Randomized phase 2 trial of NP001—a novel immune regulator: Safety and early efficacy in ALS

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ABSTRACT

Objective: To assess the safety, tolerability, and preliminary efficacy of NP001, a novel immune regulator of inflammatory monocytes/macrophages, for slowing progression of amyotrophic lateral sclerosis (ALS).

Methods: This was a phase 2 randomized, double-blind, placebo-controlled trial of NP001 in 136 patients with ALS of <3 years' duration and forced vital capacity ≥70%. Participants received NP001 2 mg/kg, NP001 1 mg/kg, or placebo for 6 months. Safety, tolerability, and inflammatory biomarkers were assessed throughout the study. Preliminary efficacy was evaluated using the ALS Functional Rating Scale-Revised (ALSFRS-R) slope and change from baseline, with and without matched historical placebo controls, after 6 months of treatment. A post hoc analysis of the percentage of patients ("responders") whose ALSFRS-R did not change from baseline was also conducted.

Results: NP001 was generally safe and well-tolerated, except for infusion site pain and dizziness. No significant slowing of decline in the primary or secondary measures was observed. However, slowing of progression was observed in the high-dose group in patients with greater inflammation (wide range C-reactive protein). Moreover, NP001 may have dose dependently halted symptom progression in a subset of patients. More than 2 times as many patients on high-dose NP001 (25%) did not progress during 6 months of treatment compared with those on placebo (11%). Most "responders" had an elevated biomarker of inflammation, interleukin-18, and were positive for lipopolysaccharide at baseline, which decreased after treatment with NP001.

Conclusion: The arresting of progression of ALS symptoms by NP001 in a subset of patients with marked neuroinflammation, as observed here, will represent a novel therapeutic approach for patients with ALS, if confirmed.

Classification of evidence: This study provides Class I evidence that for patients with ALS, NP001 is safe and did not significantly slow progression of the disease (difference in slope of the ALSFRS-R/month 0.12 favoring NP001, $p = 0.55$). The study lacks the precision to exclude an important effect of NP001. ***Neurol Neuroimmunol Neuroinflamm* 2015;2:e100; doi: 10.1212/NXI.0000000000000100**

GLOSSARY

AE = adverse event; **ALS** = amyotrophic lateral sclerosis; **ALSFRS-R** = ALS Functional Rating Scale-Revised; **CRP** = C-reactive protein; **FVC** = forced vital capacity; **IFN** = interferon; **IL** = interleukin; **LPS** = lipopolysaccharide; **MCP-1** = macrophage chemotactic protein-1; **NF-κB** = nuclear factor κB; **TEAE** = treatment-emergent adverse event; **TNF-α** = tumor necrosis factor α; **VC** = vital capacity; **wr-CRP** = wide range CRP.

Abnormal inflammatory monocytes/macrophages systemically and locally in the CNS are implicated in amyotrophic lateral sclerosis (ALS) progression, with the degree of macrophage activation related to the rate of progression.[1–5] The importance of these processes is well-established in

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both preclinical models and clinical data.[5,6] The proinflammatory state in the ALS spinal cord is mediated not only by parenchymal-activated microglia but also by activated inflammatory monocytes migrating from the blood into the spinal cord and brain, releasing factors associated with neurodegeneration.[3,5,7–11] Microglial activation results in induction and secretion of prototypic inflammatory mediators such as lipopolysaccharide (LPS), tumor necrosis factor α (TNF-α), interleukin (IL)-6, CD68, and inducible nitric oxide synthase, resulting in a "neurotoxic" milieu in ALS. Recent autopsy data showed that approximately 20% of all motor neurons are engulfed by inflammatory macrophages.[5] The secretion of cytokines by activated macrophages migrating into the CNS likely contributes to motor neuron death in ALS.[7–11]

NP001, a pH-adjusted IV formulation of purified sodium chlorite, is a novel molecule that regulates inflammation in vitro and in vivo.[12] Within monocytes/macrophages, chlorite is converted into taurine chloramine that downregulates nuclear factor κB (NF-κB) expression and inhibits production of proinflammatory cytokine IL-1β.[13–17] These mechanisms of downregulation transform inflammatory monocytes/macrophages from a proinflammatory to a basal phagocytic state.[14] A recent phase 1 controlled trial of NP001 in patients with ALS demonstrated the safety and tolerability of single ascending doses of the drug. Of importance, 24 hours after a single dose, NP001 dose dependently downregulated CD16-expressing inflammatory macrophages in blood.[12] In the current study, we examined the safety of multiple doses of NP001 and the possible efficacy in a subset of patients.

