Supplemental Material for

Title: Predictive Model of Response to Tafamidis in Hereditary ATTR Polyneuropathy

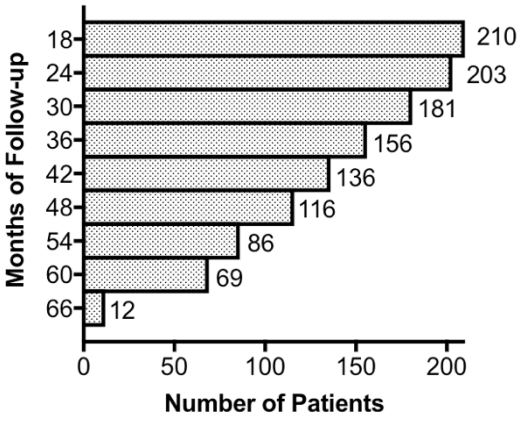
Supplemental Tables

Supplemental Methods

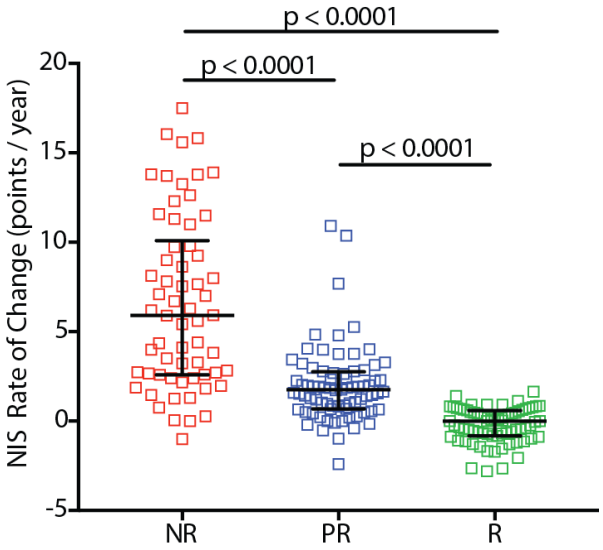
Synthesis of Tafamidis-glucuronide

Supplemental Figure 1. Number of patients according to number of follow-up months

A minimum of 2 years (24 months) for all patients, or 1.5 years (18 months) for patients that suspended therapy at 18 months, was established as the inclusion criterion. The exact number of patients in each group is shown in front of the corresponding bar.

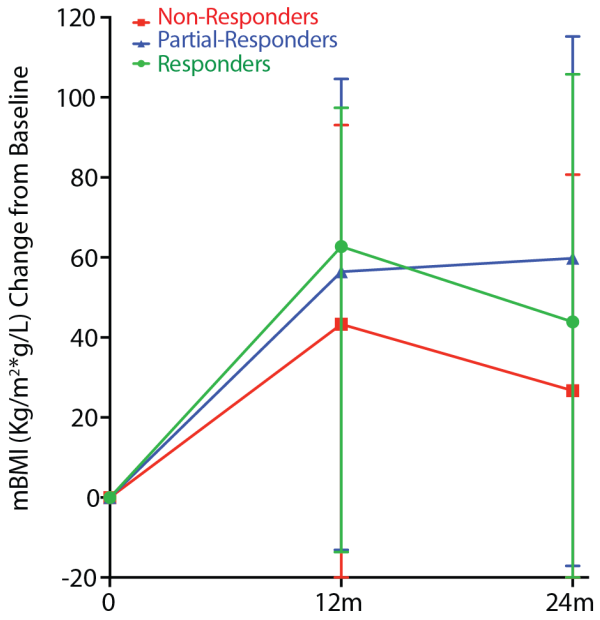


Supplemental Figure 2. Rate of NIS change according to response classification. Kruskal-Wallis Test with Dunn's correction for multiple comparison was used to calculate P values. Horizontal bars represent median and errors bar represent interquartile range.

