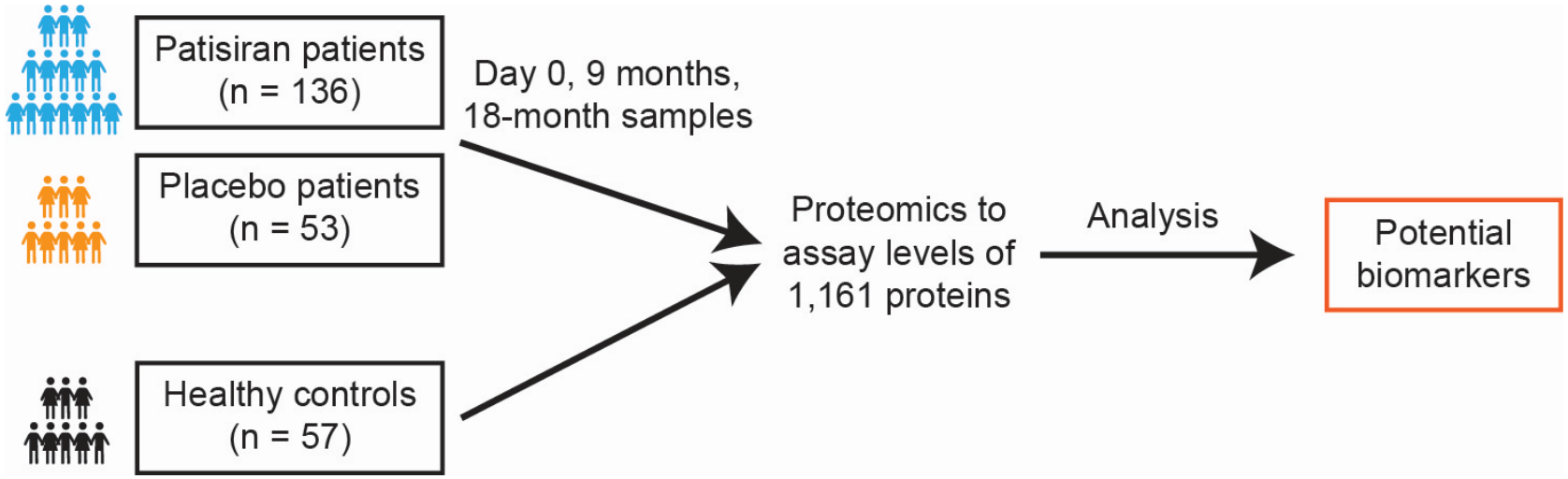
1. Ticau S et al. *Neurology* 2021;96:e412–e422

Analyses from the APOLLO Study[1]

Methods

- Post-hoc analysis of plasma samples in a subset of patients enrolled in the APOLLO study[2] who completed the study and consented to a biomarker discovery study
  - Levels of 1,161 unique proteins (including NfL) were measured semi–quantitatively (Olink® Proteomics, Watertown, MA, USA)
  - Subsequent quantitative measurements of NfL were made using an ultrasensitive single molecule array (Simoa) method
- Healthy control samples were collected separately and were age, sex, and race matched to the baseline demographics of the APOLLO patients (Dx Biosamples, LLC, San Diego, CA, USA)

Overview of the Analyses and Samples Analyzed



NfL, neurofilament light chain.

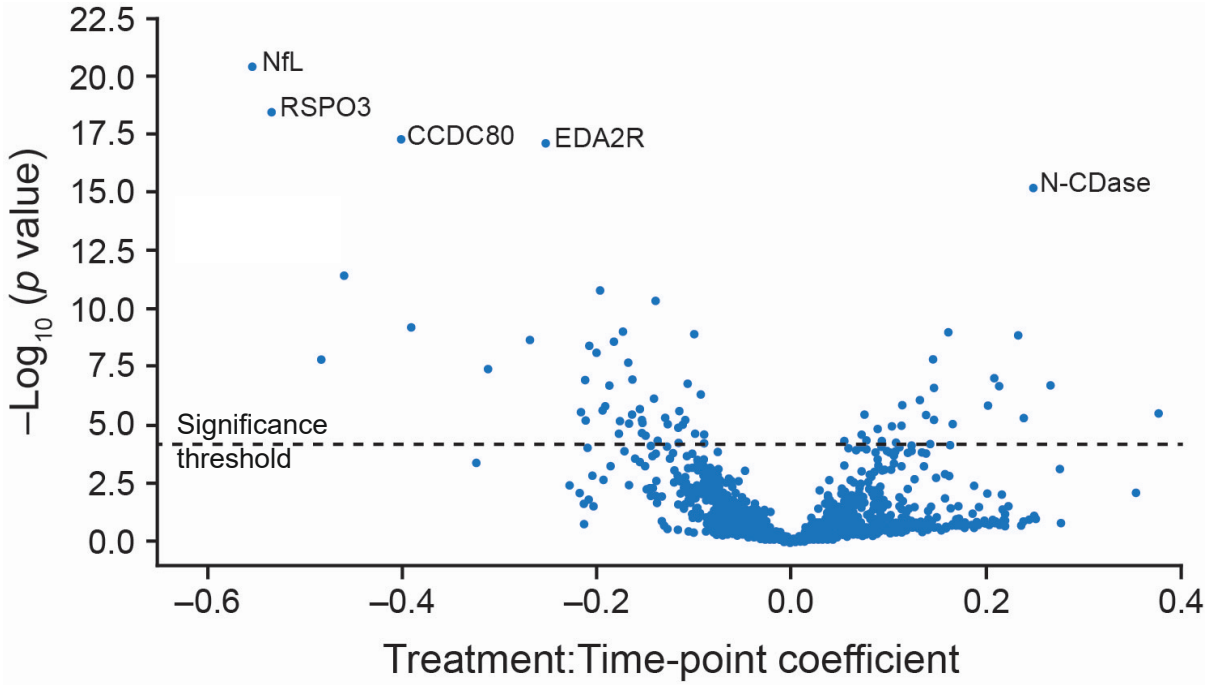
1. Ticau S et al. *Neurology* 2021;96:e412–e422; 2. Adams D et al. *N Engl J Med* 2018;379:11–21.

NfL Time Profile was Significantly Different between Patisiran and Placebo

Results: Proteomic Analysis

- A linear mixed model was used to determine the effect of patisiran treatment on the time profile of the plasma level of each protein by analyzing levels at baseline, 9 months, and 18 months
- The levels of 66 proteins, including NfL, changed significantly in patients treated with placebo versus patisiran over time ($p<4.18\times10^{-5}$)
- NfL had the **most significant change** ($p=3.95\times10^{-21}$)

Change in Levels of Proteins in Patients Treated with Patisiran vs. Placebo over 18 Months



Proteins are shown here as a volcano plot, with the strength of the association on the y–axis ($-\log_{10}$ [p value]) and the effect size on the x–axis (shown as the treatment × time point coefficient from the model).

CCDC80, coiled–coil domain containing 80; EDA2R, ectodysplasin–A2 receptor; N–CDase, neutral ceramidase; NfL, neurofilament light chain; NT–proBNP, *N*–terminal prohormone of brain–type natriuretic peptide; RSPO3, R–spondin 3.

