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Repurposing Diflunisal for Familial Amyloid Polyneuropathy: A Randomized Clinical Trial

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Abstract

Importance—Familial amyloid polyneuropathy (ATTR-FAP), a lethal genetic disease caused by aggregation of variant transthyretin, induces progressive peripheral nerve deficits and disability. Diflunisal, a non-steroidal anti-inflammatory agent, stabilizes transthyretin tetramers and prevents amyloid fibril formation *in vitro*.

Objective—To determine the effect of diflunisal on polyneuropathy progression in patients with ATTR-FAP.

Design, Setting, and Patients—We conducted an investigator-initiated international, randomized, double-blind, placebo-controlled study at amyloid centers in Sweden (Umea), Italy (Pavia), Japan (Matsumoto and Kumamoto), England (London), and the United States (Boston, New York, Rochester, MN) from 2006 through 2012. 130 ATTRFAP patients with clinically detectable peripheral or autonomic neuropathy were randomly assigned to diflunisal 250 mg or placebo twice daily for 2 years.

Main Outcome Measures—The primary endpoint, the difference in polyneuropathy progression between treatments, was measured by the Neuropathy Impairment Score plus 7 nerve tests (NIS+7) which ranges from 0 (no neurologic deficits) to 270 points (no detectable peripheral nerve function). Secondary outcomes included a quality of life questionnaire (Short Form-36 (SF-36)) and modified body mass index (mBMI).

Results—One hundred thirty randomized patients (66 placebo, 64 diflunisal) underwent serial NIS+7 evaluations over 2 years. Due to attrition, we employed likelihood based modeling and multiple imputation (MI) analysis of baseline to 2 year data. By MI, NIS+7 increased 25.0 points (95% CI, 18.4 to 31.6) among placebo and 8.7 points (95% CI, 3.3 to 14.1) in the diflunisal group, a difference of 16.3 points (95% CI, 8.1 to 24.5, p=0.001). Mean SF-36 physical scores fell 4.9 points (95% CI, −7.6 to −2.2) among placebo and rose 1.5 points (95% CI, −0.8 to 3.7) in the diflunisal group (p=0.003). SF-36 mental scores declined 1.1 (95% CI, −4.3 to 2.0) among placebo while increasing 3.7 (95% CI, 1.0 to 6.4) in the diflunisal group (p=0.022). By responder analysis, 29.7% of diflunisal and 9.4% of placebo exhibited neurologic stability at 2 years (< 2 points NIS +7 increase) (p=0.007).

Conclusions and Relevance—Among patients with ATTR-FAP, the use of diflunisal compared with placebo for 2 years reduced the rate of progression in neurologic impairment and preserved quality of life. Although longer term follow up studies are needed, these findings suggest benefit of this treatment for ATTR-FAP.

INTRODUCTION

Hereditary transthyretin amyloidosis (ATTR) is a lethal, autosomal dominant genetic disease caused by the aggregation of variant and wild type transthyretin (TTR), a thyroxine transport protein predominantly produced by the liver.[1, 2] More than 100 different mutations in the TTR gene destabilize its tetrameric structure, promoting TTR dissociation and misassembly

into oligomeric aggregates including amyloid fibrils.[3, 4] The process of TTR amyloidogenesis produces a spectrum of debilitating disease ranging from pure polyneuropathy (transthyretin-type familial amyloid polyneuropathy (ATTRFAP)) to selective heart involvement.[5, 6] In ATTR-FAP, small and large fiber injury induce sensory and autonomic deficits accompanied by motor weakness in a length dependent fashion, mimicking manifestations of diabetic polyneuropathy. Untreated, patients exhibit progressive neurologic deficits, dying 10–15 years after disease presentation.[7] Fewer than 10,000 people are estimated to be clinically affected world-wide.[8]

Orthotopic liver transplantation, standard treatment for FAP since its initial use in 1990, eliminates 95% of variant TTR from the blood and impacts the course of disease.[9, 10] However, limited organ availability, exclusion of older patients and those with advanced disease, the high costs of transplantation, the risks of life-long immunosuppression, and reports of disease progression following liver transplantation[11, 12] warrant development of alternative treatments.

Dissociation of TTR tetramers is the rate limiting step of amyloidogenesis in patients with ATTR-FAP.[13, 14] Slowing TTR tetramer dissociation by either ‘interallelic trans suppression’[13, 15] in which a second TTR gene mutation counters the destabilizing effect of the first TTR mutation, or by the binding of small molecule kinetic stabilizers to TTR tetramers appears to minimize clinical disease expression.[16, 17,18] A phase I study demonstrated that diflunisal, a generic non-steroidal anti-inflammatory drug, at 250 mg twice daily successfully complexes to the thyroxine binding site and kinetically stabilizes circulating TTR tetramers, inhibiting release of the TTR monomer required for amyloidogenesis.[16, 19]

Pursuing the NIH mission to repurpose old drugs, we conducted an investigator-initiated, international, multi-center, randomized, double-blind, placebo-controlled study to determine the effect of diflunisal on polyneuropathy progression in patients with ATTR-FAP.

METHODS

STUDY CONDUCT AND OVERSIGHT

All patients provided written informed consent. The institutional review board or ethics committee at each participating study site approved the study protocol. An NIH-appointed Data and Safety Monitoring Board regularly examined aggregate data for effect and futility, and all adverse events for evidence of patient harm. A medical monitor reviewed all serious adverse events at the time of reporting. Merck Sharp & Dohme, Inc. (Whitehouse Station, NJ) produced and donated diflunisal 250 mg tablets at the outset of the study; Bilcare, Inc. (Phoenixville, PA) over encapsulated the diflunisal tablets and generated matching capsules filled with excipient for placebo use. Stability and dissolution profiles of the over encapsulated diflunisal tablets were generated by Bilcare, Inc. at 24, 36, 48 and 60 months, and the data reviewed by the FDA Center for Drug Evaluation and Research (CDER).