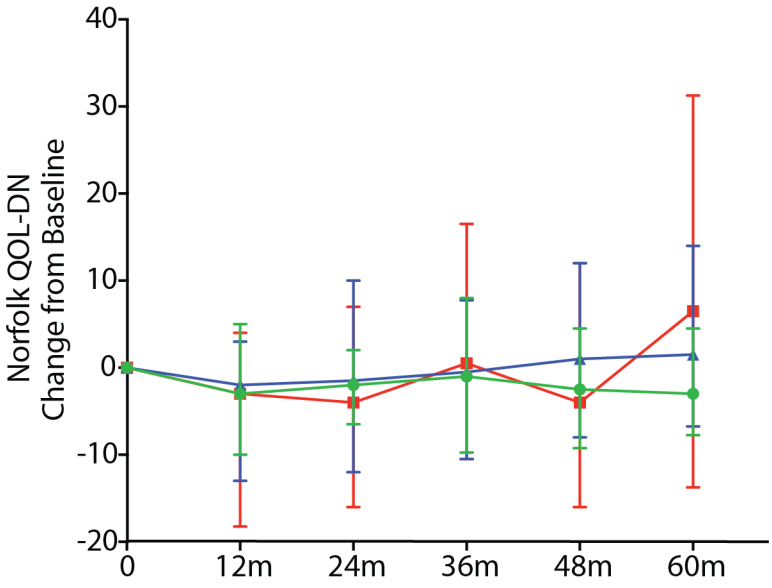


Supplemental Figure 3. Change in mBMI from baseline according to response classification.

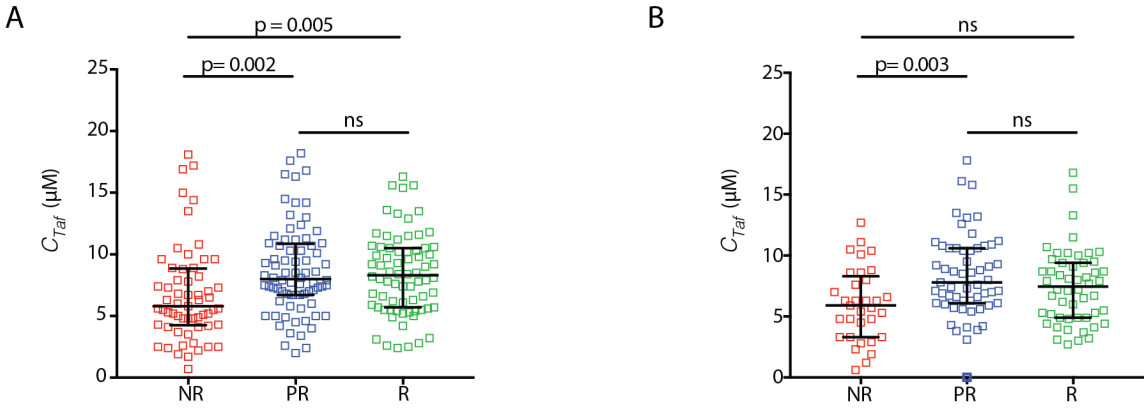
mBMI is modified body mass index (calculated as BMI multiplied by serum albumin in g/L).



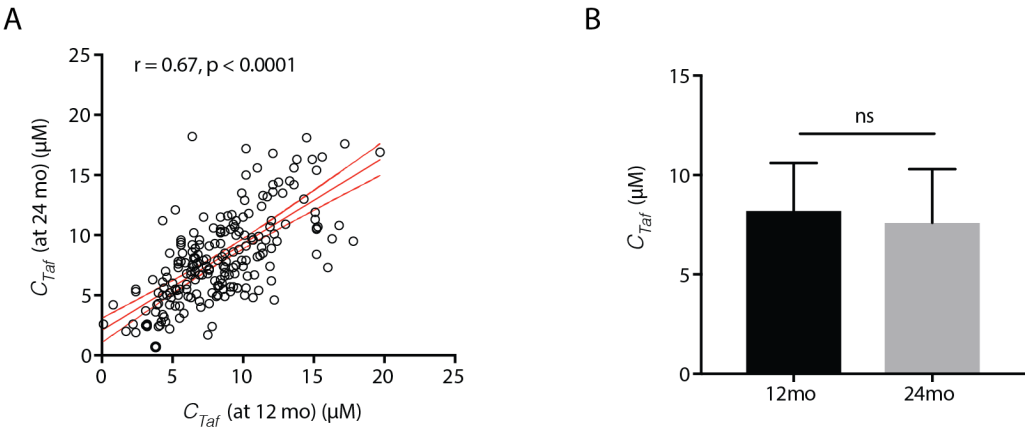
Supplemental Figure 4. Change in Norfolk QOL-DN from baseline according to response classification. No statistical difference was found in Norfolk QOL-DN between the three response groups.