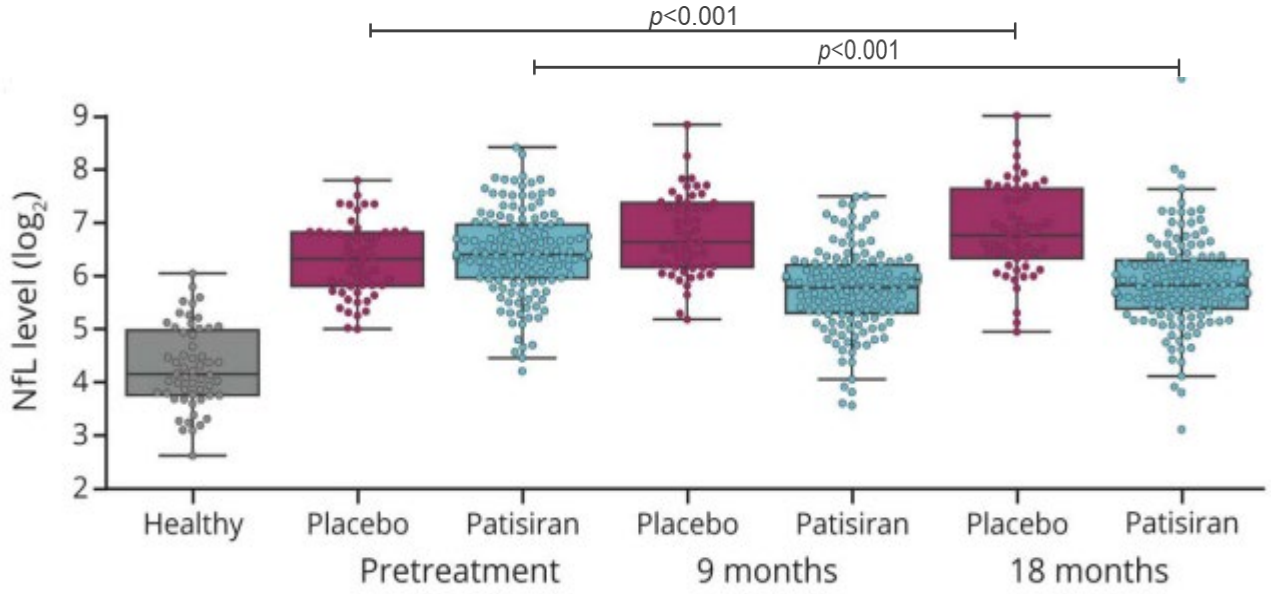
Ticau S et al. *Neurology* 2021;96:e412–e422.

NfL May Serve as a Biomarker for Disease Progression[1–3]

Results: Semi–quantitative NfL Measurements

- Patients diagnosed with hATTR amyloidosis with PN had **>4–fold higher** levels of NfL compared with healthy controls, independent of age adjustment (p<0.0001)
- NfL levels **significantly increased** in the placebo group at 18 months relative to baseline (p<0.001)
- NfL levels **significantly decreased** in the patisiran group at 18 months relative to baseline (p<0.001)

Change in NfL Levels in Patients Treated with Placebo or Patisiran over 18 Months



Levels of NfL (normalized protein expression values, which are on a $\log_2$ scale) in healthy controls and patients treated with placebo or patisiran at baseline, 9 months, or 18 months. Boxplots show the first quartile, median, and third quartile of the data. The whiskers are the minimum and maximum values within 1.5 × the interquartile range.

hATTR, hereditary transthyretin–mediated; NfL, neurofilament light chain; PN, polyneuropathy.

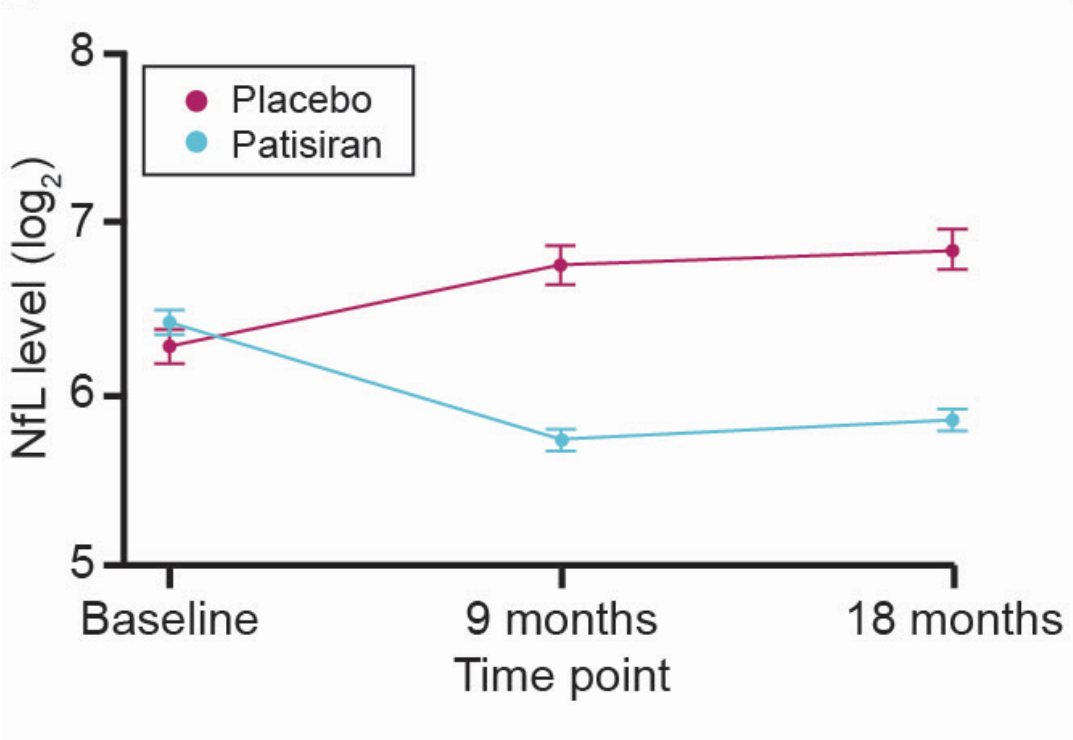
1. Ticau S et al. *Neurology* 2021;96:e412–e422; 2. Ticau S et al. *EU–ATTR* 2019; 3. Ticau S et al. *HFSA Virtual Meeting* 2020.

NfL May Serve as a Biomarker for Disease Progression[1–3]

Results: Semi–quantitative NfL Measurements

- NfL levels **significantly increased** in the placebo group at 18 months relative to baseline ($p<0.001$)
- NfL levels **significantly decreased** in the patisiran group at 18 months relative to baseline ($p<0.001$)

NfL Levels (Mean ± Standard Error) in Patients Treated with Patisiran or Placebo at Baseline, 9, and 18 Months



NfL, neurofilament light chain.