METHODS

Participants. The phase 2 trial was conducted from January 2011 through November 2012 at 17 sites in the United States. The study was fully enrolled and did not terminate early. Participants were men and women 21–80 years of age who were diagnosed with probable or definite ALS according to El Escorial criteria. Participants were required to have an onset of ALS-related weakness within 3 years, forced vital capacity (FVC) ≥70% of predicted, and a life expectancy of >6 months. Participants receiving riluzole had to be on a stable dose for >30 days. Patients on continuous positive airway pressure or bilevel positive airway pressure, those with active pulmonary disease, and those who had received recent immunotherapy were excluded.

Standard protocol approvals, registrations, and patient consents. The study was conducted in accordance with principles of Good Clinical Practice and approved by institutional review boards and each site's regulatory agency. Informed consent was obtained from all patients. The study was registered at ClinicalTrials.gov (NCT01281631).

Design. The study objectives were to evaluate the safety, tolerability, and preliminary efficacy of IV NP001. The study was randomized, double-blind, and placebo-controlled, and study drug was administered over 6 cycles. Patients were allocated in a 1:1:1 manner to NP001 1 mg/kg/infusion, NP001 2 mg/kg/infusion, or placebo using a blinded, computerized, centralized randomization schedule via an interactive voice system stratified by center and site of onset (bulbar/limb). Study drug was infused over 30 minutes by an infusion pump. Patients received a total of 20 infusions over 6 cycles during a 25-week double-blind treatment period (figure 1A). There were 4 weeks between the start of each cycle. Cycle 1 consisted of 5 consecutive daily infusions. Cycles 2, 3, 4, 5, and 6 each consisted of 3 consecutive daily infusions. The dosing regimen was based on prior data in an HIV population.[14] Four weeks after the final infusion, participants had an end-of-treatment visit (week 25). Each patient then had 3 consecutive monthly visits (weeks 29, 33, and 37). The ALS Functional Rating Scale-Revised (ALSFRS-R) score and vital capacity (VC) were determined on the first day of each dosing cycle and at weeks 25, 29, 33, and 37. Study investigators, site staff, and ALSFRS-R raters remained blinded to treatment allocation throughout the study. An independent data monitoring committee periodically evaluated safety during the trial.

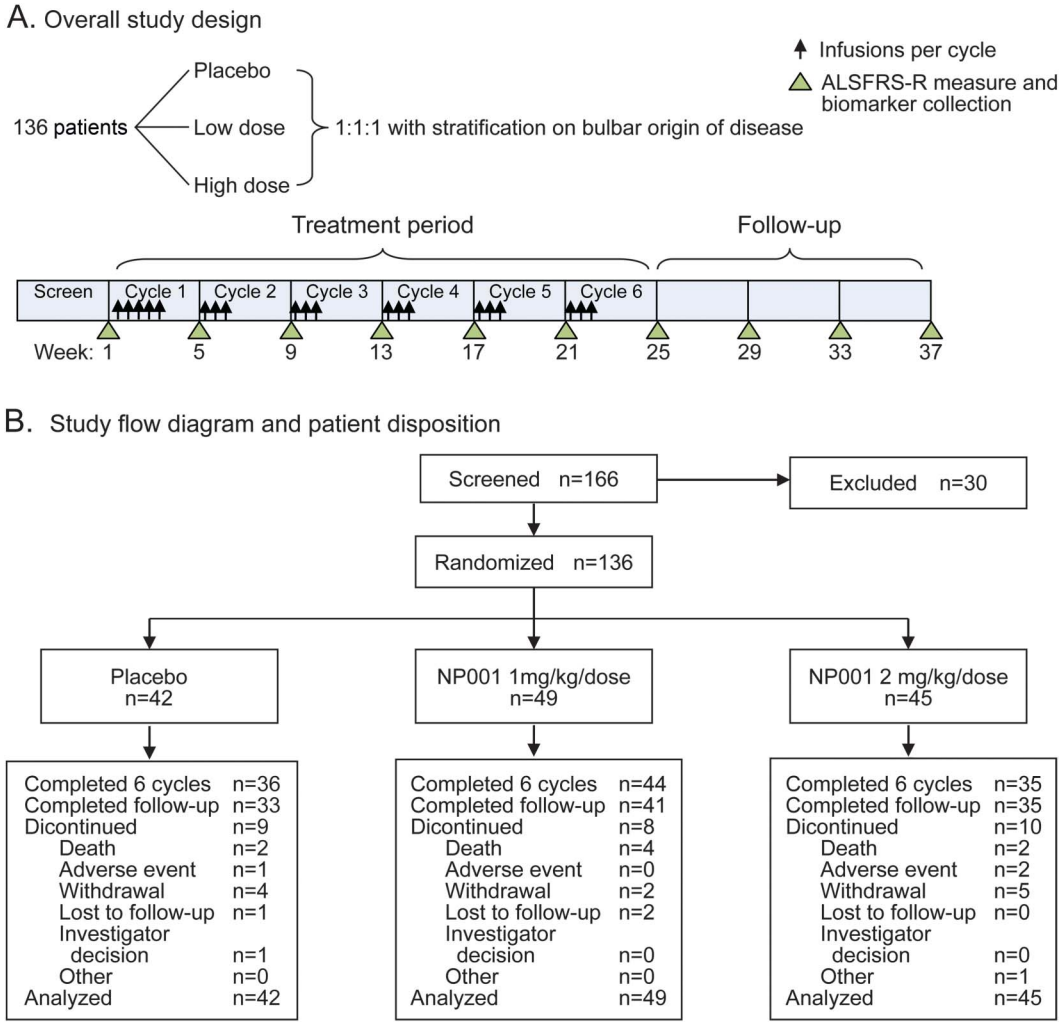
Preliminary efficacy assessments. The primary outcome measure was the ALSFRS-R slope over the 6-month treatment period. The secondary outcomes included the ALSFRS-R change from baseline through the end of the treatment period and follow-up, pulmonary function (slow VC), and participant status, including survival and time to assisted ventilation (tracheotomy). During the trial, blinded aggregate ALSFRS-R scores identified a population of patients in whom ALSFRS-R scores were not worsening, or in some cases actually improving. As such, a post hoc efficacy outcome measure of % responders in each group, defined as those patients whose ALSFRS-R was stable or improved over the 6-month treatment period, was evaluated.

