Original Investigation

Repurposing Diflunisal for Familial Amyloid Polyneuropathy
A Randomized Clinical Trial

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IMPORTANCE Familial amyloid polyneuropathy, a lethal genetic disease caused by aggregation of variant transthyretin, induces progressive peripheral nerve deficits and disability. Diflunisal, a nonsteroidal 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 familial amyloid polyneuropathy.

DESIGN, SETTING, AND PARTICIPANTS International randomized, double-blind, placebo-controlled study conducted among 130 patients with familial amyloid polyneuropathy exhibiting clinically detectable peripheral or autonomic neuropathy at amyloid centers in Sweden (Umeå), Italy (Pavia), Japan (Matsumoto and Kumamoto), England (London), and the United States (Boston, Massachusetts; New York, New York; and Rochester, Minnesota) from 2006 through 2012.

INTERVENTION Participants were randomly assigned to receive diflunisal, 250 mg (n=64), or placebo (n=66) twice daily for 2 years.

MAIN OUTCOMES AND MEASURES The primary end point, 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 neurological deficits) to 270 points (no detectable peripheral nerve function). Secondary outcomes included a quality-of-life questionnaire (36-Item Short-Form Health Survey [SF-36]) and modified body mass index. Because of attrition, we used likelihood-based modeling and multiple imputation analysis of baseline to 2-year data.

RESULTS By multiple imputation, the NIS+7 score increased by 25.0 (95% CI, 18.4-31.6) points in the placebo group and by 8.7 (95% CI, 3.3-14.1) points in the diflunisal group, a difference of 16.3 points (95% CI, 8.1-24.5 points; $P < .001$). Mean SF-36 physical scores decreased by 4.9 (95% CI, −7.6 to −2.2) points in the placebo group and increased by 1.5 (95% CI, −0.8 to 3.7) points in the diflunisal group ($P < .001$). Mean SF-36 mental scores declined by 1.1 (95% CI, −4.3 to 2.0) points in the placebo group while increasing by 3.7 (95% CI, 1.0-6.4) points in the diflunisal group ($P = .02$). By responder analysis, 29.7% of the diflunisal group and 9.4% of the placebo group exhibited neurological stability at 2 years (<2-point increase in NIS+7 score; $P = .007$).

CONCLUSIONS AND RELEVANCE Among patients with familial amyloid polyneuropathy, the use of diflunisal compared with placebo for 2 years reduced the rate of progression of neurological impairment and preserved quality of life. Although longer-term follow-up studies are needed, these findings suggest benefit of this treatment for familial amyloid polyneuropathy.

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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 [ATTR-FAP]) 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 neurological deficits, dying 10 to 15 years after disease presentation.[7] Fewer than 10 000 people are estimated to be clinically affected worldwide.[8]

ATTR *hereditary transthyretin amyloidosis*

ATTR-FAP *transthyretin-type familial amyloid polyneuropathy*

NIS+7 *Neuropathy Impairment Score plus 7 nerve tests*

NIS-LL *Neuropathy Impairment Score of the Lower Limbs*

SF-36 *36-Item Short-Form Health Survey*

TTR *transthyretin*

Orthotopic liver transplantation, standard treatment for FAP since its initial use in 1990, eliminates 95% of variant TTR from the blood and affects 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 lifelong 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—either by 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.[16-18] A phase 1 study demonstrated that diflunisal, a generic nonsteroidal anti-inflammatory drug, at a dosage of 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 National Institutes of Health (NIH) mission to repurpose old drugs, we conducted an investigator-initiated, international, multicenter, 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 produced and donated diflunisal 250-mg tablets at the outset of the study; Bilcare Inc overencapsulated the diflunisal tablets and generated matching capsules filled with excipient for placebo use. Stability and dissolution profiles of the overencapsulated diflunisal tablets were generated by Bilcare Inc at 24, 36, 48, and 60 months, and the data were reviewed by the US Food and Drug Administration Center for Drug Evaluation and Research.

Participants

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 sensorimotor or autonomic neuropathy clinically detectable by a trained neurologist, and routinely spent more than 50% of waking hours out of bed or chair (Eastern Cooperative Oncology Group performance status <3). Exclusions included alternative causes of sensorimotor polyneuropathy (eg, diabetes mellitus, vitamin B_{12} deficiency), limited survival prognosis (<2 years), prior liver transplantation, severe congestive heart failure (New York Heart Association class IV) or renal insufficiency (estimated creatinine clearance <30 mL/min), and ongoing anticoagulation. Full inclusion and exclusion criteria are provided in the eBox in the Supplement.