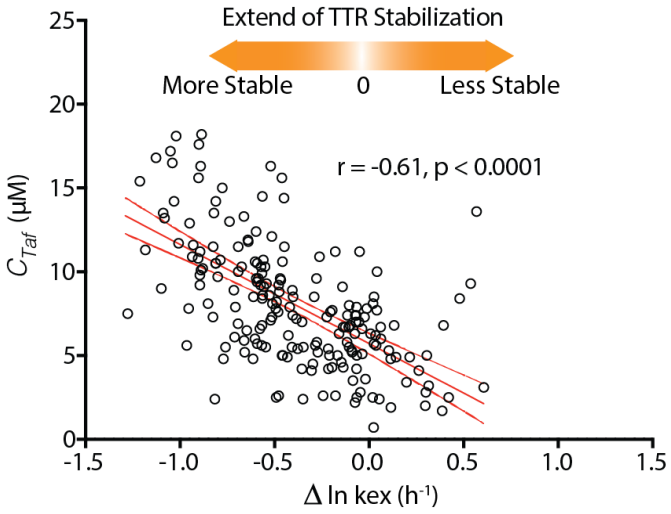
Supplemental Figure 5 Tafamidis levels (C_{Taf}) are different according to response classification. (A) C_{Taf} in plasma samples collected after 24 months of therapy with tafamidis is lower in Non-Responders (n = 61; median 5.8 μM), than in Partial-Responders (n = 76; 8.0 μM) and Responders (n = 72; 8.3 μM) **(B)** C_{Taf} in plasma samples collected at the last visit (36, 48 or 60 months) of therapy with tafamidis is lower in Non-Responders (n = 33; median 5.9 μM), than in Partial-Responders (n = 57; 7.8 μM). Although Responders have higher tafamidis levels at the last visit (n = 52; 7.5 μM) when compared to Non-Responders, this difference is not statistically significant; we believe that this lack of statistical significance is because of the low number of patients, especially Non-Responders, that had visits at longer time points. P values were calculated using Kruskal-Wallis test with Dunn's correction for multiple comparisons. Horizontal bars represent median and errors bar represent interquartile range.



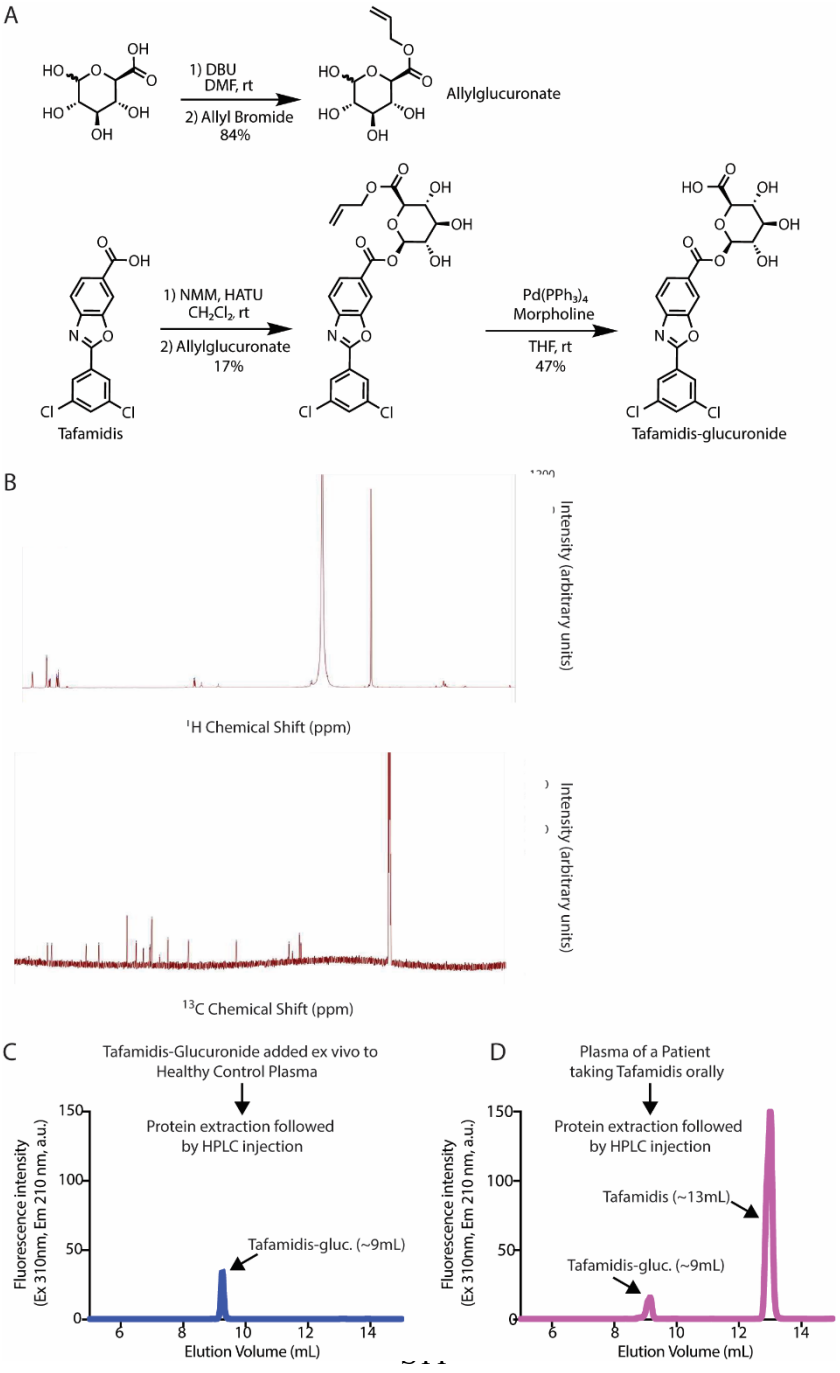
Supplemental Figure 6. Plasma tafamidis levels are overall stable within each individual throughout this study. (A) C_{Taf} at 12 months correlates well with C_{Taf} at 24 months. Each dot represents one patient (n = 207); the results acquired from the 12 months' sample are shown in the x axis; results from the 24 months' sample in the y-axis. r: Spearman correlation coefficient; red full line represents best-fit ($R^2 = 0.44$), dotted red lines represent 95% confidence intervals of the best-fit. **(B)** No statistically significant differences in C_{Taf} were found within the same individual at 12 and 24 months. P value was calculated using the Wilcoxon matched-pairs signed rank test (n = 207 paired samples); median C_{Taf} at 12 months was 8.2 μM; median C_{Taf} at 24 months was 7.6 μM.