STUDY PATIENTS

We recruited patients with ATTR-FAP from 8 amyloid centers located in 5 countries (England, Italy, Japan, Sweden, and United States). Patients were eligible for the study if they were between 18 and 75 years, had biopsy proven amyloid deposition by Congo Red staining and mutant TTR genopositivity by DNA sequence analysis, exhibited signs of peripheral or autonomic neuropathy clinically detectable by a trained neurologist, and routinely spent more than 50% of waking hours out of bed or chair (ECOG performance status < 3). Exclusions included alternative causes of sensorimotor polyneuropathy (e.g., diabetes mellitus and vitamin B12 deficiency), limited survival (< 2 years), prior liver transplantation, severe congestive heart failure (class IV New York Heart Association (NYHA)) or renal insufficiency (estimated creatinine clearance <30 mL/min), and ongoing anticoagulation. Full inclusion and exclusion criteria are provided in the Supplementary Appendix (eTable 1).

STUDY ENDPOINTS

Neuropathy Impairment Score (NIS) plus 7 nerve tests (NIS+7) combines a study neurologist's clinical assessment of muscle weakness, sensory loss, and decreased muscle stretch reflexes (NIS) with 5 nerve conduction study attributes derived from 3 lower extremity nerves (tibial nerve distal motor latency; peroneal nerve compound muscle action potential amplitude, distal motor latency and velocity; and sural nerve sensory nerve action potential amplitude); vibratory detection threshold determined by quantitative sensory testing; and heart rate variability during deep breathing (HRDB) (CASE IV, WR Medical Electronics, Maplewood, MN).[20] Higher NIS+7 scores reflect greater neurologic deficit (0 to 270 points). NIS+7 composite scoring has been validated as a neuropathy measure in longitudinal studies of diabetic polyneuropathy, a disease that mimics the clinical and histologic manifestations of ATTR-FAP.[20–22] The international Peripheral Nerve Society defined a 2-point change in NIS+7 score as the minimal clinically important difference detectable by neuromuscular experts.[23] A 2-point change in NIS+7, for example, could reflect a 25% decline in muscle strength and a 50% decrease in muscle stretch reflex, touch pressure vibration, pin prick or joint motion sensation. NIS or NIS+7 scoring has been used in clinical trials for diabetic sensorimotor polyneuropathy,[20, 24–27] monoclonal gammopathy of undetermined significance (MGUS) neuropathy,[28] and chronic inflammatory demyelinating polyradiculopathy (CIDP).[29, 30] To ensure high quality endpoint evaluation of NIS+7 we employed standardized tests with published reference values; clinical evaluations by certified neurologists and clinical neurophysiologists; extensive pre-training and certification of all clinical investigators performing quantitative sensory testing, standardized electromyography, and neurologic examinations; use of reference percentile values obtained from a healthy cohort study; and quality control in a central reading center.[31] One neurologist at each study site was designated to perform all NIS examinations, limiting interobserver variability.[32] The difference in progression of polyneuropathy between treatment groups, measured as change of mean NIS+7 scores from enrollment to 2 years treatment, constituted the primary endpoint. Patients discontinuing drug before study completion were invited to return at 24 months to complete NIS+7 testing.

Secondary endpoint measures included NIS (0–244 points) and NIS-Lower Limb (NIS-LL, 0–88 points) with higher scores indicating greater deficits, the Short Form 36 (SF-36) quality of life questionnaire (0–100 points, lower scores reflecting diminished status), an instrument used to study treatment impact in other forms of systemic amyloidosis;[33] modified body mass index (mBMI), the product of serum albumin concentration (g/L) and BMI that correlates with survival in ATTR-FAP;[34, 35] and the Kumamoto Score (0–96 points, increasing with disease severity), a clinical neurologic scale of motor, sensory, and autonomic function combined with heart and kidney end organ measures developed to track disease progression in ATTR-FAP.[36]

STUDY DESIGN

We designed and conducted a randomized, placebo-controlled clinical trial in which patients, investigators, study coordinators, and investigational pharmacists were unaware of treatment assignments. Patients were randomly assigned in 1:1 manner to receive diflunisal 250 mg or matching placebo capsules twice daily by mouth for 2 years. Randomization was performed in permuted blocks of 2 or 4 stratified for mutant TTR (non-V30M versus V30M) and study site. Study drug pre-packaged according to a computer generated randomization scheme was dispensed by independent investigational pharmacists using sequential study IDs. The randomization code was not broken at any time during the study. We assessed study drug adherence by counts of returned pills, defining compliance as > 80% pill use ([dispensed – returned study drug)/dispensed drug]*100).

NIS+7 (including NIS, NIS-LL, nerve conduction studies), Quality of Life (SF-36) questionnaires, mBMI, and Kumamoto Scale data were collected at enrollment, 12 and 24 months (study end). Patients visited their primary care providers at 1, 3, and 18 months after enrollment for vital signs, complete blood counts, occult stool testing, and serum chemistries. A 6 month study site visit with full neurologic testing in the absence of nerve conduction studies provided early monitoring for deleterious study drug effects.