Study End Points

The 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 conduction velocity; and sural sensory nerve action potential amplitude); vibratory detection threshold determined by quantitative sensory testing; and heart rate variability during deep breathing (CASE IV, WR Medical Electronics).[20] Higher NIS+7 scores reflect greater neurological deficit (score range, 0-270 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 histological 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, pinprick sensation, or joint motion sensation.

The NIS or NIS+7 has been used in clinical trials for diabetic sensorimotor polyneuropathy,[20,24-27] monoclonal gam-

mopathy of undetermined significance neuropathy,[28] and chronic inflammatory demyelinating polyradiculopathy.[29,30] To ensure high-quality end-point evaluation of the NIS+7, we used standardized tests with published reference values; clinical evaluations by certified neurologists and clinical neurophysiologists; extensive pretraining and certification of all clinical investigators performing quantitative sensory testing, standardized electromyography, and neurological 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 in mean NIS+7 scores from enrollment to 2 years, constituted the primary end point. Patients discontinuing drug before study completion were invited to return at 2 years to complete NIS+7 testing.

Secondary end-point measures included NIS (scale, 0-244 points) and NIS-Lower Limb (NIS-LL; scale, 0-88 points), with higher scores indicating greater deficits; the 36-Item Short-Form Health Survey (SF-36) quality-of -life questionnaire (scale, 0-100 points; lower scores reflect diminished status), an instrument used to study treatment effect in other forms of systemic amyloidosis[33]; modified body mass index (BMI), the product of serum albumin concentration (measured in grams per liter) and BMI (calculated as weight in kilograms divided by height in meters squared) that correlates with survival in ATTR-FAP[34,35]; and the Kumamoto score (scale, 0-96 points; higher scores reflect increasing disease severity), a clinical neurological 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

In this randomized, placebo-controlled clinical trial, patients, investigators, study coordinators, and investigational pharmacists were unaware of treatment assignments. Patients were randomly assigned in a 1:1 manner to receive diflunisal, 250 mg, or matching placebo capsules to be taken twice daily by mouth for 2 years. Randomization was performed in permuted blocks of 2 or 4 stratified for mutant TTR (non-V30M vs V30M) and study site. Study drug was prepackaged according to a computer-generated randomization scheme and 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 adherence as 80% or higher pill use ([dispensed - returned study drug)/dispensed drug] × 100).

The NIS+7 (including NIS, NIS-LL, and 7 nerve tests), quality-of-life (SF-36) questionnaires, modified BMI, and Kumamoto score data were collected at enrollment, at 1 year, and at 2 years (study end). Patients visited their primary care physicians at 1, 3, and 18 months after enrollment for vital sign measurement, complete blood counts, occult stool testing, and serum chemistries. A 6-month study site visit with full neurological testing in the absence of nerve conduction studies provided early monitoring for deleterious study drug effects.

Statistical Analysis

We performed power calculations for 2-sample, 2-sided *t* tests comparing changes in NIS+7 scores (end point - baseline) between the 2 treatment groups. In the absence of NIS+7 data in patients with ATTR-FAP, we used estimates of the variability of NIS+7 scores over time in diabetic polyneuropathy to calculate expected effect sizes, with 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 84.2% to detect a moderate effect size of 0.5 (a 1.8-point difference in NIS+7 scoring), with a 2-sided test at α=.05 in the intention-to-treat (ITT) population, defined as all randomized patients who initiated treatment. Study drug expiration limited accrual to 130 patients (65 patients per group), a sample size that provided a power of 81.4% to detect the moderate effect size previously described.

