Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Adams D, Gonzalez-Duarte A, O'Riordan WD, et al. Patisiran, an RNAi therapeutic, for hereditary transthyretin amyloidosis. N Engl J Med 2018;379:11-21. DOI: 10.1056/NEJMoa1716153

SUPPLEMENTARY APPENDIX

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Contents

APOLLO STUDY INVESTIGATORS

We would also like to acknowledge the contributions of the remaining APOLLO study investigators:

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ADDITIONAL METHODOLOGICAL DETAILS

Pharmacodynamic Assessments

Levels of serum TTR protein, vitamin A, and retinol-binding protein (RBP) were measured at baseline, pre-dose on Day 0 and on Days 21, 126, 252, 273, 399, 546, and post-dose on Day 253-272 (9 months) and Day 553–560 (18 months). Serum TTR was measured using an enzyme-linked immunosorbent assay (ELISA) and a turbidimetric assay. Serum RBP was quantified using nephelometry. Serum samples were evaluated by a high-performance liquid chromatography assay to determine vitamin A levels.

Cardiac Structure and Function Assessments

Cardiac structure and function were assessed through echocardiograms and measurement of serum levels of cardiac biomarkers, pre-dose on Day 0, and on Days 253–272 (9 months) and 553–560 (18 months). Echocardiograms were obtained according to a pre-defined protocol and analysed and interpreted in a blinded core laboratory. Select parameters reported included mean left ventricular wall thickness (normal: <12 mm, lower value indicates less cardiac amyloid involvement) and longitudinal strain (normal: -16 to -21%, lower value [more negative] indicates better systolic function). NT-proBNP (normal: <125 pg/mL, lower value indicates less cardiac stress) was assessed using an electrochemiluminescence method (Roche Diagnostics) and quantification performed at a central laboratory.

Safety Assessments

Adverse events (AEs) were assessed throughout the study and reported according to the Medical Dictionary of Regulatory Activities (MedDRA®; version 18.0). AEs were graded based on their severity (mild, moderate, or severe) and the causal relationship to study drug or premedication recorded. Clinical laboratory and serum chemistry tests, thyroid function parameters, urinalysis, anti-drug antibodies, electrocardiograms, physical and vital signs, and ophthalmology examinations (including electroretinography) were also monitored pre-dose on Day 0 and throughout the study as specified in the protocol. Serial electroretinograms (ERGs) were performed in all patients and were centrally read.

Statistical Assessments

Primary, secondary, and exploratory endpoints were analyzed using a restricted maximum likelihood (REML) based mixed-effects model repeated measures (MMRM) method. The outcome variable was change from baseline. Baseline value was included as a continuous covariate, and treatment group, visit (Month 9 or 18), treatment-by-visit interaction, age at symptom onset (<50 vs >=50), geographic region (North America, Western Europe, and Rest of World), genotype (V30M vs non-V30M) and prior stabilizer use (yes vs no) were included as fixed effect terms. Analyses were conducted using SAS PROC MIXED.

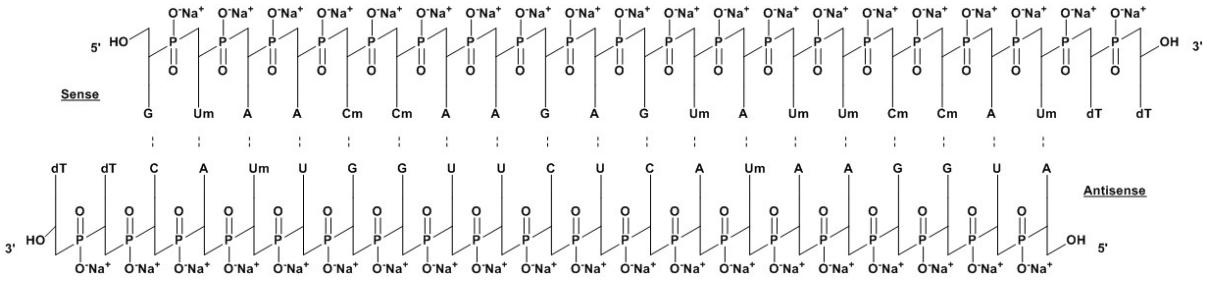
For mNIS+7, two thresholds were prespecified for binary analyses: < 10-point increase and < 0-point increase from baseline at Month 18. The numbers and percentages of patients were estimated and compared between two treatment groups using the Cochran-Mantel-Haenszel test, stratified by genotype (V30M vs non-V30M). For Norfolk QOL, NIS-W and 10-MWT gait speed, binary analyses were conducted post hoc to estimate the percentages of patients with <0-point increase (or >0 m/sec increase for gait speed) from baseline at Month 18.