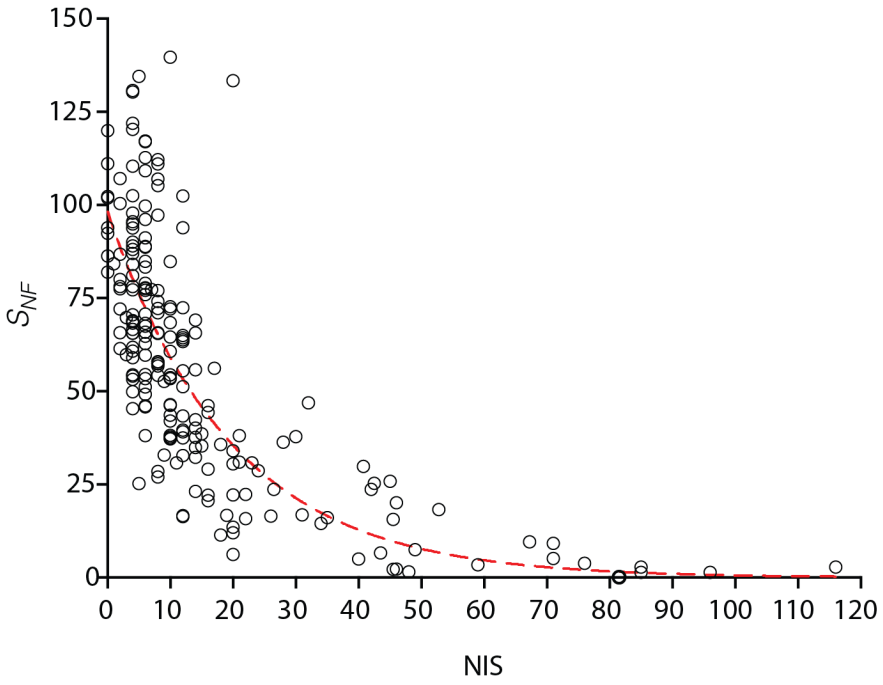
Supplemental Figure 7. Correlation between C_{Taf} at 24 months and the extent of TTR stabilization at the same time point. N = 196; r: Spearman correlation coefficient; red line represents best-fit (R^2 = 0.37), dotted red lines represent 95% confidence intervals of the best-fit.