STATISTICAL ANALYSIS

We performed power calculations for two sample, two-sided t-tests comparing changes in NIS+7 scores (endpoint – baseline) between the two groups of the trial. In the absence of NIS+7 data in ATTR-FAP patients, we used estimates of the variability of NIS+7 scores over time in diabetic polyneuropathy to calculate expected effect sizes; a 2 point difference corresponding to a moderate effect size of 0.56. We planned to enroll 70 patients per group yielding a power of 0.842 to detect a moderate effect size of 0.5 (a 1.8 point difference in NIS+7 scoring), with a two-sided test at alpha level 0.05 in the intention-totreat (ITT) population, defined as all randomized patients who initiated treatment. Study drug expiration limited accrual to 130 patients, a sample size (65 patients per group) that provided a power of 0.814 to detect the moderate effect size previously described.

We assessed baseline characteristics and comparability of the two treatment groups by the two-sample t-test for continuous variables and by Chi-square or Fisher’s exact test for categorical variables. We compared attrition across study groups by survival analyses with log-rank testing. We identified significant deviation from the assumption of missingness

completely at random (MCAR) for the data using the permutation test.[37] Given the non MCAR character of attrition, we performed likelihood based longitudinal analyses using general linear models for repeated measures of outcome data collected at baseline, 12 and 24 months to expand our primary analyses.[38] To assess the sensitivity of inferences to assumptions on the missing data, we performed sensitivity analyses using multiple imputations[39] (incorporating previous outcome values, treatment, and TTR mutation group), last observation carried forward, and a 'worst case scenario' (assigning the highest observed NIS+7 score to all missing values following dropout). We employed a categorical "responder" analysis applying extreme assumptions of 'success' (< 2 point change in NIS +7) or 'failure' (≥ 2 point change in NIS+7 or study drop out for any reason) to both treatment groups. Fisher's exact test was used to compare treatment "response" between the study groups. We calculated risk ratios (RR) for success and their 95% confidence intervals. We employed the 'responder' analysis, biased against treatment success by its stringent definition, as another 'worst case scenario' analysis. In the completers analysis, we used analysis of covariance (ANCOVA) to adjust for baseline outcome measures in the evaluation of primary and secondary endpoints. All analyses were two-sided with alpha level set at 0.05. SAS 9.2 statistical software (SAS Institute Inc., Cary, NC) was used for all computations.

RESULTS

PATIENT CHARACTERISTICS

A total of 249 patients were screened for participation in the study; 130 patients were enrolled and randomized. The most frequent reasons for ineligibility included a lack of biopsy-proven amyloid deposits (37.6%), absence of clinically detectable peripheral or autonomic neuropathy (33.9%), normal TTR DNA analyses (12.8%), other causes of sensorimotor polyneuropathy (12.8%), and current anticoagulation (11.9%) (Figure 1).

Baseline characteristics, TTR genotyping, and polyneuropathy staging were similar between treatment groups (Table 1). Non-V30M ATTR included T60A (11.5%), L58H (11.5%), F64L (3.1%), S50R (3.1%), and 17 other genotypes (eTable 2). Nearly one third (30.8%) of the patients required support when walking; 4 patients in each treatment group were wheelchair bound. Outcome measures including NIS+7, NIS, NIS lower limb (NIS-LL), Kumamoto Score, modified body mass index (mBMI), SF-36 physical and mental scores were not statistically different between groups at enrollment.

COMPLIANCE

Compliance, defined as > 80% pill use by counts of returned study drug, was 100% in the placebo and 91.8% in the diflunisal groups at 12 months. At 24 months, 86.2% of the placebo and 85.4% of the diflunisal groups were compliant.

ATTRITION

Sixty-seven patients discontinued study drug before completing the 2 year protocol, including 40 patients from the placebo group and 27 from the diflunisal group. Disease progression (23 placebo, 11 diflunisal) and orthotopic liver transplantation (9 placebo, 7

diflunisal) were the leading reasons for drop out (Figure 1). Baseline and 1 year NIS+7 scores were collected on 37 patients randomized to placebo and 50 randomized to diflunisal treatment. Baseline and 2 year NIS+7 scores were obtained in 28 patients assigned to placebo and 40 assigned to diflunisal treatment. Five patients in the placebo group and three in the diflunisal group discontinued study drug and acquired diflunisal outside the study but completed 24 month NIS+7 testing.

Survival analysis of attrition by treatment assignment revealed greater drop out in the placebo group over time (log-rank test p=0.025). There were no statistically significant differences in attrition by variant TTR (V30M versus non-V30M), age, or polyneuropathy staging (p=0.312, 0.291, and 0.355, respectively). Analysis of missingness completely at random for the primary and secondary outcomes using the permutation test indicated dependence of drop out on the outcome values. Drop out was preceded by significantly worse disease state. Those who dropped out after 12 months had significantly higher 12 month NIS+7 score (permutation test p=0.023), higher NIS and NISLL scores (p=0.032) and lower SF-36 physical score (p=0.002) than patients remaining on study drug.

EFFICACY

LONGITUDINAL ANALYSIS—Differential attrition is a prominent feature of this study. To address missingness, we applied varied statistical methods. Longitudinal analysis examined data from all 130 participants (66 placebo, 64 diflunisal) using intention-to-treat principles. By longitudinal analysis of the primary outcome measure, the change in NIS+7 over time, patients randomized to diflunisal exhibited significantly less progression of polyneuropathy than those assigned to placebo. The change in NIS+7 from baseline to 2 years was 26.3 points (95% CI, 20.2 to 32.4) in the placebo group and 8.2 points (95% CI, 2.9 to 13.6) in the diflunisal group, a difference of 18.0 points between treatment groups (95% CI, 9.9 to 26.2, p<0.001) (Table 2). The inhibitory effect of diflunisal on neuropathy progression was also detectable at 1 year. The change in NIS+7 from baseline to 1 year measured 12.5 points (95% CI, 8.6 to 16.4) in the placebo group versus 6.2 points (95% CI, 2.8 to 9.6) in the diflunisal group, a difference of 6.4 points (95% CI, 1.2 to 11.6, p=0.017). Additionally, diflunisal treatment inhibited change in NIS and NIS-LL, components of the NIS+7 composite score, from baseline to 2 years when compared to the placebo group (NIS diflunisal 6.4 points (95% CI, 1.6 to 11.2) versus placebo 23.2 points (95% CI, 17.8 to 28.5, p<0.001); NIS-LL diflunisal 3.8 points (95% CI, 0.9 to 6.6) versus placebo 12.1 points (95% CI, 8.9 to 15.3, p=0.002) (Table 2).