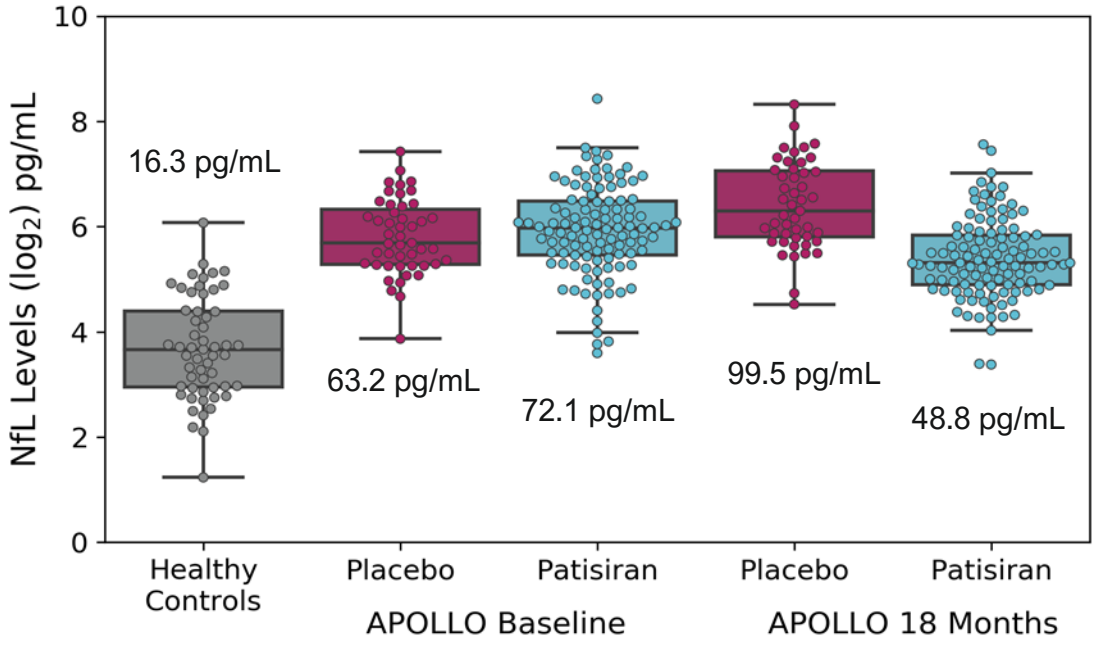
1. Ticau S et al. *Neurology* 2021;96:e412–e422; 2. Ticau S et al. *EU–ATTR* 2019; 3. Ticau S et al. *HFSA Virtual Meeting* 2020.

NfL May Serve as a Biomarker for Disease Progression[1,2]

Results: Quantitative NfL Measurements

- Patients diagnosed with hATTR amyloidosis with PN had significantly higher levels of NfL compared with healthy controls ($p<0.0001$ for both patisiran and placebo groups at baseline)
- NfL levels **significantly increased** in the placebo group at 18 months relative to baseline ($p<0.001$)
- NfL levels **significantly decreased** in the patisiran group at 18 months relative to baseline ($p<0.001$)

Levels of NfL in Healthy Controls and Patients Treated with Placebo or Patisiran at Baseline and 18 months



Numbers represent mean NfL levels. Boxplots show the first quartile, median, and third quartile of the data. The whiskers are the minimum and maximum values within 1.5 × the interquartile range. One outlier with a study-unrelated cerebral infarct at Month 17 with NfL plasma levels of 747 pg/mL in the patisiran-treated group was excluded from the calculations.

hATTR, hereditary transthyretin–mediated; NfL, neurofilament light chain; PN, polyneuropathy.

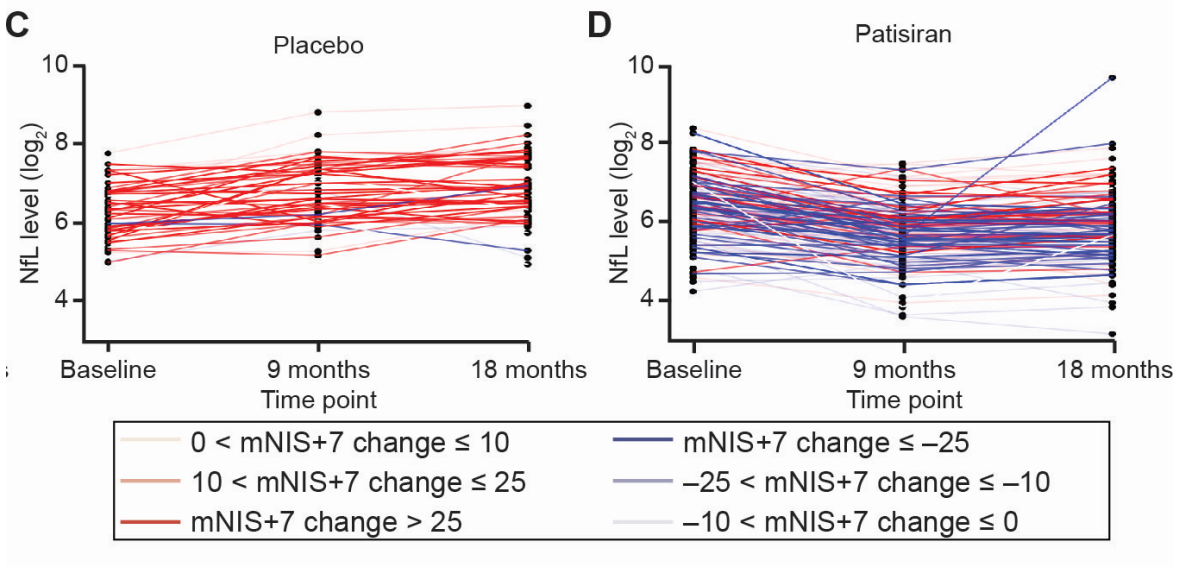
1. Ticau S et al. *Neurology* 2021;96:e412–e422; 2. Ticau S et al. *HFSA Virtual Meeting* 2020.

Changes in NfL Levels were Correlated with Changes in the Severity of PN

Results: Individual Patient Trajectories

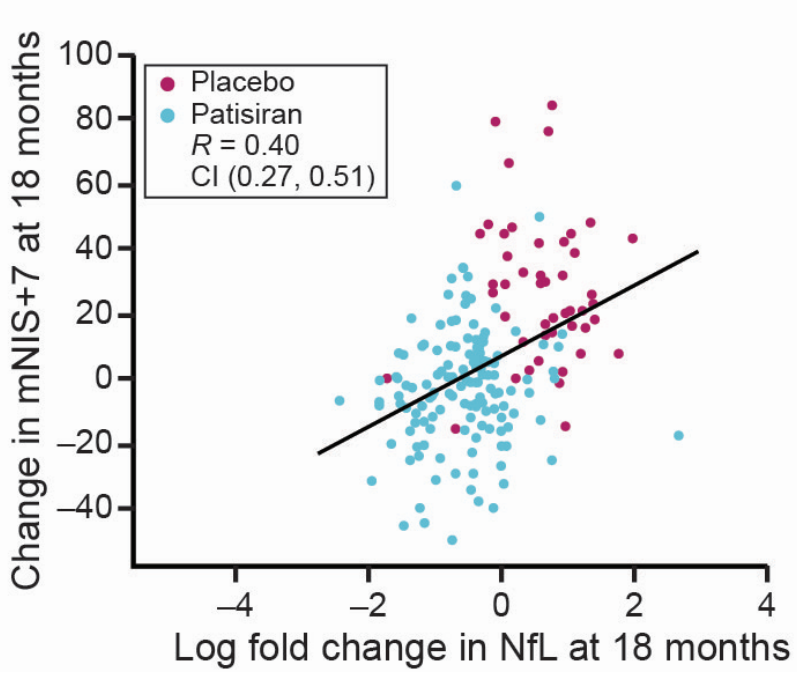
- Changes in plasma NfL levels were correlated with changes in mNIS+7 score at 18 months (R=0.4 [95% CI: 0.27–0.51]), indicating that decreasing plasma NfL levels are associated with an improvement in PN

NfL Trajectories of Patients Over Time



Trajectories of individual patients receiving placebo or patisiran over time, color-coded by their corresponding worsening (change in mNIS+7 score >0; red) or improvement (change in mNIS+7 score <0; blue) in mNIS+7 score from baseline to 18 months.

