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MONITORING EFFECTIVENESS AND SAFETY OF TAFAMIDIS IN TRANSTHYRETIN AMYLOIDOSIS IN ITALY.

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A longitudinal multicenter study in a non-endemic area

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ABSTRACT

Background. Tafamidis is a transthyretin (TTR) stabilizer able to prevent TTR tetramer dissociation. There have been a few encouraging studies on Tafamidis efficacy in early-onset inherited transthyretin amyloidosis (ATTR) due to Val30Met mutation. However, less is known about its efficacy in later disease stages and in non-Val30Met mutations.

Methods. Multi-center observational study on symptomatic ATTR patients prescribed to receive Tafamidis. We followed up patients according to a standardized protocol including general medical, cardiological and neurological assessments at baseline and every six months up to three years.

Results. 61 (42 males) patients were recruited. Only 28% of enrolled subjects had the common Val30Met mutation, mean age of onset was remarkably late (59 years) and 18% was in advanced disease stage at study entry. Tafamidis proved safe and well-tolerated. One third of patients did not show significant progression along 36 months, independently from mutation type and disease stage. Neurological function worsened particularly in the first six months but progression slowed significantly thereafter. Autonomic function remained stable in 33%, worsened in 56% and improved in 10%. Fifteen percent of patients showed cardiac disease progression and 30% new onset of cardiomyopathy. Overall, Tafamidis was not able to prevent functional progression of the disease in 23 (43%) subjects, including 16 patients who worsened in their walking ability and 12 patients who reached a higher NYHA score during the follow-up period. A higher mBMI at baseline was associated with better preservation of neurological function.

Conclusions. Neuropathy and cardiomyopathy progressed in a significant proportion of patients despite treatment. However, worsening of neurological function slowed after the first six months and also subjects with more advanced neuropathy, as well as patients with non-Val30Met mutation, benefited from treatment. Body weight preservation is an important favorable prognostic factor.

INTRODUCTION

Inherited transthyretin amyloidosis (ATTR) is an autosomal dominant disorder due to mutations of the transthyretin (*TTR*) gene. TTR is synthetized mainly by the liver and released in plasma as a tetrameric transport protein. Mutations in *TTR*, of which Val30Met (p.Val50Met) is the most common, cause transthyretin tetramer dissociation, monomer misfolding, and aggregation into insoluble fibrillar proteins in different tissues. Peripheral nerves and heart are the most frequently affected organs, but also eye, leptomeninges and kidneys can be involved [1,2].

Orthotopic liver transplantation (OLT), by removing the main site of mutated TTR production, proved able to halt or slow neurological progression and is, at present, the standard-of-care treatment in patients aged <50 with Val30Met mutation. However, mortality rate following OLT is not negligible (around 10%) [3,4]. Moreover, OLT is often not curative of ATTR, since cardiac disease tends to progress after OLT, possibly due to continued accumulation of wild type TTR[5–7]. Altogether, these observations warranted the search for new treatment options in ATTR.

Tafamidis meglumine (Vyndaqel®) is a small molecule, which kinetically stabilizes the TTR tetramer and prevents its dissociation into amyloidogenic monomers. It has been approved by EMA for the treatment of transthyretin amyloidosis in adult patients with stage 1 symptomatic polyneuropathy to delay peripheral neurologic impairment.

There have been a few encouraging studies on safety and long-term efficacy of Tafamidis in early-onset Val30Met ATTR patients [8–10]. However, less is known about its efficacy in later stages of the disease and in non-Val30Met mutations, which represent a significant proportion of ATTR genotype spectrum in Italy, as well as in other countries where ATTR is non-endemic [11,12]. Moreover, diverse studies reported different rate of response to Tafamidis treatment, possibly based on genetic and demographic heterogeneity of treated patients [8–10]. In fact, prognostic factors affecting the response to treatment are still largely unknown.