Supplemental Figure 8. Method for detection of tafamidis-glucuronide. (A) Synthetic scheme used to prepare tafamidis-glucuronide; DBU: 1,8-Diazabicyclo[5.4.0]undec-7-ene, DMF: Dimethylformamide, rt: room temperature, NMM: N-Methylmorpholine, HATU: 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate, $Pd(PPh_3)_4$: Tetrakis(triphenylphosphine)palladium(0), THF: Tetrahydrofuran. **(B)** ^{1}H-NMR (top) and ^{13}C-NMR (bottom) spectra of Tafamidis-gluc. obtained in DMSO-d_6. **(C)** Tafamidis-gluc. (synthesized as shown in panel A) was added to healthy control plasma *ex vivo*; after protein extraction and reverse-phase chromatography separation, tafamidis-gluc. was detected using the same fluorescence channel as tafamidis; HPLC: high performance liquid chromatography. **(D)** Chromatogram representing fluorescence detection of tafamidis-glucuronide and unmetabolized tafamidis in the plasma of a patient taking oral tafamidis; the two molecules elute at different volumes, allowing individual quantification.

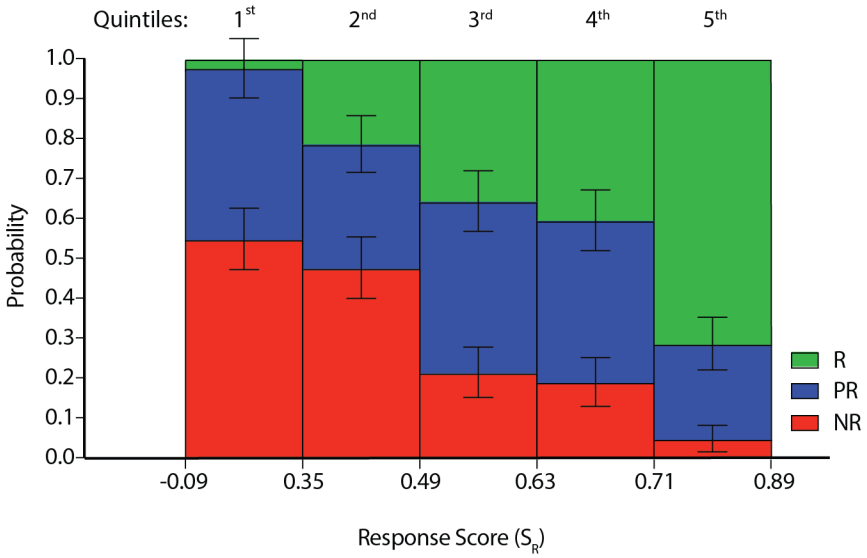


Supplemental Figure 9. Exponential relationship between S_{NF} and NIS at baseline. S_{NF} decreases exponentially as NIS increases so that S_{NF} decreases precipitously over a narrow range of small NIS values and vice versa. Nonlinear regression yields the following best-fit relationship: $S_{NF} = 98.3\ (\pm 3.8)\ e^{-0.0509\ (\pm 0.0049)\times \mathrm{NIS}}$ ($R^2 = 0.57$, $F = 275.5$, $\mathrm{P} = 8.9\times 10^{-40}$). The dotted red curve represents the best-fit nonlinear regression curve.



Supplemental Figure 10. Predictive model of response to tafamidis using NIS instead of S_{NF} (*Model-NIS).* The response score (S_R) interval of each quintile is shown in the *x* axis, and the probability for each response classification within each quintile is shown in the *y* axis. Error bars represent standard deviation. The equation for this model is shown below (Equation 3). The best fit values for the parameters are as follows: $a_0 = 0.551 \pm 0.075$, $b_{NIS} = -0.0057 \pm 0.0013$, $b_M = -0.482 \pm 0.085$, $b_{M\text{-}Taf} = 0.026 \pm 0.008\ \mu M^{-1}$, and $b_{TTR} = 0.076 \pm 0.023\ \mu M^{-1}$. AUC for Non-Responders+Partial-Responders vs. Responders (NR+PR vs R) ROC curve: 0.66; AUC for Non-Responders vs. Partial-Responders+Responders (NR vs PR+R) ROC curve: 0.77. ROC curves are not shown.