Plasma biomarker assessments. The plasma concentrations of wide range C-reactive protein (wr-CRP) and macrophage chemotactic protein-1 (MCP-1) as exploratory biomarkers were measured on the first day of each dosing cycle and after 6 months of treatment. In addition, plasma samples were collected and archived at baseline, at the beginning of each dosing cycle, and after 6 months of treatment. Plasma concentrations of inflammatory mediators/cytokines/activators (i.e., C-reactive protein [CRP], IL-1β, IL-18, IL-6, TNF-α, interferon (IFN)-γ, LPS) relevant to inflammatory macrophage activation, as well as activation of the caspase-1 and NF-κB pathways implicated in ALS onset and progression, were measured after completion of the trial.

Safety assessments. Tolerability and safety were assessed via adverse event (AE) reports, vital signs, ECGs, laboratory parameters, physical examinations, and formal phlebitis scoring.

Sample size and statistical analyses. This preliminary trial was designed to evaluate 90 completed patients. The study enrolled 136 patients, as more patients qualified for randomization in the final

Figure 1 Study design and patient flow



ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised.

weeks of the recruitment period. The final sample size had approximately 65% power to detect a 30% difference in estimated slope of decline of the ALSFRS-R (2-sided, $\alpha = 0.10$) over the 6-month treatment period. A secondary analytical approach, defined a priori, involved the use of ALSFRS-R data from a matched historical placebo database for the analysis of the present study. Placebo outcomes in patients matched for inclusion criteria and showing stable rates of decline over the past 9 years from a large database of 616 historical placebo controls from 6 recent clinical trials were used as historical controls.[18] This allowed increased power and added precision to the point estimates, resulting in 87% power to detect a 30% improvement in disease progression as assessed by the ALSFRS-R slope.

The intention-to-treat population, consisting of all randomized patients who received at least 1 infusion of study medication, was the primary population used for the efficacy and safety analyses. The analysis of ALSFRS-R slope used a general linear mixed-effects model with random effects to estimate the rate of decrease (slope) of ALSFRS-R, expressed as points per month, from baseline to completion of the treatment period. A secondary analysis of the slope endpoint involved the addition of matched historical placebo controls. Changes in ALSFRS-R scores using analyses of covariance were calculated from baseline to the end of the 25-week treatment period, from the beginning to the end of the 12-week follow-up period (weeks 25 through 37), and from baseline to the end of the follow-up period (weeks 1 through 37). Covariates of age, race, sex, riluzole use, duration, type and site of ALS onset, El Escorial criteria, baseline ALSFRS-R, and VC were utilized. Pairwise comparisons for slope and change from baseline were conducted for each dose group vs placebo group. Changes in VC for the same time periods were calculated, as well as subset analyses of slope using ALSFRS-R domain subscores, sex, site of onset, and those patients whose baseline wr-CRP or MCP-1 was greater than or equal to the baseline median values for the entire enrolled population. Descriptive statistics and percent change from baseline were used to analyze the biomarker concentrations during the treatment period. Missing data were not imputed.