We therefore designed a protocol to evaluate patients who have just been prescribed Tafamidis meglumine (and who were going to start treatment) in Italy. Study entry occurred after the decision to prescribe the drug but before its administration. *Sinsu strictu* this is a longitudinal observational study on a series of patients on treatment with Tafamidis. Aims of this multicenter study were: 1) to prospectively collect data about safety and exploratory efficacy on nerve and heart function of Tafamidis in ATTR patients, including Val30Met and non-Val30Met mutations, in early and late stages of the disease (as it was possible to prescribe tafamidis according to Italian regulation in patients in different disease stages at the beginning of this study); 2) to identify factors associated with response to treatment and better preservation of neurological function; 3) to test the usefulness of a reproducible, easy-to-administer clinical scale: the Charcot-Marie-Tooth (CMT) Neuropathy Score (CMTNS), to monitor disease progression in ATTR.

METHODS

Patients and study protocol

Patients were enrolled between October 2011 and December 2014 in eight Centers across Italy.

Inclusion criteria were: 1) Women and men aged ≥18 affected by symptomatic ATTR-related neuropathy, defined by the presence of a pathogenic *TTR* mutation and symptomatic sensory, sensory-motor and/or autonomic peripheral

neuropathy; 2) Patients starting treatment with tafamidis meglumine (20 mg once daily); 3) Written informed consent to participate in the study. Exclusion criteria were: 1) previous orthotopic liver transplantation; 2) pregnancy or breastfeeding; 3) other current anti-amyloidogenic treatment such as diflunisal, doxycycline, tauroursodeoxycholic acid.

Patients were assessed at treatment start and every six months thereafter up to three years according to a standardized protocol which included the following assessments (see also Supplementary material): Neuropathy Impairment Score (NIS) and NIS-Lower Limb subscale (NIS-LL) [8,13]; Kumamoto Scale [14] CMTNS and its clinical component CMT Examination Score (CMTES), version 2 [15]; Familial amyloid polyneuropathy (FAP) Stage [16] and polyneuropathy disability (PND) score [17]; Modified Body Mass Index (mBMI) as defined by BMI (Kg/m^2) multiplied by serum albumin (g/L), in order to compensate for possible oedema [18]; blood routine testing including N-terminal pro-hormone brain natriuretic peptide (NT-proBNP) [19]; echocardiography with measurement of inter-ventricular septum (IVS) thickness and electrocardiogram. Adverse Events (AEs) were monitored throughout the study. Ethics committees of participating centres approved the study.

Statistical analyses

Changes from baseline and from follow-up visits were compared with paired t-test or Wilcoxon signed-rank test. Pearson's correlation coefficient was used to test for correlation between scales. Their responsiveness was assessed by calculating the standardized response mean (SRM) as the mean baseline-to-12 months point change in score divided by the standard deviation (SD) of the individual's score change. The SRM threshold levels were defined as follows: ≥0.20 small, ≥0.50 moderate, and ≥0.80 good [20]. Responders were defined as patients with a less than two-point increase in NIS-LL during treatment because it is the least degree of change a physician can recognize and because it was one of the co-primary end-points in the Tafamidis registration trial [8,21]Univariate logistic regression was performed with response to treatment at 12 months as outcome variable and stage of FAP, NIS, NIS-LL, mBMI >960 (corresponding to the median mBMI in our study cohort), presence of cardiac involvement, presence of dysautonomia, type of mutation (Val30Met vs other mutations), age, age at onset, disease duration and gender as independent variables. Variables significantly associated with the response to treatment at univariate analysis were tested in an age- and gender-adjusted multivariate logistic regression model.

RESULTS

We enrolled 61 patients. One patient with alcohol-related liver cirrhosis had developed ATTR-related neuropathy following domino liver transplantation received 14 years before enrolment from a donor with Val30Met *TTR* mutation. Baseline characteristics of study population are summarized in **Table 1**. The majority were males, with remarkably late mean age of onset and mean disease duration of 3.4 years. Notably, 44 (72%) subjects had TTR mutations other than Val30Met. A summary of baseline features of patients carrying one of the 3 most common mutations (Val30Met, Glu89Gln and Phe64Leu) is provided in **supplementary table 1**. Forty-four patients (72%) were in stage 1 at study entry, whereas seventeen had already lost ambulatory independency, of whom 12 (20%) were in stage 2 and 5 (8%) in stage 3, and Tafamidis was prescribed according to the contemporary national regulation. There was a good correlation between PND score and NIS at enrolment (r=0.83), as also reported in a recent multinational study [22]. Forty-two (69%) patients had dysautonomia at enrolment, including 29 patients (48%) with diarrhea, 37 patients (61%) with orthostatic hypotension, 18 patients (30%) with urinary retention, five patients with dry eye (8%) and 15 patients with