For categorical variables, the number and percentage of patients in each category were calculated. For continuous variables, mean, median, standard deviation, and range were calculated.

Based on the therapeutic hypothesis for patisiran that TTR reduction would result in clinical benefit in patients with hATTR amyloidosis with polyneuropathy, the correlation between TTR reduction and change in mNIS+7 was evaluated using the Pearson correlation coefficient.

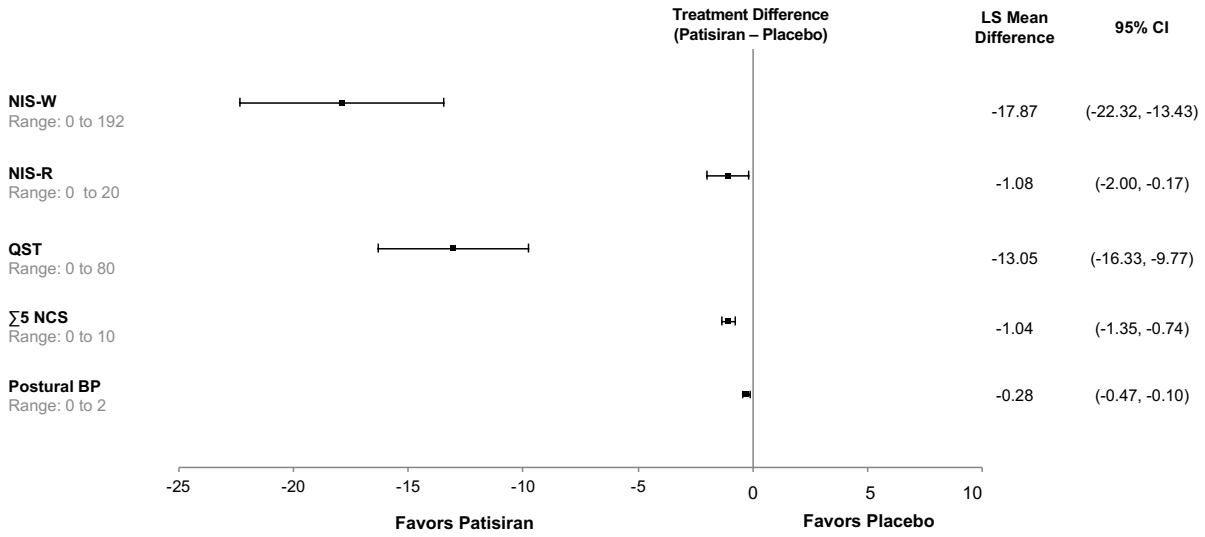
SUPPLEMENTARY FIGURES

Figure S1. Structural Formula of Patisiran. Patisiran is a double-stranded small interfering ribonucleic acid (siRNA), formulated as lipid nanoparticles for delivery to hepatocytes. Patisiran specifically binds to a genetically conserved sequence in the 3'-untranslated region (3'-UTR) of mutant and wild-type transthyretin (TTR) messenger RNA (mRNA). All of the pyrimidines in the sense strand (top) and two of the uridines in the antisense strand (bottom) contain 2'-O-methyl modified ribonucleosides with the remaining being unmodified ribonucleotides. Both sense and antisense strands contain 2'-deoxythymidine dinucleotide overhangs at their respective 3'-ends. All internucleotide linkages are natural, chemically unmodified phosphodiester linkages.