The baseline to 2 year change in secondary outcomes supported the reduced disease progression demonstrated by NIS+7 scores in the diflunisal treatment group. The clinical Kumamoto Score detected greater inhibition of disease progression at 2 years in the diflunisal treatment group (3.1 points, 95% CI, 1.1 to 5.1) than the placebo group (8.0 points, 95% CI, 5.8 to 10.3, p=0.015) (Table 2). A trend toward slower decline in mBMI from baseline to 2 years in the diflunisal group did not meet statistical significance (p=0.211) (Table 2). Physical quality of life (SF-36) stabilized from baseline to 2 years in those assigned to diflunisal treatment (1.2 points, 95% CI, −1.2 to 3.7) while falling in the placebo group (−4.9 points, 95% CI, −7.6 to −2.1, p=0.001). Although mental quality of life

at 2 years improved in the diflunisal group (3.5 points, 95% CI, 0.4 to 6.7), the difference between treatment groups was not statistically significant (−4.5 points, 95% CI, −9.2 to 0.2, p=0.062) (Table 2). The diflunisal effect on outcome measures was seen across study sites, gender, TTR mutation grouping, and neuropathy stage at entry.

SENSITIVITY ANALYSES—Sensitivity analyses including multiple imputations (MI), last observation carried forward (LOCF), and 'worst case scenario' imputation (substituting maximal NIS+7 scores for all dropout data points) substantiated our findings. As with longitudinal analysis of the data, MI identified a significant inhibitory effect of diflunisal on neuropathy progression by multiple outcome measures – including both physical and mental QOL (Table 3). Specifically, MI analysis estimated a difference in change between placebo and diflunisal groups of 16.3 points (95%CI, 8.1 to 24.5, p=0.001) for NIS+7 at 2 years and 6.1 points (95% CI, 1.1 to 11.1, p=0.017) at 1 year; 16.1 points (95% CI, 9.0 to 23.2, P<0.001) for NIS at 2 years and 5.9 points (95% CI, 1.8 to 10.0, p=0.005) at 1 year; 8.2 points (95% CI, 4.0 to 12.5, p<0.001) for NIS-LL at 2 years; 4.9 points (95% CI, 1.7 to 8.1, p=0.003) for Kumamoto Score at 2 years; −6.4 points (95% CI, −9.8 to −2.9, p<0.001) for physical QOL and −4.9 points (95% CI, −9.0 to −0.7, p=0.022) for mental QOL at 2 years. Modified BMI was the only endpoint that did not detect a favorable diflunisal effect.

LOCF analyses, biased toward the null by effectively limiting the magnitude of polyneuropathy progression assigned to drop outs, also estimated significant differences between groups of 6.6 points (95%CI, 1.3 to 11.8) at 2 years. Although the 1 year LOCF analysis was not statistically significant, the direction of effect again favored diflunisal.

A 'worst case scenario' analysis, assigning the highest observed NIS+7 scores to all missing data points following study dropout, also revealed a significant difference in NIS+7 change between treatment groups of 25.9 points (95%CI, 3.0 to 48.8, p=0.027) at 2 years and 25.0 points (95%CI, 4.1 to 45.9, p=0.019) at 1 year.

By responder analysis (assigning treatment failure to all study drop outs and patients with >2 point rise in NIS+7), the diflunisal group exhibited significantly greater neurologic stability at 2 years than the placebo group (29.7% versus 9.4%, p=0.007). Risk ratio analysis indicated a 3-fold greater probability of response in the diflunisal versus the placebo group (RR 3.2, 95%CI, 1.4 to 7.4). Greater apparent neurologic stability by responder analysis of 1 year data among patients receiving diflunisal versus placebo treatments (26.6% versus 14.1%, p=0.123 and RR 1.9, 95% CI, 0.9 to 3.9) did not meet statistical significance.

COMPLETERS (ANCOVA) ANALYSIS—Eighty-seven patients (37 placebo, 50 diflunisal) completed NIS+7 at 1 year and 68 patients (28 placebo, 40 diflunisal) completed at 2 years. We used ANCOVA analysis to examine change from baseline at 1 and 2 years for the primary (NIS+7) and secondary outcomes in those patients completing measurements ('completers'). As with the longitudinal and MI analyses, a completers analysis supported the inhibitory effect of diflunisal on ATTR-FAP neuropathy by all measures examined. At 2 years, outcomes reflecting beneficial diflunisal effect expressed as significant differences between treatment groups included NIS+7 (13.5 points, 95% CI, 6.5 to 20.6, p<0.001), NIS (13.8 points, 95% CI, 7.5 to 20.1, P<0.001), NIS-LL (7.1 points, 95% CI, 3.2 to 11.1,

p<0.001), Kumamoto score (3.9 points, 95% CI, 0.9 to 6.8, p=0.010), physical QOL (−6.9 points, 95% CI, −10.5 to −3.3, p=0.048) and mental QOL (−4.3 points, 95% CI, −8.5 to −0.2, p=0.040), and mBMI (−50.8 (95% CI, −101.1 to −0.6, p=0.048) (Table 4).