dry mouth (25%). Out of 57 patients with available echocardiography at study entry, 34 (60%) had definite cardiac involvement with IVS > 12 mm. NT-proBNP was altered in 22/53 (42%) patients. ECG was abnormal in 27 (44%) mainly due to low limb voltages and conduction abnormalities. Of the 61 enrolled patients with baseline visit, 53 were evaluated also after 6 months (M6), 37 up to 12 months (M12), 34 up to 18 months (M18), 19 up to 24 months (M24), 12 up to 30 months (M30) and seven up to 36 months (M36).

Seven patients (11%) discontinued treatment: one patient underwent OLT, one moved to a different country where Tafamidis was not licensed, the subject who received domino-liver transplant lost eligibility to Tafamidis treatment following changes of the national regulation for Tafamidis prescription, another patient with Phe64Leu mutation died of cardiac failure, and three patients discontinued because of disease progression (two patients showed cardiac disease progression at M12 and M24, respectively, and one patient discontinued because of neuropathy progression at M18).

Changes in neurological function

Both NIS and NIS-LL scores worsened as a mean during the follow-up period **(Table 2).**

In 34 patients treated for at least 18 months, NIS increased by 15.6 points (**Figure 1A**). In patients treated for longer periods the neurological function continued to deteriorate in the subsequent months (**Figure 1B**). However, when examining the 6-month interval deterioration rate, we observed that NIS increase significantly declined from 8.3 ± 10.3 during the M0-M6 interval to 1.4 ± 12 in the subsequent M6-M12 interval (p=0.025), and remained relatively stable at 18 months (**Figure 1C**). Similar considerations apply to changes of NIS-LL (**Supplementary figure 1A**). Overall, one-third of patients showed stability of the neuropathy, as defined by NIS-LL increase < 2 points from baseline, across the whole duration of the study (**Figure 1D**). The percentage of responders after 12 months of treatment tended to be higher in patients in very early disease stage, whose neurological impairment was limited to sensory disturbances, compared with patients showing motor weakness and altered walking capability, 44% vs 29% respectively, although the difference was not significant (p=0.3). Patients with non-Val30Met mutations showed changes of neurological function similar to Val30Met patients and responders were distributed in similar rates across both Val30Met and other mutations.

The mean Kumamoto score, which evaluates impairment related to sensory-motor and autonomic neuropathy as well as cardiac conduction defects, steadily worsened throughout the duration of the study **(Supplementary Figure 1B)**.

Regarding the 39 patients with evidence of dysautonomia at baseline, as assessed with the Kumamoto scale, and followed-up for at least 6 months, autonomic function remained stable in 13 (33%), worsened in 22 (56%), and improved in four (10%), including improvement of urinary retention (two cases), orthostatic hypotension (one case), eye and mouth dryness (two cases). Out of 15 patients without autonomic dysfunction at baseline, six later developed dysautonomia (orthostatic hypotension in four cases and diarrhea in two cases. Two of these patients developed additional symptoms, namely dry mouth and urinary incontinence).

CMTES also showed a steady mean increase ranging from 1.7 to 2.6 per year (**Supplementary Figure 1C**).

During the follow-up period, overall nine patients had progressed to a higher disability grade in FAP stage (seven patients progressed from stage 1 to 2, and three patients progressed from stage 2 to 3), although in six of them the progression occurred in the first six months, while only two patients progressed at M12 and one patient at M18. Seven

patients with stable FAP disease stage progressed in terms of PND score, namely five patients with sensory disturbances only at baseline developed motor impairment and two patients with unilateral aid at walking at baseline required bilateral support later.