A post hoc exploratory analysis of the percentage of patients in each group that either improved or did not progress over the 6-month treatment period ("responders"), as assessed by change from baseline in ALSFRS-R scores, was conducted.

Safety and tolerability data were assessed by counts and tabulations of treatment-emergent adverse events (TEAEs), defined as those occurring during or after the first dose and

within 30 days of the last dose of study drug, and changes from baseline in laboratory values, vital signs, physical examinations, and ECGs.

RESULTS **Patient accounting and demographics.** One hundred sixty-six patients were screened for the trial, and 30 patients were excluded, mainly for low FVC or longer disease duration (data not shown). A total of 136 patients were enrolled and randomized (figure 1B). No patient who received study drug and terminated early was replaced.

Approximately 95% of the patients in each group completed all 5 infusions planned in cycle 1. The majority of patients in each group completed 6 dosing cycles (78%–90%); however, patients in the NP001 2 mg/kg group had the smallest percentage of cycle 6 completions (78%). The majority of patients in all 3 groups completed follow-up (78%–84%). Similar percentages of patients withdrew from the study early in each treatment group (4%–9%). The most common reason was withdrawal by the patient (anecdotally reported as difficulties in traveling to the clinic).

Table 1 lists the baseline features of the patient population. The groups had similar demographics and baseline ALS characteristics, although the placebo group had a greater percentage of patients with ALSFRS-R ≥42 (28%) than the NP001 1 mg/kg (18%) and NP001 2 mg/kg (20%) groups. Baseline median wr-CRP and MCP-1 values were similar between groups. There were no significant differences in baseline characteristics between groups.

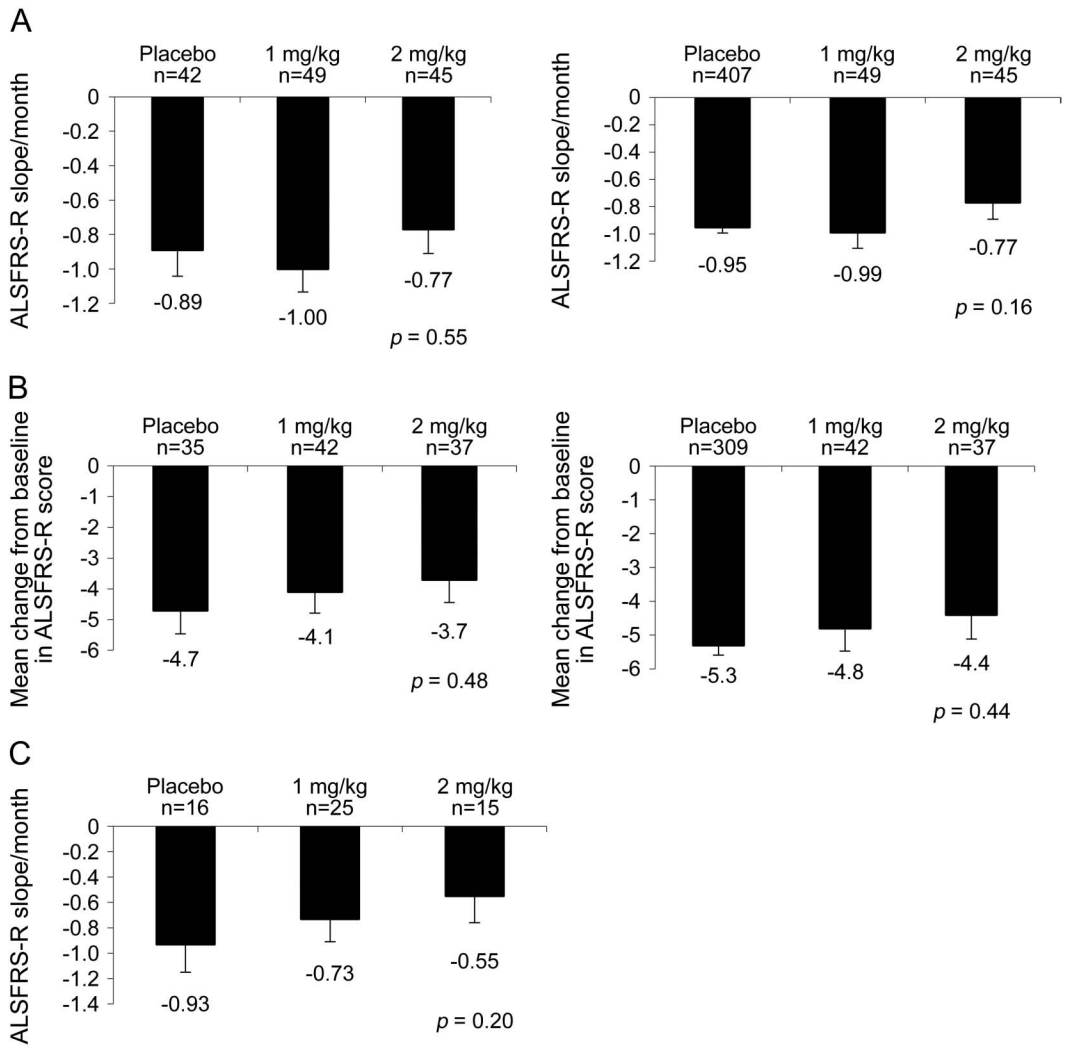
Efficacy. NP001 2 mg/kg did not have a statistically significant effect in reducing ALS progression compared with placebo, as shown by percent change in mean slope in points per month without historical placebo controls (13%, $p = 0.55$) (figure 2A). The rate of progression of the high-dose group was 19% less than the historical placebo controls ($p = 0.16$). No significant benefits were observed for change in ALSFRS-R