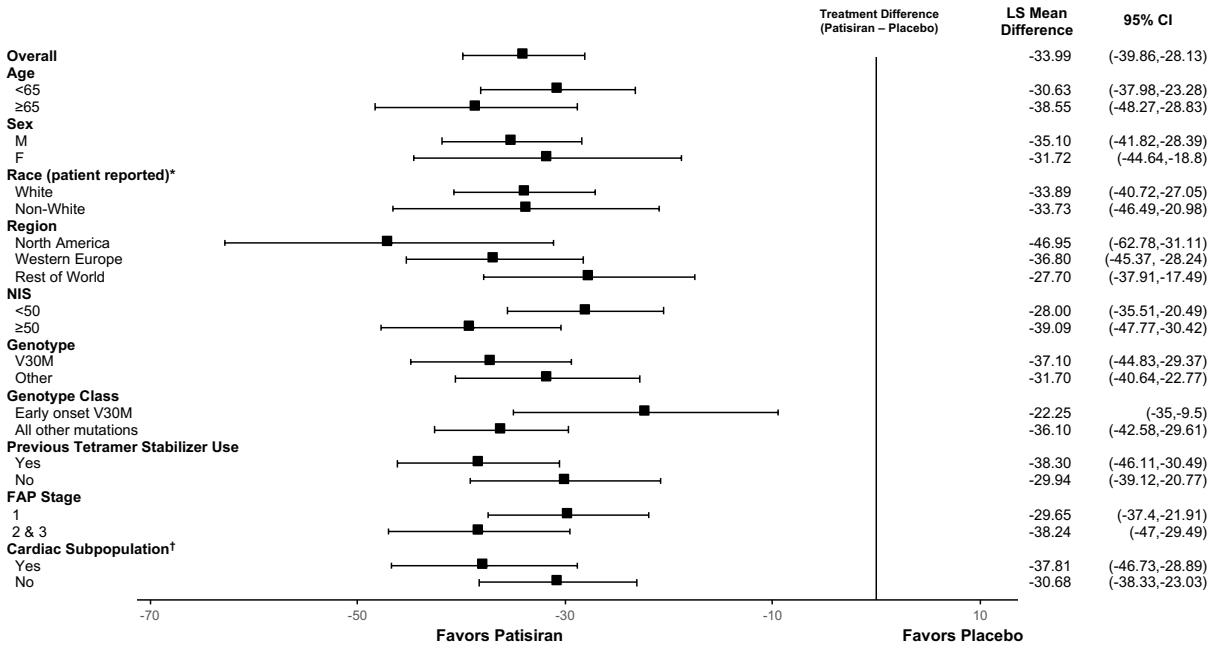
A, adenosine; C, cytidine; G, guanosine; U, uridine; Cm, 2'-*O*-methylcytidine; Um, 2'-*O*-methyluridine; dT, thymidine

Figure S2. Forest Plot Showing the LS Mean Difference Between Patisiran and Placebo Treatment Groups in Change from Baseline to Month 18 in mNIS+7 Subcomponents.



BP, blood pressure; CI, confidence interval; LS, least square; mNIS+7, modified neurologic impairment score +7; NCS, nerve conduction studies; NIS-R, Neurologic Impairment Score-reflexes; NIS-W, Neurologic Impairment Score-Weakness; QST, quantitative sensory testing.

Figure S3. Subgroup Analysis of mNIS+7. Forest plot showing the LS mean difference in mNIS+7 change from baseline to Month 18 between patisiran- and placebo-treated patients within subgroups.