There was good correlation between NIS, CMTES and Kumamoto scores. Kumamoto Scale showed the highest responsiveness to change (SRM>0.8), followed by CMTES and NIS-LL, in both ambulatory independent patients and patients requiring walking aids (**Table 3**).

Changes in cardiac function

In the 34 patient with cardiac involvement at baseline, mean IVS increased from baseline by 0.6 ± 1.6 mm at M12 and 1.05 ± 2.0 mm at M24. Five/34 (15%) patients showed echocardiographic evidence of cardiac disease progression (1 at M6, 2 at M12, 1 at M18 and 1 at M30). In three of them increase in IVS was paralleled by consistent increase of NT-proBNP by ≥30% from baseline.

Eight (35%) of 23 patients without cardiac involvement at baseline later developed cardiomyopathy, as defined by IVS>12 mm (see supplementary methods), (four at M6, two at M12, one at M18, one at M30) and three showed a concomitant increase of NT-proBNP by≥30%.

Overall, 12 patients progressed to a higher NYHA heart failure class (one at M6, four at M12, five at M18, one at M24 and one at M30). One patient required pace-maker implantation after 18 months of treatment.

mBMI

Remarkably, mBMI did not change during the 36 months of observation while on treatment (**Supplementary Figure 1D**).

Adverse events

AEs were reported by eight (13%) patients: two had urinary tract infections, one diarrohea, one gastroenteritis, and one angular stomatitis. Three patients had serious AEs: one patient died of cardiac failure and two had rapid cardiac function worsening, consistent with disease-associated cardiac morbidity and mortality and unlikely to be related to treatment, which led to Tafamidis discontinuation in one of them. No patient discontinued because of treatment-related AE.

Predictors of response to treatment

A higher mBMI at baseline, but not disease stage, NIS, NIS-LL, presence of cardiac involvement, presence of dysautonomia, mutation type, onset age, disease duration, age or gender, was significantly associated (p=0.02) with response to treatment after 1 year. In a multivariable age- and gender-adjusted logistic regression model, mBMI >960 was associated with a 7-fold higher probability of stability of NIS-LL after 12 months of treatment compared with subjects with lower mBMI [OR 6.8, p= 0.02, CI 1.3-34.7].

DISCUSSION

To date, one double-blind placebo-controlled study [8] and three single-arm interventional or observational studies [8–10] provided information about safety and efficacy of Tafamidis for ATTR **(table 4).**

In the first 18-month trial, Tafamidis slowed neurologic impairment progression in Val30Met patients, although the primary endpoints were not reached. Overall, 45% of treated patients had stable NIS-LL after 18 months. Nutritional status significantly improved in Tafamidis-treated subjects, but worsened in placebo-controls [8].

The beneficial treatment effect was maintained in the following 12-month open-label extension study, and no significant concern about its safety ensued. NIS-LL steadily increased by 0.96-1.32 points/year, with higher benefit when treatment started early [9].

However, a much lower responder rate, with only two out of 37 patients (7%) showing stable NIS-LL after 12 months and 55% of patients progressing to a higher disability stage, was observed in a single center open-label study in Val30Met patients with more advanced neuropathy [10].

Tafamidis effectively stabilized TTR tetramers in plasma from patients with both Val30Met and non-Val30Met mutations [11]. Nonetheless, after 12 months of treatment, benefit on neurological function in non-Val30Met subjects [11] was less marked than in Val30Met patients [8].

Our study provides long-term follow-up data in a large cohort of patients with ATTR from a non-endemic area, including subjects with more advanced disease and non-Val30Met mutations.

Since 2010 and until drug registration, Tafamidis was available in Italy under a special accession program for the treatment of ATTR with symptomatic polyneuropathy, whatever the disease stage and mutation type, and patients started treatment, making the current study possible. The distribution of mutations in recruited patients, with 72% of non-Val30Met mutation, of which Phe64Leu and Glu89Gln were the most common, reflects the high genetic heterogeneity of ATTR in Italy [23–27]]. Of note, patients with Glu89Gln mutation had earlier onset of the disease and more prominent heart involvement, while patients with Phe64Leu mutation showed more aggressive neuropathy (**supplementary table 1**). Such data are in keeping with recent observation in a partly overlapping cohort of patients with ATTR from endemic regions in Sicily, thus confirming earlier heart dysfunction in Glu89Gln mutation and more severe peripheral neuropathy in Phe64Leu mutation [23].