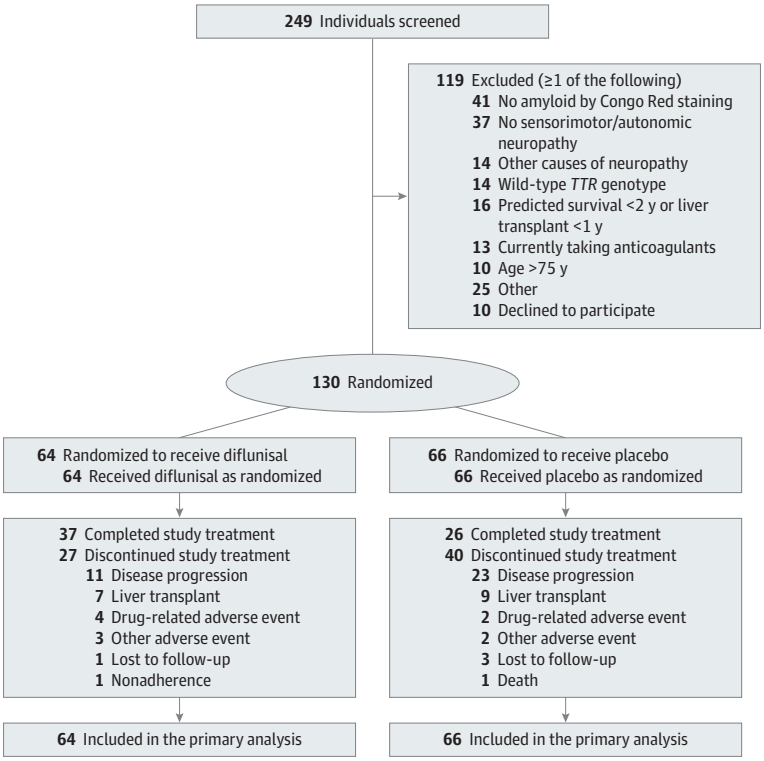
We assessed baseline characteristics and comparability of the 2 treatment groups by the 2-sample *t* test for continuous variables and by χ^2 or Fisher exact test for categorical variables. We compared attrition across study groups by survival analyses with log-rank testing. We identified significant deviation from the assumption of missingness completely at random for the data using the permutation test.[37] Given that the character of attrition does not entail missingness completely at random, we performed likelihood-based longitudinal analyses using general linear models for repeated measures of outcome data collected at baseline, 1 year, and 2 years.[38] To assess the sensitivity of inferences to assumptions on the missing data, we performed sensitivity analyses using multiple imputation[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 used 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 dropout for any reason) to both treatment groups. The Fisher exact test was used to compare treatment "response" between the study groups. We calculated risk ratios for success and their 95% confidence intervals. We used 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 to adjust for baseline outcome measures in the evaluation of primary and secondary end points. All tests were 2-sided with α=.05. SAS software, version 9.2 (SAS Institute Inc), 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-

Figure. Participant Flow



Among the 63 participants who completed study treatment, analyzable primary outcome data were obtained for 60 (placebo, n=23; diflunisal, n=37); 3 in the placebo group had inadmissible data for the Neuropathy Impairment Score plus 7 nerve tests (NIS+7). Among the 67 in whom study drug was discontinued prior to 2 years (placebo, n=40; diflunisal, n=27), 2-year primary outcome data (NIS+7) were obtained for 8 participants (placebo, n=5; diflunisal, n=3).

proven amyloid deposits (37.6%), absence of clinically detectable peripheral or autonomic neuropathy (33.9%), wild-type TTR DNA results (12.8%), other causes of sensorimotor polyneuropathy (12.8%), and current anticoagulation (11.9%) (**Figure**).

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 1 in the Supplement). Nearly a 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-LL, Kumamoto score, modified BMI, and SF-36 physical and mental scores were not statistically different between groups at enrollment.

Treatment Adherence

Adherence, defined as 80% or more pills taken based on counts of returned study pills, was 100% in the placebo group and 91.8% in the diflunisal group at 1 year. At 2 years, 86.2% of the placebo group and 85.4% of the diflunisal group were adherent.

Attrition

Sixty-seven patients discontinued study treatment before completing the 2-year protocol, including 40 patients from the placebo group and 27 from the diflunisal group. Disease progression (placebo, n= 23; diflunisal, n=11) and orthotopic liver transplantation (placebo, n=9; diflunisal, n=7) were the leading reasons for dropout (Figure). Baseline and 1-year NIS+7 scores were collected for 37 patients randomized to placebo and 50 randomized to diflunisal treatment. Baseline and 2-year NIS+7 scores were obtained for 28 patients assigned to placebo and 40 assigned to diflunisal treatment. Five patients in the placebo group and 3 in the diflunisal group discontinued study drug and acquired diflunisal outside the study but completed 2-year NIS+7 testing.

Survival analysis of attrition by treatment assignment revealed greater dropout in the placebo group over time (P = .03 by log-rank test). There were no statistically significant differences in attrition by variant TTR (V30M vs non-V30M; P = .31), age (P = .29), or polyneuropathy staging (P = .36). Analysis of missingness completely at random for the primary and secondary outcomes using the permutation test indicated dependence of dropout on the outcome values. Dropout was preceded by significantly worse disease state. Those who dropped out after 1 year had significantly higher 1-year NIS+7 scores (P = .02 by permutation test), higher NIS and NIS-LL scores (P = .03), and lower SF-36 physical scores (P = .002) than patients continuing study treatment.