ADVERSE EVENTS

A complete listing of adverse events by patient is provided in Supplemental Tables (eTable 3). GI, renal, cardiac, and blood adverse events occurred in similar numbers by treatment groups. Independent of relatedness, adverse events in the musculoskeletal and general disorders categories occurred more frequently in the diflunisal group, however drug-related adverse events by patient did not differ between groups. No differences in serious adverse events by patient were reported between treatment groups. Drug-related adverse events led to study drug discontinuation in 4 patients from the diflunisal group (gastrointestinal bleed, congestive heart failure, glaucoma, nausea) and 2 patients from the placebo group (headache, renal failure). Thirteen deaths (9 placebo group, 4 diflunisal group) were reported by 24 months with 12 occurring off study drug.

DISCUSSION

In this investigator-initiated, international, randomized, double-blind, placebo-controlled trial, diflunisal 250 mg taken twice daily for 2 years inhibited progression of polyneuropathy in patients with ATTR-FAP. A 2- to 3-fold beneficial diflunisal effect was detected by multiple measures at 2 years including a quantitative composite neuropathy primary endpoint (NIS+7), a qualitative neuropathy and end organ scale developed for ATTR-FAP (Kumamoto Score), and modified BMI, a predictor of survival in ATTR-FAP. As a 2 point change in NIS+7 identifies a minimal clinically detectable change in polyneuropathy progression,[23] the 16.3 point NIS+7 difference between treatment groups at 2 years by MI analysis in this study signals a clinically meaningful diflunisal effect. Confining neurologic deficits to lower limb muscle function, a 16 point NIS+7 difference might represent a 50% decline of knee extensor and flexor strength plus ankle dorsiflexion in the placebo group with no change occurring in the treatment group – approximating the ability to rise from a chair or walk unaided. The magnitude of polyneuropathy progression measured over 2 years by NIS+7 in the placebo group (25 points) far exceeded the 2 year progression reported in diabetics (1.70 points),[20] quantifying the devastating nature of ATTR-FAP. The NIS+7 finding extended across TTR mutations (V30M and non-V30M), gender, neuropathy severity (PND staging), and major study sites. Importantly, diflunisal affected not only the progression of neuropathy but also the quality of life for FAP patients, a critical element when considering the impact of new treatments. Although our study design targeted 2 years observations, a clinically significant diflunisal effect (2-fold less polyneuropathy progression by NIS+7 versus the placebo group) was evident after 1 year of treatment, supporting shorter observation periods in future drug trials.

A recent clinical study initiated after our trial began examined the effect of a proprietary kinetic stabilizer (tafamidis®) on ATTR-FAP disease progression.[17] Enrollment was limited to patients with one TTR mutation (V30M) and early polyneuropathy. By intention to treat (ITT) analysis, tafamidis treatment did not meet statistical significance for its co-primary

endpoints, NIS-LL and a quality of life questionnaire (Norfolk Quality of Life Diabetic Neuropathy). Limiting analysis to patients completing the 18-month protocol, however, revealed a statistically significant drug effect.[17] In contrast, our study is the first to involve a cohort representative of ATTR-FAP disease and report a treatment effect that met its primary and secondary end points (and NIS-LL) by ITT, LOCF, multiple imputations, and sensitivity/responder analyses.

In addition to demonstrating by multiple measures that diflunisal inhibits progression of debilitating polyneuropathy in patients with ATTR-FAP, the Diflunisal Trial is pivotal for several reasons. It is the first randomized controlled trial involving a broad cross section of the spectrum of disease and the most prevalent genotypes for ATTR patients with polyneuropathy. It provides invaluable natural history data on the rate of neurologic disease progression (NIS+7 12–13 points/year) in an inclusive and heterogeneous ATTR-FAP population that will be the foundation of future clinical trial designs for this disease. It supports the use of a composite quantitative neuropathy score (NIS+7) in monitoring progression of polyneuropathies involving large and small fiber disease, correlating clinically detectable change with impact on quality of life. It establishes that diflunisal is well tolerated by ATTR-FAP patients with a spectrum of neuropathy often compounded by amyloid cardiomyopathy. It suggests that the diflunisal effect may extend to patients with advanced polyneuropathy, a population often deemed ineligible for orthotopic liver transplantation. It provides a low cost treatment by re-purposing a drug that had lost its clinical relevance as a non-steroidal anti-inflammatory agent. Finally this study provides proof-of-concept that kinetically stabilizing an amyloidogenic precursor protein (transthyretin) translates to successfully modifying amyloid-related neurologic disease progression.

Attrition, a limitation of this study, occurred unequally across treatment groups as might be expected when dealing with a neurologically progressive disorder and a disease-altering treatment. Indeed, disease progression, the predominant cause for drop out, occurred 50% more frequently in the placebo group and explained the attrition differences between treatment groups. Reasons for significant drop out included a) the unexpected rapidity of neurologic decline during the 2 year observation period (more than 10 times the rate of diabetic polyneuropathy), b) existence of a validated alternative treatment (liver transplantation), and c) wide-spread availability of diflunisal outside the study. By assigning a final NIS+7 score at drop out for those lost to follow up, early drop out predominantly limited recorded neurologic decline in the placebo group, minimizing NIS+7 differences between the treatment groups. Despite these limitations, our data reveal statistically significant diflunisal effect on ATTR-FAP by multiple measures of neurologic function and quality of life attributes. We performed multiple statistical analyses to address attrition, including a ‘worst case scenario’ analysis that assigned the highest possible NIS+7 score to all data points occurring after patient dropout. These analyses did not materially alter our findings or conclusions. Moreover, dichotomous responder analysis, assigning treatment failure to all study withdrawals regardless of cause and to patients with even the smallest clinically detectable worsening of composite neurologic score (NIS+7 > 2 points), revealed significantly greater neurologic stability (success) at 2 years in the diflunisal group than the placebo group.