Tafamidis proved safe and well-tolerated over 36 months and no patient had treatment-related serious AEs. In keeping with previous reports [18], we did not observe significant changes of mBMI over 3 years of treatment. More than one third of patients showed no meaningful NIS-LL increase along 36 months of treatment, independently from mutation type. Notably, both patients in early and late disease stages responded to treatment, although patients in very early stage without motor impairment (PND1) tended to have a higher responder rate.

This observation confirms previous understanding of a higher efficacy of anti-amyloidogenic treatments if given early in disease course suggesting that treatment should be started as soon as possible [11]. Moreover, our study suggests a possible benefit of Tafamidis also in ATTR patients with more advances disease.

Notably, the worsening of neurologic function, according to NIS, NIS-LL and PND, occurred mainly in the first 6 months of treatment and became subsequently less prominent. Such observation, in keeping with previous experience by Lozeron *et al*. [10] would encourage continuing Tafamidis administration for at least 1 year. In fact, although tetramer stabilization if often reached by 6 weeks of treatment [11], we observed that it can take up to 12 months before clinically significant changes are detected, thus making inappropriate an earlier discontinuation based on apparent inefficacy.

Overall, Tafamidis was not able to prevent functional progression of the disease in 23 (43%) subjects, including 16 patients who worsened in their walking ability and 12 patients who reached a higher NYHA score during the follow-up period. Two-thirds of subjects increased their neurological impairment according to NIS-LL, 17% reached a higher disability FAP stage and in 30% the PND score worsened. Similarly, one-third of patients without heart involvement developed amyloid-related cardiac disease, septal thickness significantly increased in 15% of patients with cardiomyopathy at baseline, and 22% of patients reached a higher NYHA score. Mean NIS-LL increase after 18 months was 3-fold higher in comparison with the progression rate reported by Coelho *et al.*[8] **(table 4)**.

The discrepancy between the two studies reflects fundamental differences in the characteristics of the patient cohorts, with a majority of early-onset slowly-progressive Val30Met patients from endemic regions for ATTR in the Tafamidis registration trial [8] compared to a large proportion of late-onset and non-Val30Met patients with faster progression in our study. In fact, both later age of onset and presence of non-Val30Met mutation are known negative prognostic factors associated with a more aggressive disease course [2,27–29].

Neurologic deterioration in non-responders could be due to limited tetramer stabilization, suggesting a role for tetramer stabilization assay in non-responding patients who may possibly benefit from higher doses of the drug. Other pathogenic mechanisms, including continued deposition of transthyretin and toxicity of amyloid precursors, template-effect of deposited amyloid fibrils by exposure of aggregation-prone regions, impaired amyloid clearance, and sustained activation of endoplasmic reticulum stress may also be involved in disease progression [29,30]. Remarkably, also in Amyloid Light-chain amyloidosis nerve damage can progress despite complete hematologic response to chemotherapy and sustained M-protein reduction (31).

Efficacy of Tafamidis on dysautonomia was also partial with 26/54 patients (48%) showing normal or stable autonomic function at last follow up.

Overall, such observations confirm that OLT, which proved to be able to stop neurologic progression in 76% of stage 1 patients with Val30Met mutation, is still an important option in the treatment of selected cases of ATTR [5,32]. Moreover, it highlights the need of further research on treatments for FAP, including RNA interference-based therapies (anti-sense oligonucleotides and small interfering RNAs).

We next investigated which factors could predict a better response to treatment. We tested whether NIS-LL change after 12 months of treatment was influenced by mutation type, onset age, symptoms duration, neurological, cardiologic and nutritional status at baseline. In our study, the only factor able to predict treatment response and preservation of neurologic function was a higher mBMI at treatment onset. The odds of having a stable NIS-LL after 1 year of treatment was 7-fold higher in patients with mBMI >960 as compared to patients with lower mBMI.