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 (placebo, n=66; diflunisal, n=64) using intention-to-treat principles. By longitudinal analysis of the primary out-

Table 1. Baseline Demographic and Clinical Characteristics[a]

Characteristics	Overall (N = 130)	Placebo (n = 66)	Diflunisal (n = 64)
Age, mean (SD), y	59.7 (11.9)	59.2 (12.2)	60.3 (11.7)
Sex, No. (%)			
Male	87 (66.9)	44 (66.7)	43 (67.2)
Female	43 (33.1)	22 (33.3)	21 (32.8)
Race, No. (%)			
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
Multiracial	7 (5.4)	4 (6.1)	3 (4.7)
ATTR genotype, No. (%)			
Met30	71 (54.6)	35 (53)	36 (56.3)
Non-met30	59 (45.4)	31 (47)	28 (43.8)
Serum albumin level, mean (SD), g/dL	4.1 (0.4)	4.1 (0.4)	4.1 (0.4)
Postural systolic blood pressure change, mean (SD), mm Hg	−11 (18.9)	−13.2 (20.2)	−8.8 (17.4)
Disease stage based on PND, No. (%)[b]			
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 score[c]			
Mean (SD)	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 score[d]			
Mean (SD)	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 score[e]			
Mean (SD)	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, mean (SD)[f]	16 (12.2)	16.7 (13.5)	15.3 (10.8)
Modified BMI, mean (SD)[g]	1021.7 (240.4)	1019 (255)	1024.4 (226.3)
SF-36 physical component score, mean (SD)[h]	35.4 (11.3)	34.8 (11)	35.9 (11.6)
SF-36 mental component score, mean (SD)[i]	46.6 (12.9)	46.5 (11.8)	46.6 (14.1)

Abbreviations: ATTR, hereditary transthyretin amyloidosis; BMI, body mass index; NIS, Neuropathy Impairment Score; NIS-LL, Neuropathy Impairment Score of the Lower Limbs; PND, Polyneuropathy Disability; SF-36, 36-Item Short-Form Health Survey.

[a] There were no significant differences between the groups. Percentages may not sum to 100 because of rounding.

[b] A PND stage of I indicates sensory disturbances but preserved walking capability; stage II indicates impaired walking ability without need for a stick; stage IIIA indicates walking only with the help of one stick; stage IIIB indicates walking with the help of 2 sticks; stage IV indicates confined to a wheelchair or bedridden.

[c] The NIS+7 (NIS plus 7 nerve tests) ranges from 0 to 270, with higher scores indicating greater neurologic deficits.

[d] The NIS ranges from 0 to 244, with higher scores indicating greater neurologic deficits.

[e] The NIS-LL ranges from 0 to 88, with higher scores indicating greater neurologic deficits.

[f] The Kumamoto score ranges from 0 to 102, with higher scores indicating more severe polyneuropathy.

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

[h] Physical component scores of the SF-36 range from 0 to 100, with higher scores indicating greater physical quality of life.

[i] Mental component scores of the SF-36 range from 0 to 100, with higher scores indicating greater mental quality of life.

come measure, change in NIS+7 score over time, patients randomized to diflunisal exhibited significantly less progression of polyneuropathy than those assigned to placebo. The change in NIS+7 score from baseline to 2 years was 26.3 points (95% CI, 20.2-32.4 points) in the placebo group and 8.2 points (95% CI, 2.9-13.6 points) in the diflunisal group, a difference of 18.0 points between treatment groups (95% CI, 9.9-26.2 points; $P < .001$) (**Table 2**). The inhibitory effect of diflunisal on neuropathy progression was also detectable at 1 year. The change in NIS+7 score from baseline to 1 year was 12.5 points (95% CI, 8.6-16.4 points) in the placebo group vs 6.2 points (95% CI, 2.8-9.6 points) in the diflunisal group, a difference of 6.4 points (95% CI, 1.2-11.6 points; $P = .02$). Additionally, diflunisal treatment inhibited change in NIS and NIS-LL scores, components of the NIS+7 composite score, from baseline to 2 years compared with the placebo group (change in NIS score: diflunisal, 6.4 points [95% CI, 1.6-11.2 points] vs placebo, 23.2 points [95% CI, 17.8-28.5 points]; $P < .001$; change in NIS-LL score: diflunisal, 3.8 points [95% CI, 0.9-6.6 points] vs placebo, 12.1 points [95% CI, 8.9-15.3 points]; $P < .001$) (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, 1.1-5.1 points) than the placebo group (8.0 points; 95% CI, 5.8-10.3 points; $P = .002$) (Table 2). A trend toward slower decline in modified BMI from baseline to 2 years in the diflunisal group did not meet statistical significance ($P = .21$) (Table 2). Physical quality of life (SF-36 scores) stabilized from baseline to 2 years in those assigned to diflunisal treatment (1.2 points; 95% CI, −1.2 to 3.7 points) while decreasing in the placebo group (−4.9 points; 95% CI, −7.6 to −2.1 points; $P = .001$). Although mental quality of life at 2 years improved in the diflunisal group