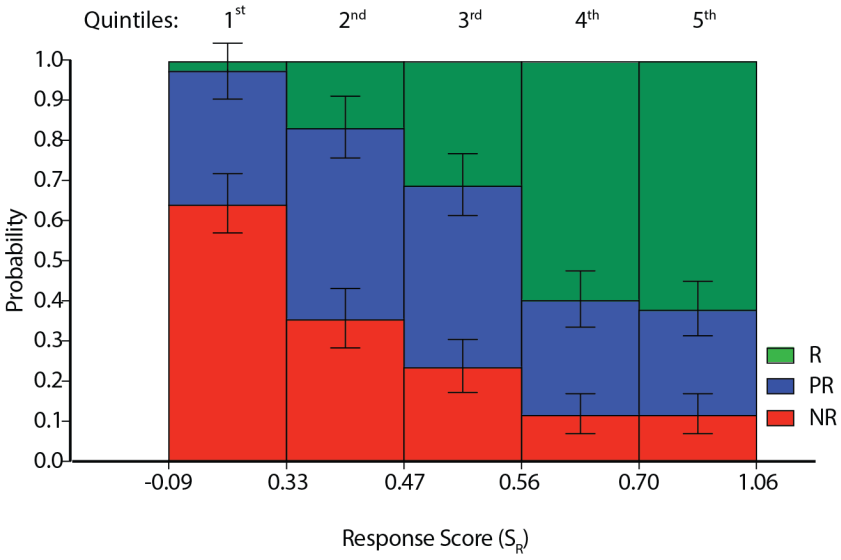
$$S_R = a_0 + b_{NIS} \times NIS + b_M \times M + b_{M\text{-}Taf} \times M \times C_{Taf} + b_{TTR} \times C_{TTR} \quad (3)$$



Supplemental Figure 11. Simplified version of the predictive model of response to tafamidis using only S_{NF} and sex (*Model-S.)*

The response score (S_R) interval of each quintile is shown in the *x* axis, and the probability for each response classification within each quintile is shown in the *y* axis. Error bars represent standard deviation. The equation for this model is shown below (Equation 4). The best fit values for the parameters are as follows: $a_0 = 0.335 \pm 0.06$, $b_{NF} = 0.0052 \pm 0.0007$, $b_M = -0.199 \pm 0.048$. AUC for Non-Responders+Partial-Responders vs. Responders (NR+PR vs R) ROC curve: 0.81; AUC for Non-Responders vs. Partial-Responders+Responders (NR vs PR+R) ROC curve: 0.77.

$$S_R = a_0 + b_{NF} \times S_{NF} + b_M \times M \quad (4)$$



Supplemental Table 1. Reasons for exclusion from the study population

Exclusion Criteria	Number of patients
Patients previously included in the tafamidis clinical trials [Clinicaltrials.gov: Fx-005 (NCT00409175) and Fx-006 (NCT00791492)]	44
Patients with co-morbidities that might compromise neurological evaluation (i.e., diseases with concomitant peripheral nervous system, central nervous system or psychiatric manifestations), including: Diabetes Mellitus (7) History of neoplasia with chemotherapy or radiotherapy treatment (5) Heavy alcohol consumption (2) Infection with Hepatitis Virus C (2) Infection with Human Immunodeficiency Virus (1) Hereditary spastic paraparesis (1) Severe anxiety syndrome (1) Intellectual disability (1)	20
No baseline or subsequent plasma samples collected	7
Concomitant use of siRNA investigational drug Patisiran (patients included in the phase 2 study and the phase 2 open label extension study; Clinicaltrials.gov: NCT01617967 and NCT01961921)	6
Poor compliance (determined by the assistant neurologist based on information given by the patient and / or pharmacy)	5
Interruption before completing two years of therapy for pregnancy	5
Lost to follow-up	4
Early stop of tafamidis (at or before completing 18 months of therapy) because of disease progression	3
Death (unknown cause)	1
Refusal to participate in study	1
Total	96

Supplemental Table 2. Baseline characteristics that are not significantly different between response groups. Yrs: years. BMI: modified body mass index, mBMI: modified body mass index (calculated as BMI multiplied by serum albumin), eGFR: estimated glomerular filtration rate [calculated using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) Equation]. Measurements were obtained for all patients (n = 210) unless otherwise noted.