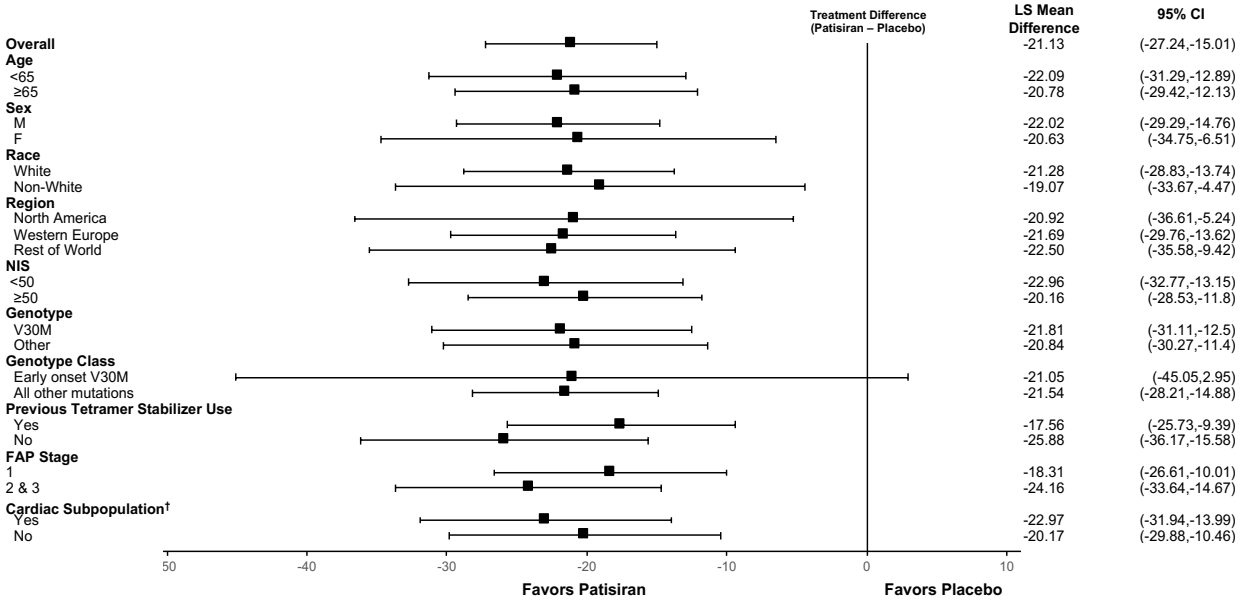


CI, confidence interval; F, female; FAP, familial amyloid polyneuropathy; LS, least squares; M, male; mNIS+7 modified Neurologic Impairment Score +7; NIS, Neurologic Impairment Score.

*Race was patient reported, Non-White subgroup: patients who identified themselves as Asian, American Indian or Alaska Native, Black or African American, Native Hawaiian or other Pacific Islander, or Other; patients who did not report race or reported more than one race were not counted in either subgroup.

†Cardiac subpopulation: patients with a baseline left ventricular wall thickness of 13 mm or more in the absence of a history of aortic valve disease or hypertension.

Figure S4. Subgroup Analysis of Norfolk QOL-DN. Forest plot showing the LS mean difference in Norfolk QoL-DN change from baseline to Month 18 between patisiran- and placebo-treated patients within subgroups.



CI, confidence interval; F, female; FAP, familial amyloid polyneuropathy; LS, least squares; M, male; mNIS+7; NIS, Neurologic Impairment Score; Norfolk QOL-DN denotes Norfolk Quality of Life-Diabetic Neuropathy.

*Race was patient reported, Non-White subgroup: patients who identified themselves as Asian, American Indian or Alaska Native, Black or African American, Native Hawaiian or other Pacific Islander, or Other; patients who did not report race or reported more than one race were not counted in either subgroup.

†Cardiac subpopulation: patients with a baseline left ventricular wall thickness of 13 mm or more in the absence of a history of aortic valve disease or hypertension.

SUPPLEMENTARY TABLES

Table S1. *TTR* Genotype of Patients Enrolled.

Genotype — no. (%)	Placebo (N=77)	Patisiran (N=148)	Total (N=225)
V30M	40 (51.9)	56 (37.8)	96 (42.7)
A97S	11 (14.3)	10 (6.8)	21 (9.3)
T60A	4 (5.2)	12 (8.1)	16 (7.1)
E89Q	4 (5.2)	10 (6.8)	14 (6.2)
S50R	3 (3.9)	9 (6.1)	12 (5.3)
S77Y	2 (2.6)	5 (3.4)	7 (3.1)
D38A	1 (1.3)	4 (2.7)	5 (2.2)
F64L	1 (1.3)	4 (2.7)	5 (2.2)
I107V	0	5 (3.4)	5 (2.2)
E89K	0	3 (2.0)	3 (1.3)
G47A	0	3 (2.0)	3 (1.3)
L58H	0	3 (2.0)	3 (1.3)
T49A	0	3 (2.0)	3 (1.3)
G47E	0	2 (1.4)	2 (0.9)
G47V	0	2 (1.4)	2 (0.9)
K35N	1 (1.3)	1 (0.7)	2 (0.9)
S77F	0	2 (1.4)	2 (0.9)
V122I	1 (1.3)	1 (0.7)	2 (0.9)
Y114C	1 (1.3)	1 (0.7)	2 (0.9)
A36P	0	1 (0.7)	1 (0.4)
A45T	1 (1.3)	0	1 (0.4)