Table 2. Longitudinal Intention-to-Treat Analyses of Primary (NIS+7) and Secondary Outcomes[a]

Outcomes	Mean (95% CI)			*P* Value
	Placebo Change From Baseline	Diflunisal Change From Baseline	Difference, Placebo−Diflunisal	
NIS+7 score				
At 1 year	12.5 (8.6 to 16.4)	6.2 (2.8 to 9.6)	6.4 (1.2 to 11.6)	.02
At 2 years	26.3 (20.2 to 32.4)	8.2 (2.9 to 13.6)	18.0 (9.9 to 26.2)	<.001
NIS score				
At 1 year	10.1 (6.9 to 13.3)	4.1 (1.2 to 6.9)	6.0 (1.7 to 10.3)	.007
At 2 years	23.2 (17.8 to 28.5)	6.4 (1.6 to 11.2)	16.8 (9.6 to 24.0)	<.001
NIS-LL score				
At 1 year	6.0 (3.9 to 8.2)	3.2 (1.3 to 5.2)	2.8 (−0.1 to 5.7)	.06
At 2 years	12.1 (8.9 to 15.3)	3.8 (0.9 to 6.6)	8.3 (4.1 to 12.6)	<.001
Kumamoto score				
At 1 year	4.1 (2.1 to 6.2)	1.9 (0.1 to 3.7)	2.3 (−0.5 to 5)	.10
At 2 years	8.0 (5.8 to 10.3)	3.1 (1.1 to 5.1)	5.0 (1.9 to 8.0)	.002
Modified BMI[b]				
At 1 year	−38.5 (−74.9 to −2.1)	−18.7 (−51.6 to 14.1)	−19.8 (−68.8 to 29.2)	.43
At 2 years	−67.9 (−108.1 to −27.7)	−33.7 (−69.3 to 1.8)	−34.1 (−87.8 to 19.5)	.21
SF-36 physical component score				
At 1 year	−1.9 (−3.9 to 0.2)	0.7 (−1.1 to 2.5)	−2.6 (−5.3 to 0.1)	.06
At 2 years	−4.9 (−7.6 to −2.1)	1.2 (−1.2 to 3.7)	−6.1 (−9.8 to −2.5)	.001
SF-36 mental score				
At 1 year	0.8 (−2 to 3.6)	2.5 (0.0 to 5.1)	−1.7 (−5.5 to 2.1)	.37
At 2 years	−0.9 (−4.4 to 2.5)	3.5 (0.4 to 6.7)	−4.5 (−9.2 to 0.2)	.06

Abbreviations: BMI, body mass index; NIS, Neuropathy Impairment Score; NIS+7, NIS plus 7 nerve tests; NIS-LL, Neuropathy Impairment Score of the Lower Limbs; SF-36, 36-Item Short-Form Health Survey.

[a] Linear models for repeated measures of outcome data were used. Means were calculated for change from baseline to 12 and 24 months for primary and secondary outcome measures by treatment groups. *P* values address the differences between treatment groups in change over 12 and 24 months for each outcome measure. See Table 1 footnotes for explanation of score ranges.

[b] Modified BMI is the product of BMI (weight in kilograms divided by the square of height in meters) and serum albumin (g/L).

(3.5 points; 95% CI, 0.4-6.7 points), the difference between treatment groups was not statistically significant (−4.5 points; 95% CI, −9.2 to 0.2 points; *P* = .06) (Table 2). The diflunisal effect on outcome measures was seen across study sites, sex, TTR mutation grouping, and neuropathy stage at entry.