	Responders (R)	Partial-Responders (PR)	Non-Responders (NR)
Demographic characteristics			
Age of onset (yrs)	35.7 (29.9 – 46.7)	33.3 (28.1 – 40.8)	33.1 (29.0 – 42.7)
Age at baseline (yrs)	37.9 (33.2 – 48.8)	36.1 (30.1 – 42.7)	35.9 (31.8 – 45.0)
Disease duration (yrs)	1.7 (1.0 – 3.4)	2.1 (1.4 – 3.6)	2.5 (1.4 – 3.6)
Nutritional status and Renal Function			
Weight (Kg)	61 (53 – 73)	65 (57 – 74)	64 (57 – 72)
BMI (Kg/m^2)	22.7 (20.0 – 26.1)	21.8 (19.6 -25.7)	21.9 (19.2 – 25.2)
mBMI (Kg/m^2 x g/dL)	1010.8 (868.6 – 1179.5)	1019.9 (875.4 – 1149.2)	933.4 (804.4 – 1104.5)
eGFR (mL/min/1.73 m^2) (n=208)	112.9 (100.6 – 117.9)	112.3 (100.9 – 121.2)	112.7 (101.2 – 119.4)
Albuminuria (n=205) (mg urinary albumin/g creatinine)	7.8 (3.5 -34.3)	7.6 (3.8 – 35.1)	8.0 (4.3 – 53.1)
Proteinuria (g/L) (n=205)	0.11 (0.08 – 0.19)	0.12 (0.08 – 0.25)	0.14 (0.09 – 0.30)
Liver function			
Total Bilirubin (mg/dL) (n=188)[a]	0.45 (0.36 – 0.61)	0.50 (0.37 – 0.72)	0.53 (0.39 – 0.68)
AP (IU/L) (n=141)[a]	52 (42 – 69)	60 (53 – 70)	65 (55 – 75)
AST (IU/L) (n=205)	19 (15 – 23)	19 (17 – 23)	20 (17 – 26)
ALT (IU/L) (n=205)	16 (12 – 24)	18 (14 – 26)	20 (14 – 30)
GGT (IU/L) (n=205)	16 (12 – 23)	16 (13 -24)	16 (12 – 29)
Albumin (g/dL) (n=210)	4.42 (4.11 – 4.72)	4.56 (4.31 – 4.80)	4.41 (4.18 – 4.69)
Total protein (g/dL) (n=210)	6.96 (6.61 – 7.20)	6.92 (6.64 – 7.27)	6.82 (6.32 – 7.15)
Lipid profile			
Total cholesterol (mg/dL) (n=207)	190 (164 – 218)	180 (157 – 210)	180 (149 – 201)
Triglycerides (mg/dL) (n=209)	77 (60 – 108)	73 (51 – 94)	80 (63 – 101)

[a]The missing values for these variables are equally represented for Responders, Partial-Responders, and Non-Responders.

Supplemental Table 2 (Continuation)

	Responders (R)	Partial-Responders (PR)	Non-Responders (NR)
Thyroid function			
Free thyroxine (ng/dL) (n=148)	1.2 (1.1 – 1.3)	1.2 (1.1. – 1.3)	1.2 (1.1 – 1.3)
TSH (mU/L) (n=148)	1.8 (1.3 – 2.8)	1.7 (1.3 – 2.2)	1.8 (1.2 – 2.7)
Complete blood count (n=210)			
Leucocytes ($x10^3/\mu L$)	7.1 (6.3 – 8.5)	7.3 (6.0 – 8.8)	7.4 (6.1 – 8.4)
Erythrocytes ($x10^3/\mu L$)	4.6(4.4 – 4.9)	4.8 (4.5 – 5.0)	4.8 (4.4 – 5.1)
Platelets ($x10^3/\mu L$)	244 (211 – 292)	237 (199 – 280)	231 (199 – 264)
Hemoglobin (g/dL)	14.0 (13.0 – 14.4)	14.4 (13.7 – 15.1)	14.4 (12.8 – 15.3)
General inflammatory markers			
C Reactive Protein (mg/L) (n=78)	1.02 (0 – 3.19)	1.01 (0 – 2.80)	0.81 (0 – 2.13)
ESV (n=78) (mm/hour)	10 (5 – 17.5)	7 (4 – 18.5)	10 (5 – 30)
Others			
ProBNP (pg/mL) (n=185)	77.9 (43.3 – 134.1)	73.4 (35.0 – 149.2)	110.2 (41.6 – 277.7)
Uric acid (mg/dL) (n=209)	4.1 (3.3 – 5.3)	4.9 (4 – 5.5)	5.4 (4.4 – 6.3)
Iron (µg/dL) (n=144)	90 (71 – 106)	96 (65 – 117)	105 (88 – 124)