CONCLUSION

Among patients with ATTR-FAP, the use of diflunisal compared with placebo for 2 years reduced the rate of progression in neurologic impairment and preserved quality of life. Although longer term follow up studies are needed, these findings suggest benefit of this treatment for ATTR-FAP. These findings support the NIH mission of repurposing old drugs for new indications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Appendix

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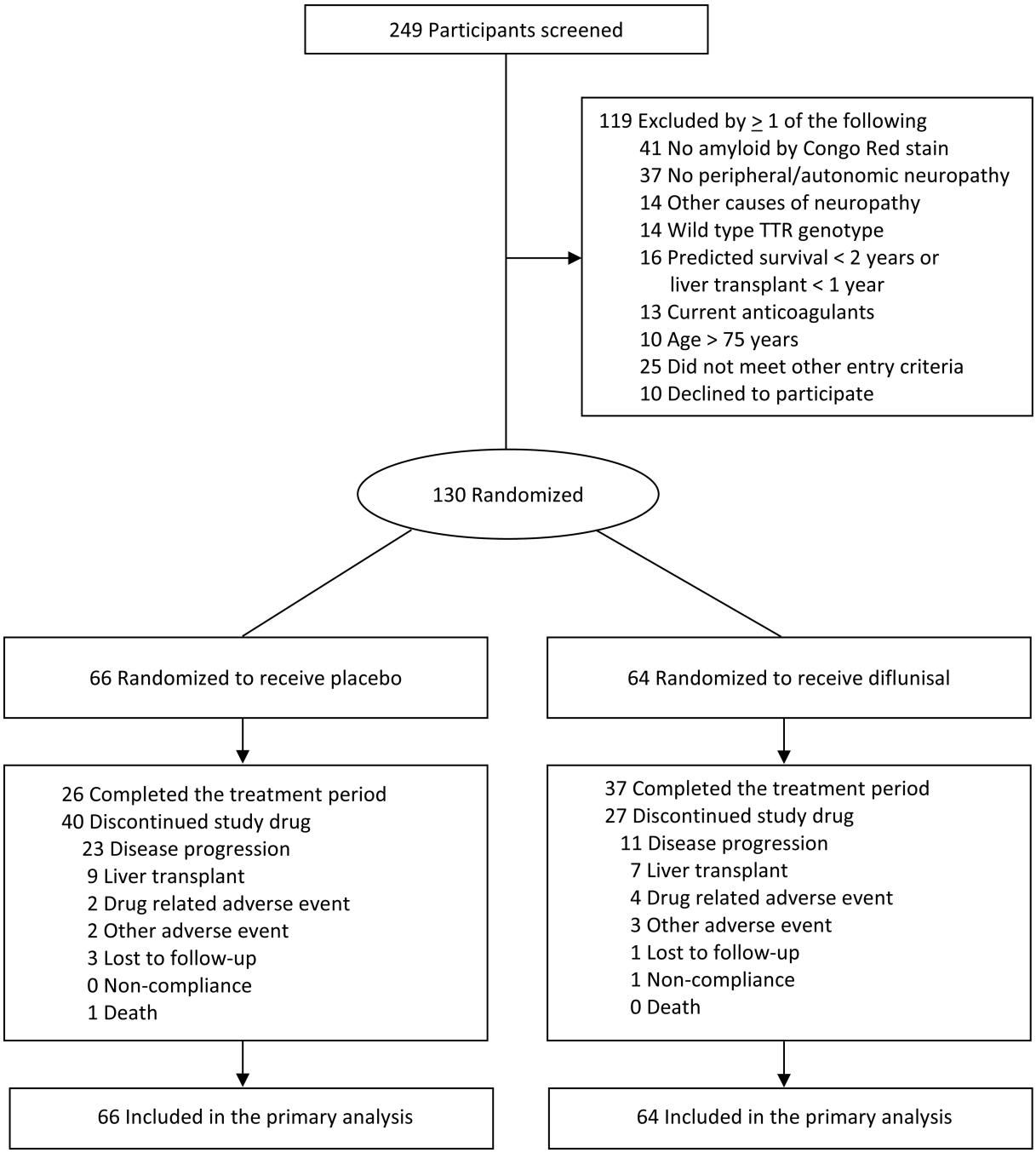

Figure 1. Screening, Randomization and Follow-up

63 patients (26 placebo, 37 diflunisal) remained on drug for 24 months. Analyzable primary outcome data were obtained on 60 of these patients (23 placebo, 37 diflunisal); 3 placebo patients had inadmissible NIS+7 data. Study drug was discontinued prior to 24 months in 67 patients (40 placebo, 27 diflunisal); 24 month primary outcome data (NIS+7) were obtained on 8 of these patients (5 placebo, 3 diflunisal).

Table 1
Baseline Demographics and Clinical Characteristics of the Patients

Data are means ± SD.; NIS data are medians (range).