Correlation Between Change in NfL Levels from Baseline to 18 Months and the Corresponding Change In mNIS+7



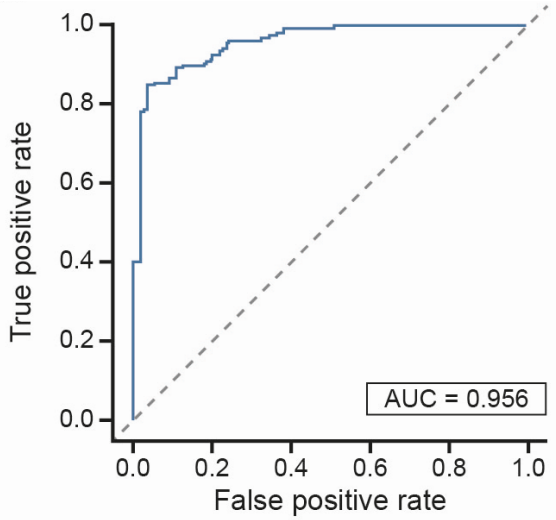
CI, confidence interval; mNIS, modified Neuropathy Impairment Score; NfL, neurofilament light chain; PN, polyneuropathy.

Ticau S et al. *Neurology* 2021;96:e412–e422.

NfL Levels Differentiated Patients with hATTR Amyloidosis from Healthy Controls

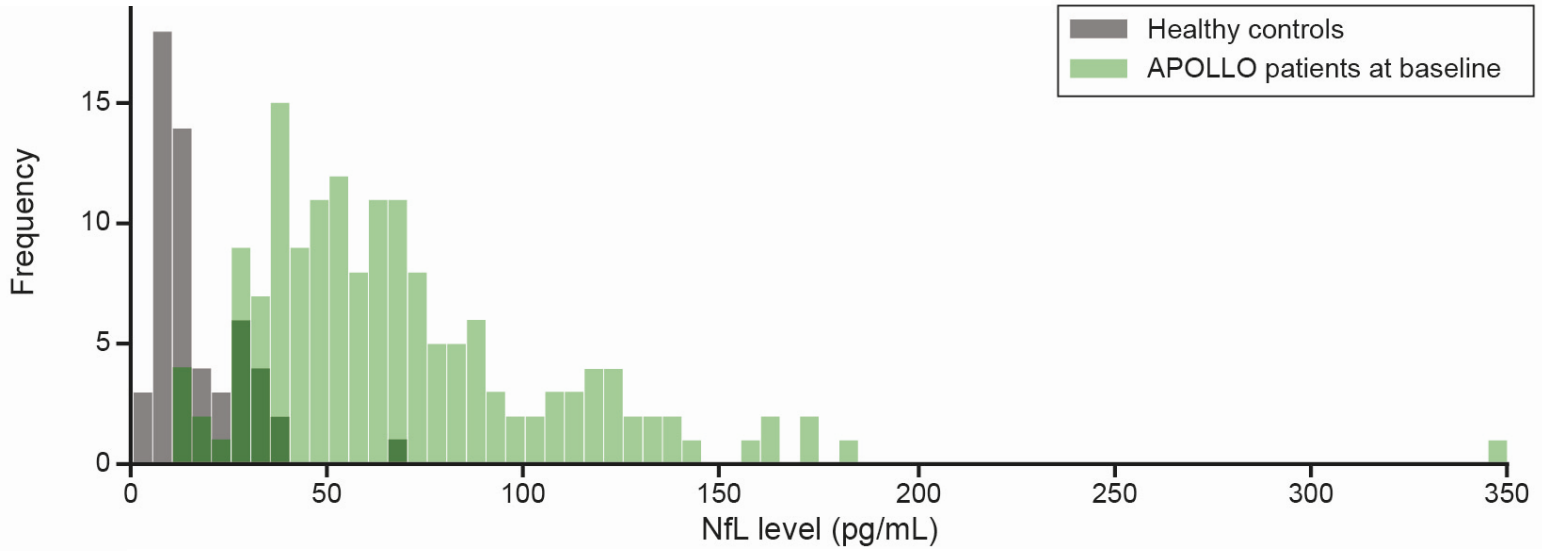
- Receiver–operating characteristic analysis determined that plasma NfL levels could discriminate between healthy controls and patients with hATTR amyloidosis with PN (AUC: 0.956)

ROC curve analysis of NfL plasma levels



ROC curve analysis of NfL plasma levels in healthy controls versus patients with hATTR amyloidosis with PN

Distributions of NfL Concentrations in Healthy Controls and APOLLO Patients



Histograms showing the distributions of NfL concentrations in healthy controls (dark gray) and patients with hATTR amyloidosis at baseline (green).

One outlier with a study–unrelated cerebral infarct at Month 17 with NfL plasma levels of 747 pg/mL in the patisiran–treated group was excluded from the calculations.

AUC, area under the curve; hATTR, hereditary transthyretin–mediated; NfL, neurofilament light chain; PN, polyneuropathy; ROC, receiver operator characteristic.

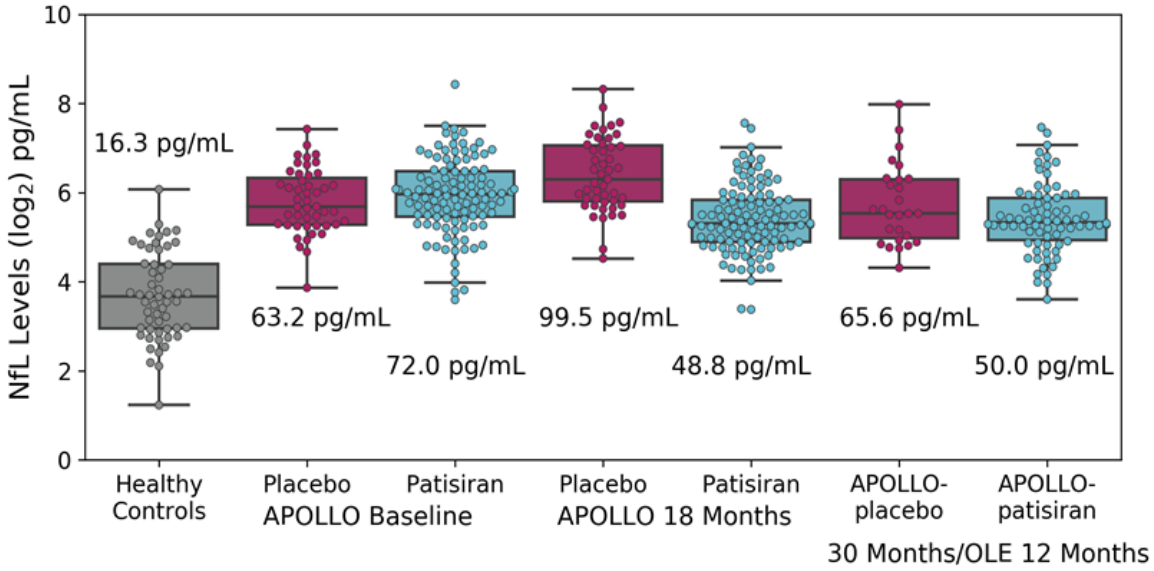
Ticau S et al. *Neurology* 2021;96:e412–e422.