Supplemental Table 3. Results of regression and predictive power of truncated predictive models based on Equation (2). In each truncated model, a single variable has been excluded from Equation (2) to determine its contribution to the predictive power of Equation (2). The quantity a_0 is the constant of regression, while b_{NF}, b_M, $b_{M\text{-}Taf}$, and b_{TTR} are the regression coefficients. R^2 is the coefficient of determination for the best fit of Equation (2) and its truncated variants to the numericized response category data (S_R). AUC is the area under the ROC curves (not shown) for Equation (2) and its truncated variants. Two types of ROC curves are considered, one that characterizes discrimination between the combined Non-Responder and Partial-Responder categories vs. the Responder category (NR+PR vs. R) and one that characterizes discrimination between the Non-Responder category vs. the combined Partial-Responder and Responder categories (NR vs. PR+R).”

	Parameters						**AUC**	
Excluded variable	a_0	b_{NF}	b_M	$b_{M\text{-}Taf}$	b_{TTR}	R^2	**NR+PR vs. R**	**NR vs PR+R**
None	0.195 (0.076)	0.0046 (0.0007)	−0.447 (0.083)	0.027 (0.008)	0.068 (0.022)	0.35	0.82	0.81
S_{NF}	0.429 (0.073)	0	−0.543 (0.089)	0.031 (0.008)	0.092 (0.024)	0.22	0.75	0.72
M	0.088 (0.078)	0.0053 (0.0007)	0	−0.0085 (0.0048)	0.062 (0.024)	0.26	0.78	0.75
$M \times C_{Taf}$	0.180 (0.078)	0.0048 (0.0007)	−0.213 (0.048)	0	0.069 (0.023)	0.31	0.81	0.77
C_{TTR}	0.348 (0.058)	0.0050 (0.0007)	−0.436 (0.084)	0.028 (0.008)	0	0.32	0.81	0.79

Synthesis of Tafamidis-glucuronide

Synthesis of Allyl-*D*-Glucuronate: 388 mg of *D*-glucuronic acid was dissolved in 10 mL of DMF at 25˚C. 329 µL of DBU was added dropwise to the solution. After stirring for 15 min, 208 µL of allyl bromide was added dropwise to the solution. The reaction was stirred for 18 hr at 25˚C. After reaction was completed according to TLC, the solvent was removed via vacuum distillation at 50˚C. The resulting residue was purified by flash chromatography, using acetone as the eluent. This yielded 393 mg of allyl-*D*-glucuronate as a white solid (Yield 84%).

Synthesis of Tafamidis-β-Glucuronide Allyl Ester((1)): 31 mg of Tafamidis (free acid), 38 mg of HATU, and 22 µL of N-methylmorpholine were suspended in 1 mL of CH_2Cl_2. This suspension was stirred at 25˚C for 1 hr. 23 mg of allyl-*D*-glucuronate was added to the suspension and the reaction was stirred for 24 hr at 25˚C. The resulting precipitate was filtered off, and the reaction was purified by flash chromatography using 5% MeOH in CH_2Cl_2. This produced 9 mg of Tafamidis-β-glucuronide allyl ester as a white solid (Yield 17%).

Synthesis of Tafamidis-β-Glucuronide: 9 mg of Tafamidis-β-glucuronide allyl ester was dissolved in 1 mL of THF at 25˚C. 15 µL of morpholine and 1 mg of $Pd(PPh_3)_4$ were added to the solution. The reaction was stirred for 2 hours at 25˚C. The solvent was removed under reduced pressure, and the resulting residue was dissolved in 900 µL of MeOH and purified via reverse-phase HPLC using an Agilent 1260 Infinity HPLC. Removing the solvent under reduced pressure and temperature yielded 4 mg of Tafamidis-β-glucuronide as a white solid (Yield 47%).

1. Perrie JA, Harding JR, Holt DW, Johnston A, Meath P, and Stachulski AV. Effective synthesis of 1beta-acyl glucuronides by selective acylation. *Organic letters.* 2005;7(13):2591-4.