D38V	0	1 (0.7)	1 (0.4)
E42G	0	1 (0.7)	1 (0.4)
E54D	0	1 (0.7)	1 (0.4)
E54Q	0	1 (0.7)	1 (0.4)
E61K	1 (1.3)	0	1 (0.4)
F33L	1 (1.3)	0	1 (0.4)
F44S	0	1 (0.7)	1 (0.4)
G42D	0	1 (0.7)	1 (0.4)
H88R	0	1 (0.7)	1 (0.4)
I84T	1 (1.3)	0	1 (0.4)
I107V	1 (1.3)	0	1 (0.4)
P24S	1 (1.3)	0	1 (0.4)
S50I	0	1 (0.7)	1 (0.4)
S52P	1 (1.3)	0	1 (0.4)
T49I	0	1 (0.7)	1 (0.4)
T59K	1 (1.3)	0	1 (0.4)
V71A	0	1 (0.7)	1 (0.4)
Y78F	0	1 (0.7)	1 (0.4)

TTR, transthyretin.

Table S2. Serious Adverse Events (Occurring in ≥2% in Either Treatment Group).

Preferred AE Term — no. of patients (%)	Placebo (N=77)	Patisiran (N=148)
At least one serious AE	31 (40.3)	54 (36.5)
Diarrhea	1 (1.3)	8 (5.4)
Pneumonia	3 (3.9)	3 (2.0)
Cardiac failure	2 (2.6)	3 (2.0)
Cardiac failure congestive	2 (2.6)	3 (2.0)
Acute kidney injury	4 (5.2)	1 (0.7)
Orthostatic hypotension	1 (1.3)	3 (2.0)
Dehydration	3 (3.9)	1 (0.7)
Vomiting	3 (3.9)	1 (0.7)
Urinary tract infection	4 (5.2)	0 (0.0)
Atrioventricular block complete	0 (0.0)	3 (2.0)
Constipation	2 (2.6)	0 (0.0)
Hereditary neuropathic amyloidosis	2 (2.6)	0 (0.0)
Hyponatremia	2 (2.6)	0 (0.0)
Pneumonia aspiration	2 (2.6)	0 (0.0)

AE, adverse event

Table S3. Summary of IRR Events.		
Preferred AE Term — no. of patients (%)	**Placebo (N=77)**	**Patisiran (N=148)**
Number of patients with any IRR	7 (9.1)	28 (18.9)
Number of patients with IRR Symptoms		
Back pain	0	9 (6.1)
Flushing	6 (7.8)	6 (4.1)
Nausea	0	5 (3.4)
Abdominal pain	0	5 (3.4)
Headache	1 (1.3)	4 (2.7)
Arthralgia	0	3 (2.0)
Dyspnea	0	3 (2.0)
Number of patients with IRR leading to infusion interruption	0	7 (4.7)
Number of patients with IRR leading to treatment discontinuation	0	1 (0.7)
Number of infusions interruption or discontinuation due to IRRs	0	17 of 3740 infusions (0.5%)

AE, adverse event; IRR, infusion-related reaction.

Table S4. Summary of Deaths	
Treatment Group	**Causes of Death**[a]
Patisiran (n=7; 4.7%)	
Patient 1	Cardiac arrest, cardiac failure congestive
Patient 2	Sudden cardiac death[b]
Patient 3	Sudden cardiac death
Patient 4	Cardiac failure, acute pulmonary edema
Patient 5	Cardiac arrest
Patient 6	Pulseless electrical activity
Patient 7	Cardiac failure
Placebo (n=6; 7.8%)	
Patient 1	Subarachnoid hemorrhage
Patient 2	Staphylococcal sepsis
Patient 3	Anemia, gastrointestinal hemorrhage (complicated by heart failure)[b]
Patient 4	Acute kidney failure, urinary tract infection, bacteremia
Patient 5	Colorectal cancer metastatic[b]
Patient 6	Ischemic stroke

[a] All deaths were considered unlikely or not related to study drug by the investigators

[b] Patient off treatment for more than 30 days