Characteristic	Overall (N=130)	Placebo (N=66)	Diflunisal (N=64)
Demographics			
Age	59.7±11.9	59.2±12.2	60.3±11.7
Gender – no. of patients (%)			
Male	87 (66.9%)	44 (66.7%)	43 (67.2%)
Female	43 (33.1%)	22 (33.3%)	21 (32.8%)
Race – no. of patients (%)			
Asian	14 (10.8%)	6 (9.1%)	8 (12.5%)
Black	6 (4.6%)	5 (7.6%)	1 (1.6%)
White	102 (78.5%)	50 (75.8%)	52 (81.3%)
Other	1 (0.8%)	1 (1.5%)	0 (0.0%)
Multiracial	7 (5.4%)	4 (6.1%)	3 (4.7%)
ATTR Genotypes – no. of patients (%)			
Met30	71 (54.6%)	35 (53%)	36 (56.3%)
Non-Met30	59 (45.4%)	31 (47%)	28 (43.8%)
Lab Data			
Serum Albumin (g/dL)	4.1±0.4	4.1±0.4	4.1±0.4
Postural Systolic Blood Pressure Change (mmHg)	−11±18.9	−13.2±20.2	−8.8±17.4
Outcomes			
Disease Stage based on PND– no. of patients (%) #			
I	49 (37.7%)	21 (31.8%)	28 (43.8%)
II	41 (31.5%)	23 (34.8%)	18 (28.1%)
IIIA	19 (14.6%)	8 (12.1%)	11 (17.2%)
IIIB	13 (10%)	10 (15.2%)	3 (4.7%)
IV	8 (6.2%)	4 (6.1%)	4 (6.3%)
NIS+7 ¶	55.3±46.5	59±50	51.6±42.8
Median (Range)	41.4 (0–181.6)	42.3 (0–176.1)	39.3 (3.6–181.6)
NIS §	42.5±43.2	45.4±46.3	39.4±39.9
Median (Range)	27.9 (0–164.8)	30.8 (0–160.3)	23.5 (0–164.8)
NIS-LL //	26.1±23.2	27.2±24.5	24.9±22
Median (Range)	20 (0–79.9)	21.5 (0–79.8)	17.8 (0–79.9)
Kumamoto Score ◊	16±12.2	16.7±13.5	15.3±10.8
mBMI **	1021.7±240.4	1019±255	1024.4±226.3
SF36 Physical †	35.4±11.3	34.8±11	35.9±11.6
SF36 Mental ‡	46.6±12.9	46.5±11.8	46.6±14.1

[*] Plus-minus values are means ±SD. NIS scores are also expressed as medians with ranges. There were no significant differences between the groups. Percentages may not sum to 100 because of rounding.

[#] A Polyneuropathy Disability (PND) score of I indicates sensory disturbances but preserved walking capability; a grade of II indicates impaired walking, ability without need for a stick; a grade of IIIA indicates walking only with the help of one stick; a grade of IIIB indicates walking with the help of two sticks; a grade of IV indicates confined to a wheelchair or bedridden.

[¶] Neuropathy Impairment Score plus 7 nerve tests (NIS+7) ranges from 0–270 with higher scores indicating greater neurologic deficits.

[§] Neuropathy Impairment Score (NIS) ranges from 0–244 with higher scores indicating greater neurologic deficits.

[//] Neuropathy Impairment Score of the Lower Limbs (NIS-LL) ranges from 0–88 with higher scores indicating greater neurologic deficits.

[◊] Kumamoto Score ranges from 0 to 102 with higher scores indicating more severe polyneuropathy.

[**] Modified body-mass index (mBMI) is the product of BMI (weight in kilograms divided by the square of the height in meters) and serum albumin (g/L).

[†] Physical component scores of the 36-Item Short-Form Health Survey (SF-36) range from 0 to 100, with higher scores indicating greater physical quality of life.

[‡] Mental component scores of the 36-Item Short-Form Health Survey (SF-36) range from 0 to 100, with higher scores indicating greater mental quality of life.

Table 2
Longitudinal Analyses of Primary (NIS+7) and Secondary Outcomes

General linear models for repeated measures of outcome data were used. Data are means of change from baseline to 12 and 24 months for primary and secondary outcome measures by treatment groups, including 95% CI. P values address the differences between treatment groups in change over 12 and 24 months for each outcome measure.

	Placebo Change from Baseline	Diflunisal Change from Baseline	Difference Placebo-Diflunisal	P-value
Characteristic	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	
NIS+ND7				
Month 12	12.5 (8.6,16.4)	6.2 (2.8,9.6)	6.4 (1.2,11.6)	0.017
Month 24	26.3 (20.2,32.4)	8.2 (2.9,13.6)	18.0 (9.9,26.2)	<0.001
NIS				
Month 12	10.1 (6.9,13.3)	4.1 (1.2,6.9)	6.0 (1.7,10.3)	0.007
Month 24	23.2 (17.8,28.5)	6.4 (1.6,11.2)	16.8 (9.6,24.0)	<0.001
NISLL				
Month 12	6.0 (3.9,8.2)	3.2 (1.3,5.2)	2.8 (−0.1,5.7)	0.056
Month 24	12.1 (8.9,15.3)	3.8 (0.9,6.6)	8.3 (4.1,12.6)	<0.001
Kumamoto Score				
Month 12	4.1 (2.1,6.2)	1.9 (0.1,3.7)	2.3 (−0.5,5)	0.103
Month 24	8.0 (5.8,10.3)	3.1 (1.1,5.1)	5.0 (1.9,8.0)	0.002
mBMI				
Month 12	−38.5 (−74.9,−2.1)	−18.7 (−51.6,14.1)	−19.8 (−68.8,29.2)	0.426
Month 24	−67.9 (−108.1,−27.7)	−33.7 (−69.3,1.8)	−34.1 (−87.8,19.5)	0.211
SF36 Physical				
Month 12	−1.9 (−3.9,0.2)	0.7 (−1.1,2.5)	−2.6 (−5.3,0.1)	0.059
Month 24	−4.9 (−7.6,−2.1)	1.2 (−1.2,3.7)	−6.1 (−9.8,−2.5)	0.001
SF36 Mental				
Month 12	0.8 (−2,3.6)	2.5 (0.0,5.1)	−1.7 (−5.5,2.1)	0.367
Month 24	−0.9 (−4.4,2.5)	3.5 (0.4,6.7)	−4.5 (−9.2,0.2)	0.062

Table 3
Multiple Imputation Analysis of Primary (NIS+7) and Secondary Outcomes

Imputation incorporating previous outcome values, treatment, and TTR mutation group was used for missing values. Data are means of change from baseline to 12 and 24 months for primary and secondary outcome measures by treatment groups, including 95% CI. P values address the differences between treatment groups in change over 12 and 24 months for each outcome measure.