Sensitivity Analyses

Sensitivity analyses including multiple imputation, last observation carried forward, 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, multiple imputation identified a significant inhibitory effect of diflunisal on neuropathy progression by multiple outcome measures, including both physical and mental quality of life (**Table 3**). Specifically, multiple imputation analysis estimated a difference in change between the placebo and diflunisal groups of 16.3 points (95% CI, 8.1-24.5 points; *P* < .001) for NIS+7 score at 2 years and 6.1 points (95% CI, 1.1-11.1 points; *P* = .02) at 1 year; 16.1 points (95% CI, 9.0-23.2 points; *P* < .001) for NIS score at 2 years and 5.9 points (95% CI, 1.8-10.0 points; *P* = .005) at 1 year; 8.2 points (95% CI, 4.0-12.5; *P* < .001) for NIS-LL score at 2 years; 4.9 points (95% CI, 1.7-8.1 points; *P* = .003) for Kumamoto score at 2 years; and −6.4 points (95% CI, −9.8 to −2.9 points; *P* < .001) for physical quality-of-life and −4.9 points (95% CI, −9.0 to −0.7 points; *P* = .02) for mental quality-of-life scores at 2 years. Modified BMI was the only end point that did not detect a favorable diflunisal effect.

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

The 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, 3.0-48.8 points; *P* = .03) at 2 years and 25.0 points (95% CI, 4.1-45.9 points; *P* = .02) at 1 year.

By responder analysis (assigning treatment failure to all study dropouts and patients with a ≥2-point increase in NIS+7 score), the diflunisal group exhibited significantly greater neurological stability at 2 years than the placebo group (29.7% vs 9.4%; *P* = .007). Risk ratio analysis indicated a 3-fold greater probability of response in the diflunisal vs the placebo group (risk ratio, 3.2; 95% CI, 1.4-7.4). Greater apparent neurological stability by responder analysis of 1-year data among patients receiving diflunisal vs placebo treatments (26.6% vs 14.1%; *P* = .12; risk ratio, 1.9; 95% CI, 0.9-3.9) did not meet statistical significance.

Completers Analysis (Analysis of Covariance)

Eighty-seven patients (placebo, n=37; diflunisal, n=50) completed the NIS+7 at 1 year and 68 patients (placebo, n=28; diflunisal, n=40) completed at 2 years. We used analysis of covariance to examine change from baseline at 1 and 2 years for the primary (NIS+7 score) and secondary outcomes in pa-

Table 3. Multiple Imputation Analysis of Primary (NIS+7) and Secondary Outcomes[a]

Outcomes	Mean (95% CI)			*P* Value
	Placebo Change From Baseline	Diflunisal Change From Baseline	Difference, Placebo−Diflunisal	
NIS+7 score				
At 1 year	12.5 (8.6 to 16.4)	6.4 (3.1 to 9.6)	6.1 (1.1 to 11.1)	.02
At 2 years	25.0 (18.4 to 31.6)	8.7 (3.3 to 14.1)	16.3 (8.1 to 24.5)	<.001
NIS score				
At 1 year	10.1 (6.9 to 13.3)	4.2 (1.5 to 7.0)	5.9 (1.8 to 10.0)	.005
At 2 years	22.8 (17.2 to 28.4)	6.7 (1.9 to 11.4)	16.1 (9.0 to 23.2)	<.001
NIS-LL score				
At 1 year	6.0 (3.9 to 8.2)	3.3 (1.4 to 5.1)	2.8 (0.0 to 5.6)	.05
At 2 years	12.1 (8.7 to 15.5)	3.8 (1.0 to 6.7)	8.2 (4.0 to 12.5)	<.001
Kumamoto score				
At 1 year	4.1 (1.9 to 6.4)	1.9 (0.0 to 3.7)	2.3 (−0.6 to 5.2)	.12
At 2 years	8.1 (5.7 to 10.6)	3.2 (1.1 to 5.3)	4.9 (1.7 to 8.1)	.003
Modified BMI[b]				
At 1 year	−40.3 (−75.4 to −5.2)	−19.7 (−54.1 to 14.7)	−20.6 (−69 to 27.9)	.41
At 2 years	−65.1 (−107.4 to −22.7)	−35.2 (−73.6 to 3.3)	−29.9 (−85.7 to 25.9)	.29
SF-36 physical component score				
At 1 year	−1.9 (−3.8 to −0.1)	0.8 (−0.9 to 2.5)	−2.8 (−5.2 to −0.3)	.03
At 2 years	−4.9 (−7.6 to −2.2)	1.5 (−0.8 to 3.7)	−6.4 (−9.8 to −2.9)	<.001
SF-36 mental component score				
At 1 year	0.6 (−1.7 to 3.0)	2.3 (0.1 to 4.5)	−1.7 (−4.9 to 1.5)	.30
At 2 years	−1.1 (−4.3 to 2.0)	3.7 (1.0 to 6.4)	−4.9 (−9.0 to −0.7)	.02