NfL Levels Decreased when Placebo Patients Started Patisiran

12 Months in the Global OLE: APOLLO Patients

- NfL levels **remained steady after 12 months in the Global OLE** for the APOLLO-patisiran patients (mean 48.8 pg/mL vs 50.0 pg/mL)
- NfL levels **decreased significantly after 12 months of patisiran treatment** in APOLLO-placebo patients (mean 99.5 pg/mL vs 65.6 pg/mL; p<0.001)

Plasma NfL Levels of Healthy Controls and Patients from the APOLLO and Global OLE Studies at Baseline: 18 Months and 30 Months*



Numbers represent mean NfL levels. Boxplots show the first quartile, median, and third quartile of the data. The whiskers are the minimum and maximum values within 1.5 × the interquartile range.

*One individual with NfL levels of 747 pg/mL at 18 months and a cerebral stroke at 17 months was excluded from the analysis.

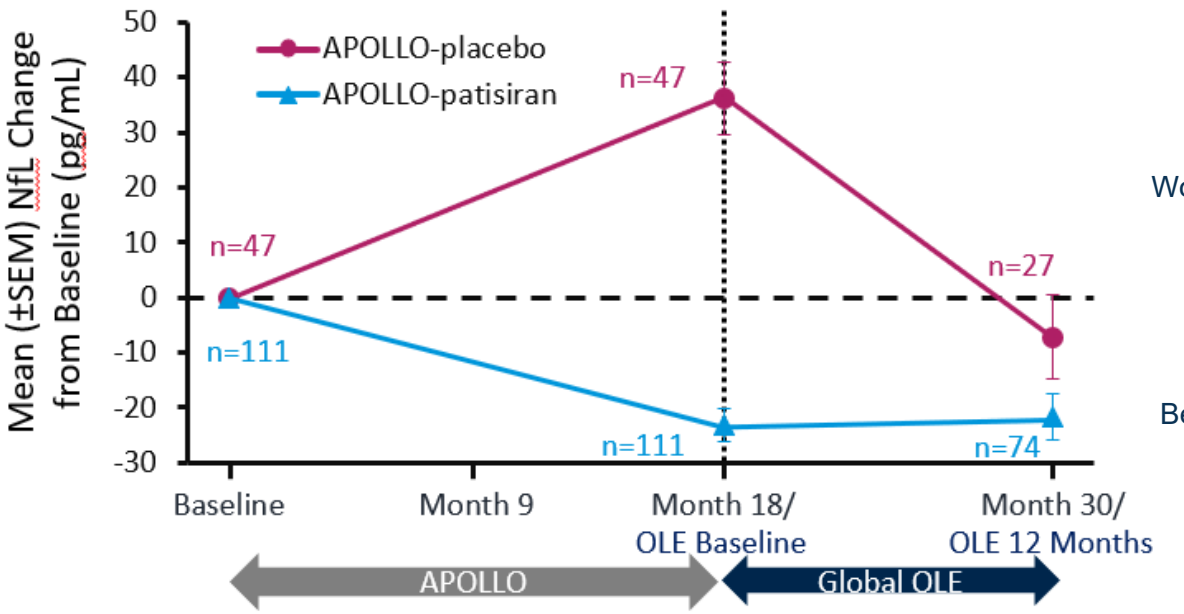
NfL, neurofilament light chain; OLE, open–label extension.

Ticau S et al. *PNS Virtual Meeting* 2020.

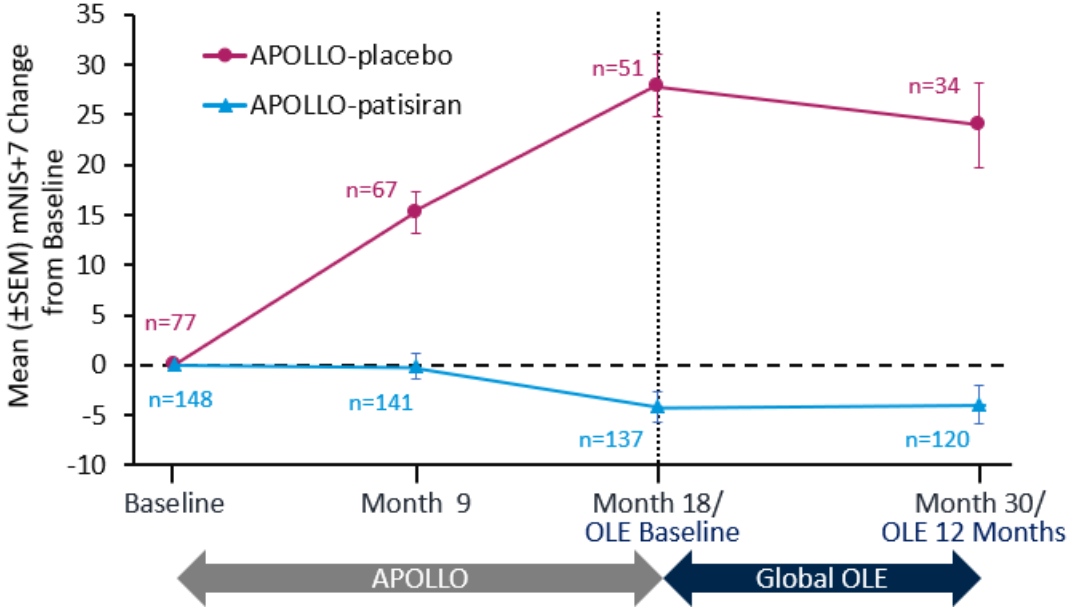
NfL Levels Decreased when Placebo Patients Started Patisiran[1]

12 Months in the Global OLE: APOLLO Patients[1,2]

Integrated Change in NfL during APOLLO and Global OLE*



Integrated Change in mNIS+7 during APOLLO and Global OLE



*One individual with NfL levels of 747 pg/mL at 18 months and a cerebral stroke at 17 months was excluded from the analysis.

mNIS+7, modified Neuropathy Impairment Score +7; NfL, neurofilament light chain; OLE, open–label extension; SEM, standard error of the mean.