	Placebo Change from Baseline	Diflunisal Change from Baseline	Difference Placebo-Diflunisal	P-value
Characteristic	**Mean (95% CI)**	**Mean (95% CI)**	**Mean (95% CI)**	
NIS+ND7				
Month 12	12.5 (8.6,16.4)	6.4 (3.1,9.6)	6.1 (1.1,11.1)	0.017
Month 24	25 (18.4,31.6)	8.7 (3.3,14.1)	16.3 (8.1,24.5)	<0.001
NIS				
Month 12	10.1 (6.9,13.3)	4.2 (1.5,7.0)	5.9 (1.8,10.0)	0.005
Month 24	22.8 (17.2,28.4)	6.7 (1.9,11.4)	16.1 (9.0,23.2)	<0.001
NISLL				
Month 12	6.0 (3.9,8.2)	3.3 (1.4,5.1)	2.8 (0.0,5.6)	0.051
Month 24	12.1 (8.7,15.5)	3.8 (1.0,6.7)	8.2 (4.0,12.5)	<0.001
Kumamoto Score				
Month 12	4.1 (1.9,6.4)	1.9 (0,3.7.0)	2.3 (−0.6,5.2)	0.121
Month 24	8.1 (5.7,10.6)	3.2 (1.1,5.3)	4.9 (1.7,8.1)	0.003
mBMI				
Month 12	−40.3 (−75.4,−5.2)	−19.7 (−54.1,14.7)	−20.6 (−69,27.9)	0.406
Month 24	−65.1 (−107.4,−22.7)	−35.2 (−73.6,3.3)	−29.9 (−85.7,25.9)	0.293
SF36 Physical				
Month 12	−1.9 (−3.8,−0.1)	0.8 (−0.9,2.5)	−2.8 (−5.2,−0.3)	0.030
Month 24	−4.9 (−7.6,−2.2)	1.5 (−0.8,3.7)	−6.4 (−9.8,−2.9)	<0.001
SF36 Mental				
Month 12	0.6 (−1.7,3.0)	2.3 (0.1,4.5)	−1.7 (−4.9,1.5)	0.300
Month 24	−1.1 (−4.3,2.0)	3.7 (1.0,6.4)	−4.9 (−9.0,−0.7)	0.022

Table 4

Completers Analysis of Primary (NIS+7) and Secondary Outcomes

Data are Least Squares Means of change from baseline to 12 and 24 months for primary and secondary outcome measures by treatment groups, including 95% CI. The difference between treatment groups at 12 and 24 months is expressed as placebo-diflunisal LSM with 95% CI. Corresponding p values were calculated by ANCOVA models.

	Placebo Change from Baseline		Diflunisal Change from Baseline		Difference Placebo-Diflunisal Mean (95% CI)	P-value
Characteristic	N	Mean (95% CI)	N	Mean (95% CI)		
NIS+ND7						
Month 12	37	12.8 (8.9,16.7)	50	6.3 (2.9,9.6)	6.5 (1.4,11.7)	0.013
Month 24	28	20.1 (14.7,25.6)	40	6.6 (2.0,11.1)	13.5 (6.5,20.6)	<0.001
NIS						
Month 12	39	10.1 (6.9,13.3)	50	4.1 (1.3,6.9)	6.0 (1.7,10.2)	0.006
Month 24	30	18.9 (14.1,23.7)	40	5.1 (0.9,9.2)	13.8 (7.5,20.1)	<0.001
NISLL						
Month 12	39	6.1 (4.0,8.3)	50	3.3 (1.4,5.2)	2.8 (−0.1,5.7)	0.055
Month 24	30	9.9 (6.9,12.8)	40	2.7 (0.2,5.3)	7.1 (3.2,11.1)	<0.001
Kumamoto Score						
Month 12	38	4.4 (2.4,6.4)	49	2.0 (0.2,3.8)	2.4 (−0.3,5.1)	0.082
Month 24	29	6.6 (4.4,8.9)	39	2.8 (0.9,4.7)	3.9 (0.9,6.8)	0.010
mBMI						
Month 12	40	−46.6 (−82.2,−11.1)	50	−20.7 (−52.5,11.1)	−25.9 (−73.6,21.8)	0.287
Month 24	29	−76.5 (−114.6,−38.4)	39	−25.7 (−58.5,7.2)	−50.8 (−101.1,−0.6)	0.048
SF36 Physical						
Month 12	38	−2.5 (−4.4,−0.6)	50	0.4 (−1.2,2.1)	−2.9 (−5.4,−0.4)	0.025
Month 24	30	−5.6 (−8.3,−2.9)	38	1.3 (−1.1,3.7)	−6.9 (−10.5,−3.3)	<0.001
SF36 Mental						
Month 12	38	0.0 (−2.4,2.4)	50	1.6 (−0.6,3.7)	−1.6 (−4.8,1.7)	0.343
Month 24	30	−1.8 (−4.9,1.3)	38	2.5 (−0.2,5.3)	−4.3 (−8.5,−0.2)	0.040