There is no obvious explanation for this observation. Weight loss is common in ATTR although its ultimate cause is unknown. A low BMI was associated with reduced survival in patients with ATTR following liver transplant [7,32]. The effect of a reduced mBMI on neuropathy progression was not previously reported. It is possible that malabsorption plays a role, i.e. by negatively affecting Tafamidis bioavailability. Also, a detrimental effect of poor nutritional status on unfolded protein response mediated cellular stress may be hypothesized [33].

Concerning the different employed scales, CMTES and CMTNS showed good correlation with NIS and Kumamoto scale, and CMTES and Kumamoto scale proved to be more responsive to changes of neurological function after 1 year, which may warrant its consideration in future trials in ATTR.

The main limitation of our observational study is that we have no control population for direct comparison which, together with the contemporary unavailability of data about pre-treatment progression rate in treated patients, limits the evaluation of treatment efficacy.

In conclusion, neuropathy and cardiomyopathy progressed in a significant proportion of patients with ATTR despite treatment. However, after the first 6 months of treatment the worsening of neurological function slowed across all stages for the entire study duration. This observation, together with the experience of patients with non-Val30Met mutation and high disability who remained neurologically stable even at long-term follow-up, entail that also subjects with more advanced neuropathy, as well as patients with non-Val30Met mutation, may benefit from Tafamidis treatment. Body weight preservation is an important favorable prognostic factor.

Disclosures

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TABLES

Table 1. Patients' demographics and baseline data

	N=61	
Age (years)	62 ± 11 (31-81)	
Age at onset (years)	59 ± 11 (30-79)	
Disease duration (years)	3.4 ± 2.1 (0.6-10)	
Male gender	42 (69%)	
***TTR* genotype**		
Val30Met	17 (28%)	
Phe64Leu	16 (26%)	
Glu89Gln	14 (23%)	
Thr49Ala	5 (8%)	
Tyr78Phe	3 (5%)	
Ala120Ser	2 (3%)	
Glu54Gln	1 (2%)	
Ile68Leu	1 (2%)	
Ser77Tyr	1 (2%)	
Ala45Thr	1 (2%)	
Stage at enrolment		
Stage 1: walk unaided	44 (72%)	
-sensory disturbances only (PND I)	26 (43%)	
-sensory disturbances and motor weakness (PND II)	18 (29%)	
Stage 2: use of a cane/walker	12 (20%)	
- walking only with the help of one stick or crutch (PND IIIA)	9 (15%)	
- walking with the help of two sticks or crutches (PND IIIB)	3 (5%)	
Stage 3: wheelchair-reliant (PND IV)	5 (8%)	
	Mean ± SD	**Median (min-max)**
NIS (0-244)	53 ± 38	44 (0-138.5)
NIS-LL (0-88)	28 ± 20	23 (0-74)
CMTES (0-28)	12 ± 7	11 (0-26)
CMTNS (0-36)	16 ± 9	15.5 (1-33)
Kumamoto Score (0-96)	20 ± 12	20 (2-53)
IVS (mm)	13.6 ± 3	13 (7-24)
NT-pro-BNP (pg/mL)	1047 ± 1576	260 (5-7136)
mBMI	978 ± 195	959 (568-1445)

Patients' characteristics at baseline are reported as number and percentage for categorical variable. Continuous variables are reported as mean±standard deviation or median (min, max), as appropriate. CMTES: Charcot-Marie-Tooth Examination Score; CMTNS: Charcot-Marie-Tooth Neuropathy Score; IVS: inter-ventricular septum; mBMI:

modified Body Mass Index; NIS: Neuropathy Impairment Scale; NIS-LL: NIS-Lower Limb. PND: Polyneuropathy Disability Score

Table 2. change of neurological function after 12 and 18 months of Tafamidis treatment

	Change after 12 months of treatment (N=37)	**Change after 18 months of treatment (N=34)**
NIS	10.3 ± 17	15.6 ± 25.7
NIS-LL	5.9 ± 9.3	8 ± 13.7
CMTES	2.1 ± 3	3.4 ± 3.9
CMTNS	2.3 ± 3.8	NA
Kumamoto Scale	5.4 ± 6.1	7.3 ± 6.4

CMTES: Charcot-Marie-Tooth Examination Score; CMTNS: Charcot-Marie-Tooth Neuropathy Score; NIS: Neuropathy Impairment Scale; NIS-LL: NIS-Lower Limb. NA: not applicable

Table 3. Sensitivity to change of neurological function after 12 months of treatment expressed as SRM, standardized response mean.