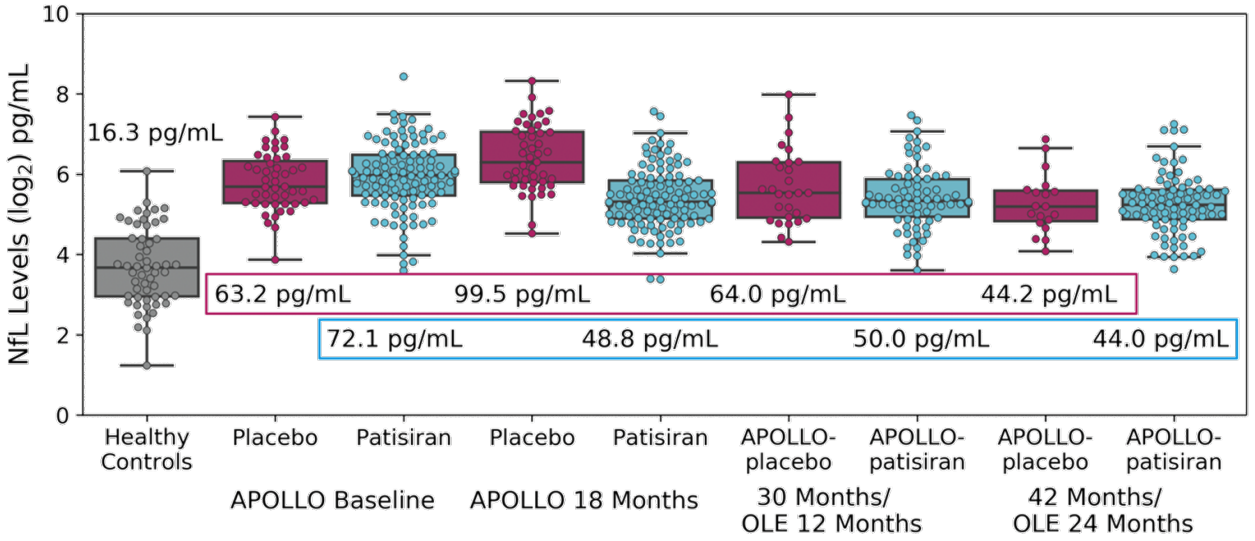
1. Ticau S et al. *PNS Virtual Meeting* 2020; 2. Adams D et al. *Lancet Neurol* 2021;20:49–59.

NfL Levels Remained Reduced with Continued Patisiran Treatment

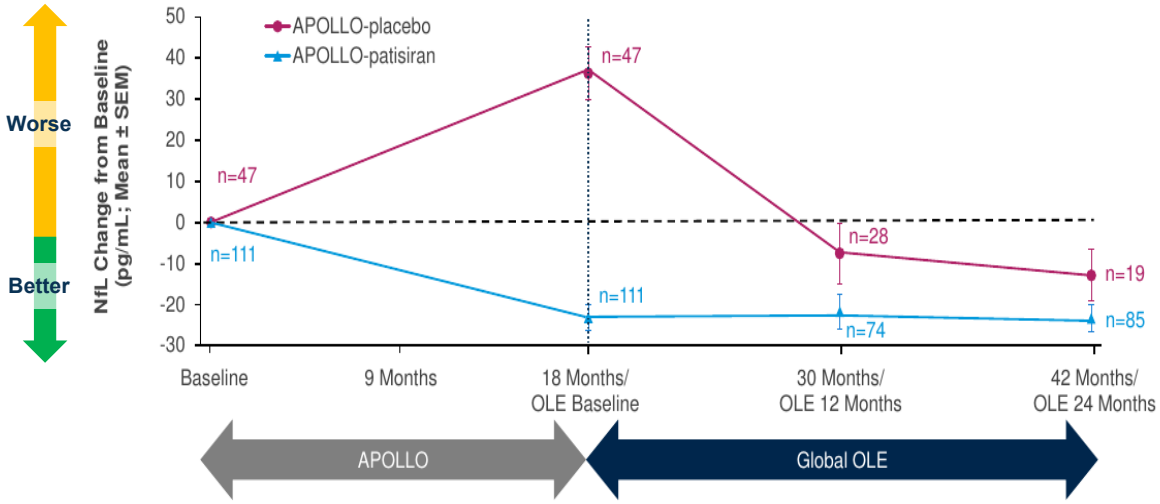
24 Months in the Global OLE: APOLLO Patients

- At Month 24 of the Global OLE, the reduction in NfL levels from parent study baseline in the APOLLO–patisiran group **continued to be significant** (mean 44.0 pg/mL, p<0.001)
- In the APOLLO–placebo group, the reduction of NfL levels observed at the end of Month 12 of the Global OLE continued at 24 months (mean 44.2 pg/mL) to reach levels **below the APOLLO baseline** and to a **similar level to the APOLLO-patisiran group**

NfL Levels during APOLLO and the Global OLE



Change in NfL from Parent Study Baseline



Numbers represent mean NfL levels

NfL, neurofilament light chain; OLE, open–label extension; SEM, standard error of the mean.

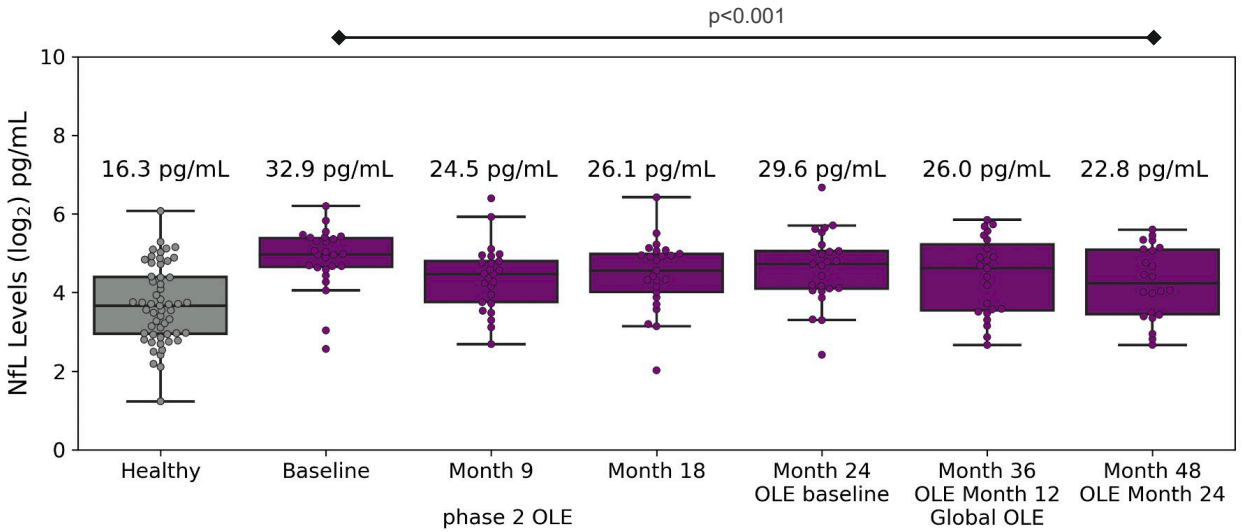
Polydefkis M et al. *AAN Virtual Meeting* 2021.