Abbreviations: BMI, body mass index; NIS, Neuropathy Impairment Score; NIS+7, NIS plus 7 nerve tests; NIS-LL, Neuropathy Impairment Score of the Lower Limbs; SF-36, 36-Item Short-Form Health Survey.

[a] Imputation incorporating previous outcome values, treatment, and TTR mutation group was used for missing values. Means were calculated for change from baseline to 12 and 24 months for primary and secondary outcome measures by treatment groups. *P* values address the differences between treatment groups in change over 12 and 24 months for each outcome measure. See Table 1 footnotes for explanation of score ranges.

[b] Modified BMI is the product of BMI (weight in kilograms divided by the square of height in meters) and serum albumin (g/L).

tients completing measurements (completers). As with the longitudinal and multiple imputation 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 score (13.5 points; 95% CI, 6.5-20.6 points; $P < .001$), NIS score (13.8 points; 95% CI, 7.5-20.1 points; $P < .001$), NIS-LL score (7.1 points; 95% CI, 3.2-11.1 points; $P < .001$), Kumamoto score (3.9 points; 95% CI, 0.9-6.8 points; $P = .01$), SF-36 physical quality-of-life score (−6.9 points; 95% CI, −10.5 to −3.3 points; $P < .001$), SF-36 mental quality-of-life score (−4.3 points; 95% CI, −8.5 to −0.2 points; $P = .04$), and modified BMI (−50.8; 95% CI, −101.1 to −0.6; $P = .048$) (**Table 4**).

Adverse Events

A complete listing of adverse events and drug-related adverse events by patient is provided in eTable 2 and eTable 3 in the Supplement. Gastrointestinal, renal, cardiac, and blood-related adverse events occurred in similar numbers by treatment group. 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 bleeding, congestive heart failure, glaucoma, nausea) and 2 patients from the placebo group (headache, renal failure). Thirteen deaths (9 in the placebo group; 4 in the diflunisal group) were reported by 2 years, with 12 occurring after discontinuation of 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 end point (NIS+7 score), a qualitative neuropathy and end organ scale developed for ATTR-FAP (Kumamoto score), and modified BMI, a predictor of survival in ATTR-FAP. A 2-point change in NIS+7 score identifies a minimal clinically detectable change in polyneuropathy progression,[23] so the 16.3-point NIS+7 difference between treatment groups at 2 years by multiple imputation analysis in this study signals a clinically meaningful diflunisal effect. Confining neurological 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 the NIS+7 in the placebo group (25 points) far exceeded the 2-year progression reported in patients with diabetes (1.70 points),[20] quantifying the

Table 4. Completers Analysis of Primary (NIS+7) and Secondary Outcomes[a]