	Stage 1	**Stage 2 & 3**
NIS	0.56	0.67
NIS-LL	0.61	0.66
CMTES	0.7	0.75
Kumamoto Scale	0.83	0.89

CMTES: Charcot-Marie-Tooth Examination Score; CMTNS: Charcot-Marie-Tooth Neuropathy Score; NIS-LL: Neuropathy Impairment Score-Lower Limb

Table 4. Comparison with previous Tafamidis studies for patients' mutation type and NIS-LL progression rate

	Tafamidis				**Placebo**
	Present study	**Coelho et al**(8)	**Lozeron et al**(10)	**Merlini et al**(11)	**Coelho et al**(8)
N	61	64	37	21	64
Type of study	observational	RCT	Open-label	Open-label	RCT
Non-Val30Met	72%	0%	0%	100%	0%
Age at onset (years)	59 ± 11	39.8 ± 12.7	62.8 (16)	63.1 ± 9.9	38.4 ± 12.9
Disease duration (months)	40.8 ± 25.2	47.0 ± 48.40	48.4 (30.4)	64.7 ± 60.8	34.7 ± 32.88
NIS-LL					
baseline	28 ± 5	8.4 ± 11.40	27.6 ± 17.2	27.6 ± 24.7	11.4 ± 13.5
month 6	+4.5 ± 6.3; 62%	NA	+4.8 ± 3.1	NA	NA
month 12	+5.9 ± 9.3; 65%	NA	+6.6	+2.5 (-1.2, 6.2)	NA
month 18	+8.0 ± 13.7; 65%	+2.81; 54.7%	NA	NA	+5.83; 71.5 %

Continuous data are reported as mean ± standard deviation or median (95% confidence interval); when information is available, patients showing neurologic progression (NIS-LL≥2) are reported in %. NA: not available. NIS-LL: Neuropathy Impairment Score-Lower Limb. RCT: randomized controlled trial.

FIGURE LEGENDS

Figure 1. Changes of neurological function during Tafamidis treatment.
(A) Change from baseline of NIS over 18 months of treatment in early (stage 1, N=21) and late (stage 2&3, N=13) stages of the disease. **(B)** Progression of neurological impairment in patients followed up over 36 months of treatment (note that patients with longer follow up had more severe disease due to inclusion of patients in stage 2-3 in the early phases of the study). (C) Six-month change of NIS over 18 months of treatment (N=35). **(D)** Percentage of responders (change of NIS from baseline NIS-LL<2 points) over 36 months of treatment**.**
NIS-LL: Neuropathy Impairment Score. NIS-LL: NIS-Lower Limb. F-U: follow-up.

Supplementary figure 1
(A-C) Change from baseline of NIS-LL **(A),** Kumamoto Score **(B)** and CMTES **(C)** over 18 months of treatment in early (stage 1, N=21) and late (stage 2&3, N=13) stages of the disease. **(D)** Preservation of mBMI over 36 months of treatment.
CMTES: Charcot-Marie-Tooth Examination Score, mBMI: modified Body Mass Index, NIS-LL: Neuropathy Impairment Score-Lower Limb.

Supplementary table 1: Patients' demographics and baseline features in patients with Val30Met, Glu89Gln and Phe64Leu mutations

Supplementary table 2: Patients' progression of neurological and cardiological disability. ACM: amyloid cardiomyopathy, NA: not applicable (only baseline data available), NE: not evaluated; NYHA: New York Heart Association, PND: Polyneuropathy Disability.