NfL Levels Decreased with Patisiran Treatment

24 Months in the Global OLE: Phase 2 OLE Patients

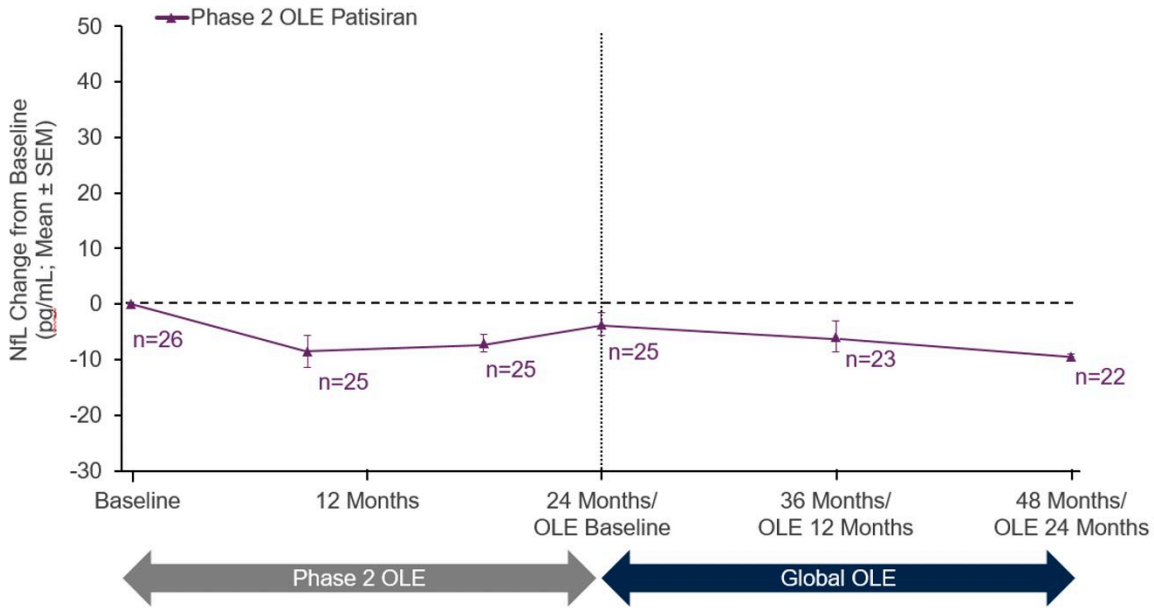
- Reduction in NfL levels from parent study baseline was significant at Month 24 of the Global OLE ($p<0.001$) in Phase 2 OLE patients

NfL Levels in Healthy Controls and Patients Treated with Patisiran at Baseline, during Phase 2 OLE, and Global OLE



Numbers represent mean NfL levels.

Mean Change from Baseline in NfL Levels in Patients Treated with Patisiran in the Phase 2 OLE and Global OLE



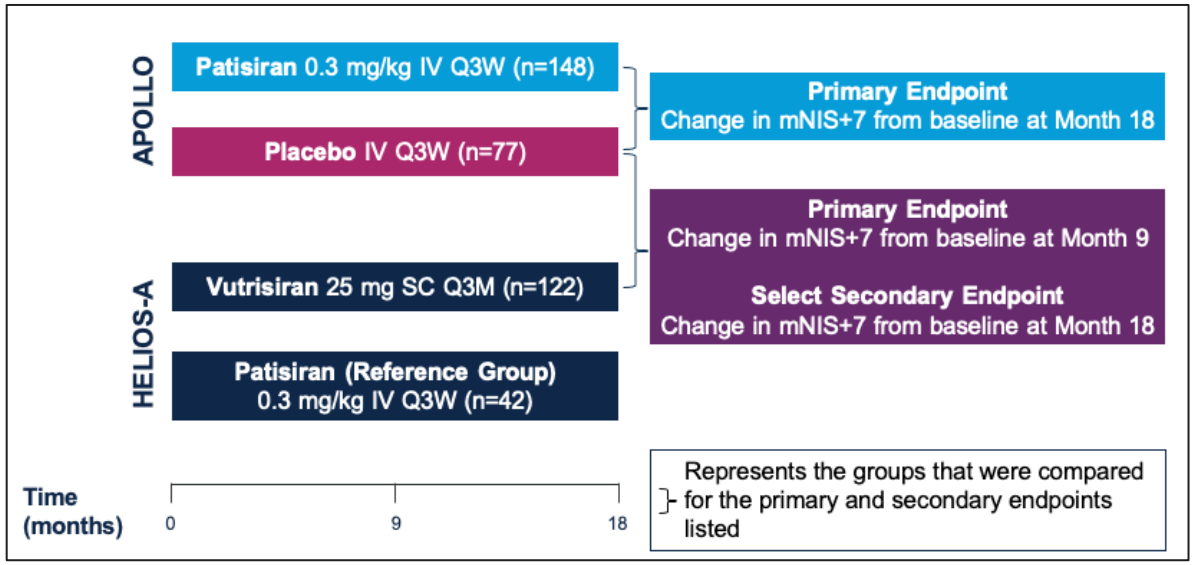
NfL, neurofilament light chain; OLE, open–label extension; SEM, standard error of the mean.

Poldefkis M et al. *AAN Virtual Meeting* 2021.

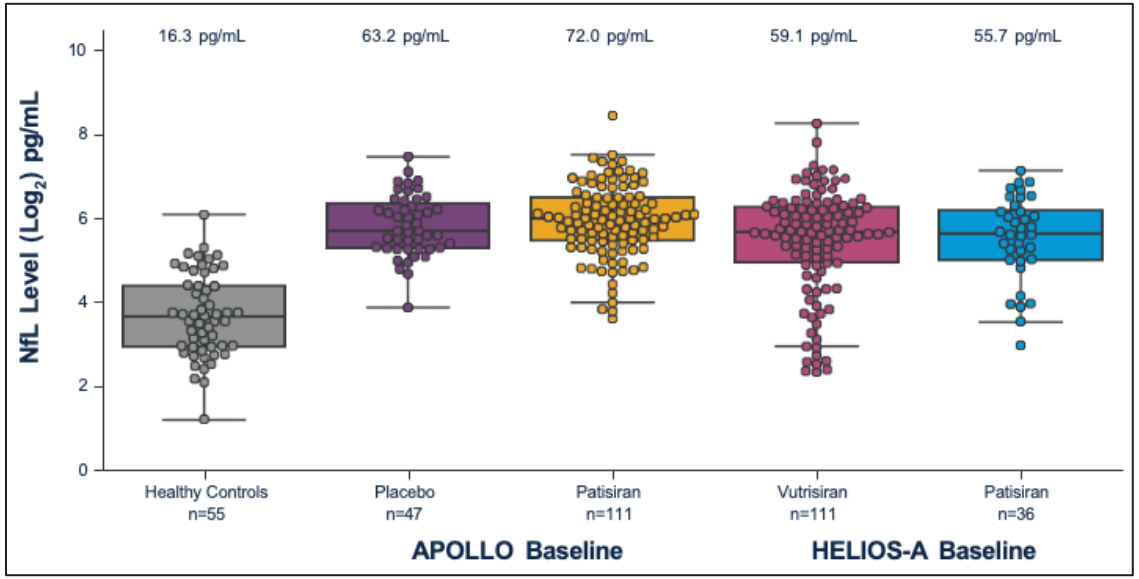
HELIOS-A Results Support that NfL Levels are Increased in hATTR Amyloidosis Patients with PN

- Post-hoc analyses demonstrated that baseline NfL levels in the patient populations of the APOLLO and HELIOS-A studies were significantly higher than in healthy controls ($p<0.001$)