Characteristics	Placebo		Diflunisal		Difference, Placebo-Diflunisal, Mean (95% CI)	*P* Value
	No. of Participants	Change From Baseline, Mean (95% CI)	No. of Participants	Change From Baseline, Mean (95% CI)		
NIS+7 score						
At 1 year	37	12.8 (8.9 to 16.7)	50	6.3 (2.9 to 9.6)	6.5 (1.4 to 11.7)	.01
At 2 years	28	20.1 (14.7 to 25.6)	40	6.6 (2.0 to 11.1)	13.5 (6.5 to 20.6)	<.001
NIS score						
At 1 year	39	10.1 (6.9 to 13.3)	50	4.1 (1.3 to 6.9)	6.0 (1.7 to 10.2)	.006
At 2 years	30	18.9 (14.1 to 23.7)	40	5.1 (0.9 to 9.2)	13.8 (7.5 to 20.1)	<.001
NIS-LL score						
At 1 year	39	6.1 (4.0 to 8.3)	50	3.3 (1.4 to 5.2)	2.8 (−0.1 to 5.7)	.06
At 2 years	30	9.9 (6.9 to 12.8)	40	2.7 (0.2 to 5.3)	7.1 (3.2 to 11.1)	<.001
Kumamoto score						
At 1 year	38	4.4 (2.4 to 6.4)	49	2.0 (0.2 to 3.8)	2.4 (−0.3 to 5.1)	.08
At 2 years	29	6.6 (4.4 to 8.9)	39	2.8 (0.9 to 4.7)	3.9 (0.9 to 6.8)	.01
Modified BMI[b]						
At 1 year	40	−46.6 (−82.2 to −11.1)	50	−20.7 (−52.5 to 11.1)	−25.9 (−73.6 to 21.8)	.29
At 2 years	29	−76.5 (−114.6 to −38.4)	39	−25.7 (−58.5 to 7.2)	−50.8 (−101.1 to −0.6)	.05
SF-36 physical component score						
At 1 year	38	−2.5 (−4.4 to −0.6)	50	0.4 (−1.2 to 2.1)	−2.9 (−5.4 to −0.4)	.03
At 2 years	30	−5.6 (−8.3 to −2.9)	38	1.3 (−1.1 to 3.7)	−6.9 (−10.5 to −3.3)	<.001
SF-36 mental component score						
At 1 year	38	0.0 (−2.4 to 2.4)	50	1.6 (−0.6 to 3.7)	−1.6 (−4.8 to 1.7)	.34
At 2 years	30	−1.8 (−4.9 to 1.3)	38	2.5 (−0.2 to 5.3)	−4.3 (−8.5 to −0.2)	.04

Abbreviations: BMI, body mass index; NIS, Neuropathy Impairment Score; NIS+7, NIS plus 7 nerve tests; NIS-LL, Neuropathy Impairment Score of the Lower Limbs; SF-36, 36-Item Short-Form Health Survey.

[a] Least-square means were calculated for change from baseline to 12 and 24 months for primary and secondary outcome measures by treatment group. The difference between treatment groups at 12 and 24 months is expressed as the placebo minus diflunisal least-square mean. Corresponding *P* values were calculated by analysis of covariance. See Table 1 footnotes for explanation of score ranges.

[b] Modified BMI is the product of BMI (weight in kilograms divided by the square of height in meters) and serum albumin (g/L).

devastating nature of ATTR-FAP. The NIS+7 finding extended across TTR mutations (V30M and non-V30M), sex, neuropathy severity (Polyneuropathy Disability stage), and major study sites. Importantly, diflunisal affected not only the progression of neuropathy but also the quality of life for patients with FAP, a critical element when considering the effect of new treatments. Although our study design targeted 2 years of observations, a clinically significant diflunisal effect (2-fold less polyneuropathy progression by NIS+7 score vs 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 1 TTR mutation (V30M) and early polyneuropathy. By intention-to-treat analysis, tafamidis treatment did not meet statistical significance for its co-primary end points, NIS-LL score and a quality-of-life questionnaire (Norfolk Quality-of-Life Questionnaire-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 intention-to-treat, last-observation-carried-forward, multiple imputation, and sensitivity/responder analyses.

In addition to demonstrating by multiple measures that diflunisal inhibits progression of debilitating polyneuropathy in patients with ATTR-FAP, our trial is pivotal for several reasons. It is the first randomized clinical 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 neurological disease progression (NIS+7 score increase of 12-13 points per 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 effect 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

repurposing a drug that had lost its clinical relevance as a nonsteroidal anti-inflammatory agent. Finally, this study provides proof of concept that kinetically stabilizing an amyloidogenic precursor protein (transthyretin) translates to successfully modifying amyloid-related neurological 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 dropout, occurred 50% more frequently in the placebo group and explained the attrition differences between treatment groups. Reasons for significant dropout included (1) the unexpected rapidity of neurological decline during the 2-year observation period (more than 10 times the rate of diabetic polyneuropathy); (2) existence of a validated alternative treatment (liver transplantation); and (3) widespread availability of diflunisal outside the study. By assigning a final NIS+7 score at dropout for those lost to follow-up, early dropout predominantly limited recorded neurological decline in the placebo group, minimizing NIS+7 differences between the treatment groups. Despite these limitations, our data reveal a statistically significant diflunisal effect on ATTR-FAP by multiple measures of neurological 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 neurological score (NIS+7 score ≥2 points), revealed significantly greater neurological stability (success) at 2 years in the diflunisal group than the placebo group.

Conclusions

Among patients with ATTR-FAP, the use of diflunisal compared with placebo for 2 years reduced the rate of progression in neurological 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.

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