Treatment Arms of the APOLLO and HELIOS-A Studies



Baseline NfL Levels in APOLLO and HELIOS-A Studies

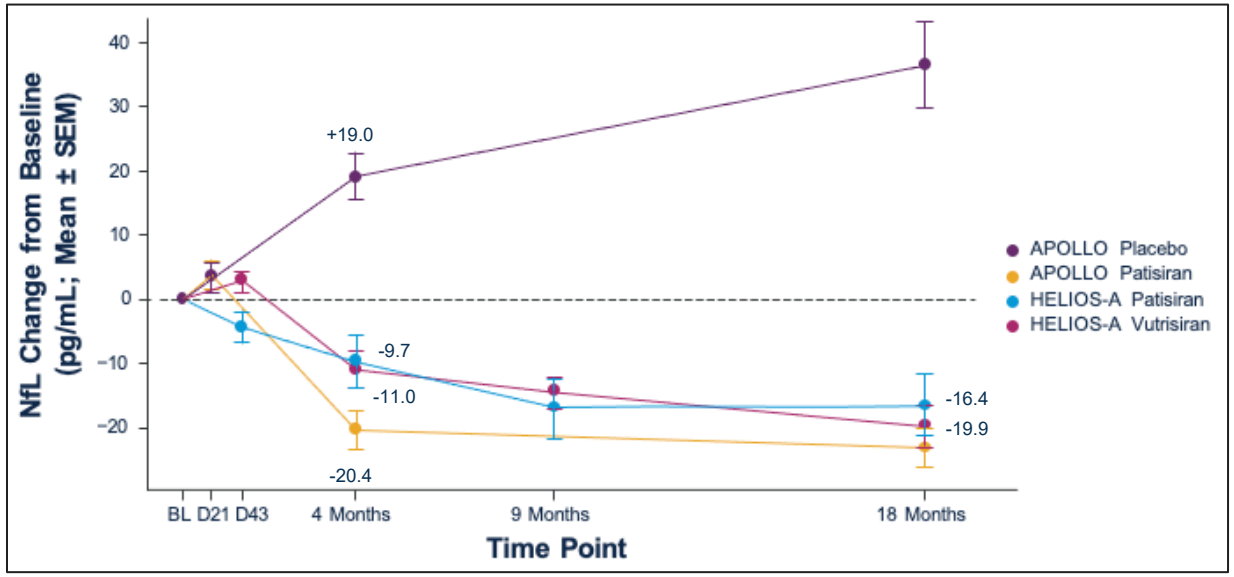


Aldinc E et al. *ANA Annual Meeting* 2022.

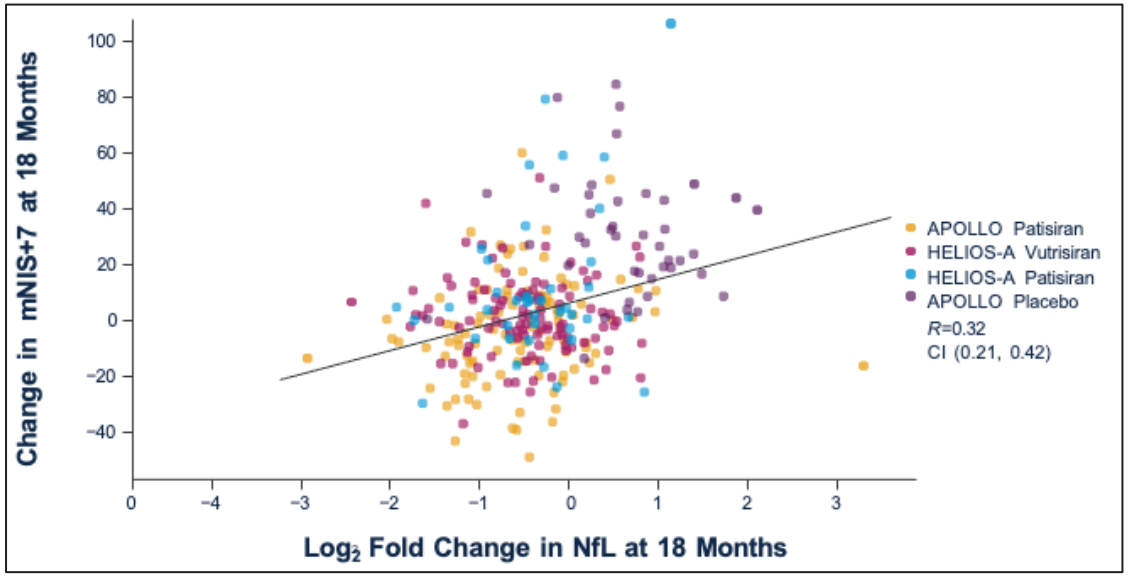
HELIOS-A Results Support that NfL Levels Significantly Decreased Following Treatment with RNAi Therapeutics

- In HELIOS-A, NfL levels in both the vutrisiran and patisiran groups decreased significantly from baseline as early 4 months ($p<0.05$), and these were maintained to 18 months post-treatment initiation ($p<0.01$)
  - These results are consistent with the results in patisiran-treated patients from APOLLO
- A positive moderate correlation (R=0.32) was observed between change in NfL levels and change in mNIS+7 in HELIOS-A and APOLLO at 18 months

Change in NfL Levels from Baseline in APOLLO and HELIOS-A Studies



Correlation between Change in mNIS+7 and Change in NfL Levels from Baseline in APOLLO and HELIOS-A



Aldinc E et al. *ANA Annual Meeting* 2022.

Summary

- NfL is a **well–studied biomarker for neuroaxonal injury** in various central and peripheral nervous system diseases[1–4]
- NfL levels **are found to be elevated** in patients with PN of hATTR amyloidosis versus healthy controls[5–7]
- Treatment with RNAi therapeutics **significantly lowered levels of NfL** in patients with hATTR amyloidosis with polyneuropathy.[5,9] This **occurred as early as Day 126 and remained low over time**[8,9]

CM, cardiomyopathy; hATTR, hereditary transthyretin–mediated; NfL, neurofilament light chain; PN, polyneuropathy; PND, polyneuropathy disability.

1. Thebault S et al. *Mult Scler* 2022; 28(10): 1491-1497; 2. Lewczuk P et al. *Alzheimers Res Ther* 2018;10(1) :71; 3. Bischof A et al. *Ann Rheum Dis* 2018;77(7):1093–1094; 4. Van Lieverloo GGA et al. *J Peripher Nerv Sys* 2019;24(2):187–194; 5. Ticau S et al. *Neurology* 2021;96:e412–e422; 6. Kapoor M et al. *J Peripher Nerv Syst* 2019;24(4):314–319. 7. Maia LF et al. *Amyloid* 2020;27(2):97–102; 8. Alnylam Pharmaceuticals. Data on file; 9. Aldinc E et al. *ANA Annual Meeting* 2022.

Conclusions

- NfL is a **well–studied biomarker** for neuroaxonal injury[1–4]
- NfL may be a useful biomarker for **monitoring disease progression and response to treatment** in patients with PN of hATTR amyloidosis[5–8]

ATTR, acquired transthyretin–mediated; hATTR, hereditary transthyretin–mediated; NfL, neurofilament light chain.

1. Thebault S et al. *Mult Scler* 2022; 28(10): 1491-1497; 2. Lewczuk P et al. *Alzheimers Res Ther* 2018;10:71; 3. Bischof A et al. *Ann Rheum Dis* 2018;77:1093–1094; 4. van Lieverloo GGA et al. *J Peripher Nerv Syst* 2019;24:187–194; 5. Ticau S et al. *Neurology* 2021;96:e412–e422; 6. Kapoor M et al. *J Peripher Nerv Syst* 2019;24:314–319; 7. Maia LF et al. *Amyloid* 2020;27:97–102; 8. Aldinc E et al. *ANA Annual Meeting* 2022.