Table 1 Baseline demographics and disease characteristics

Variable[a]	Placebo (N = 42)	NP001 1 mg/kg (N = 49)	NP001 2 mg/kg (N = 45)
Sex, n (%)			
Female	13 (31.0)	13 (26.5)	14 (31.1)
Male	29 (69.0)	36 (73.5)	31 (68.9)
Race, n (%)			
White	41 (97.6)	48 (98.0)	43 (95.6)
Black	1 (2.4)	0 (0.0)	0 (0.0)
Other	0 (0.0)	0 (0.0)	1 (2.2)
Age at enrollment, y	53.7 (9.52)	54.4 (12.4)	53.6 (10.1)
Duration of ALS symptoms, mo	17.19 (8.9)	21.88 (9.4)	17.38 (8.3)
Type of ALS, n (%)			
Familial	5 (11.9)	2 (4.1)	2 (4.4)
Sporadic	37 (88.1)	47 (95.9)	43 (95.6)
Site of ALS onset, n (%)			
Bulbar	7 (16.7)	9 (18.4)	8 (17.8)
Limb	35 (83.3)	40 (81.6)	37 (82.2)
El Escorial criteria for ALS, n (%)			
Probable	19 (45.2)	29 (59.2)	23 (51.1)
Definite	21 (50.0)	20 (40.8)	20 (44.4)
Concurrent riluzole use, n (%)	29 (69.0)	38 (77.6)	32 (71.1)
ALSFRS-R score at baseline	38.2 (5.6)	37.6 (5.5)	37.6 (5.0)
Baseline MCP-1, pg/mL[b]	178.4 (111.8, 388.6)	170.0 (22.5, 327.4)	179.4 (106.0, 305.2)
Baseline wr-CRP, ng/mL[b]	1,009.0 (1.69, 12,730.0)	1,298.0 (1.69, 15,710.0)	1,064.0 (1.69, 15,585)
Vital capacity at baseline, L	3.77 (1.03)	3.76 (0.82)	3.80 (0.88)

Abbreviations: ALS = amyotrophic lateral sclerosis; ALSFRS-R = ALS Functional Rating Scale-Revised; MCP-1 = macrophage chemotactic protein-1; wr-CRP = wide range C-reactive protein.
All values are mean ± SD unless otherwise indicated.
[a] n = number of randomized patients.
[b] Median and range are reported.

Figure 2 ALSFRS-R changes



(A) Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised (ALSFRS-R) slope after 6 months of treatment without (left) and with (right) historical controls. (B) Mean change from baseline in ALSFRS-R score at week 25 without (left) and with (right) historical controls. (C) ALSFRS-R slope after 6 months of treatment in patients with baseline wide range C-reactive protein (wr-CRP) greater than or equal to the baseline median wr-CRP. Error bars represent standard error.

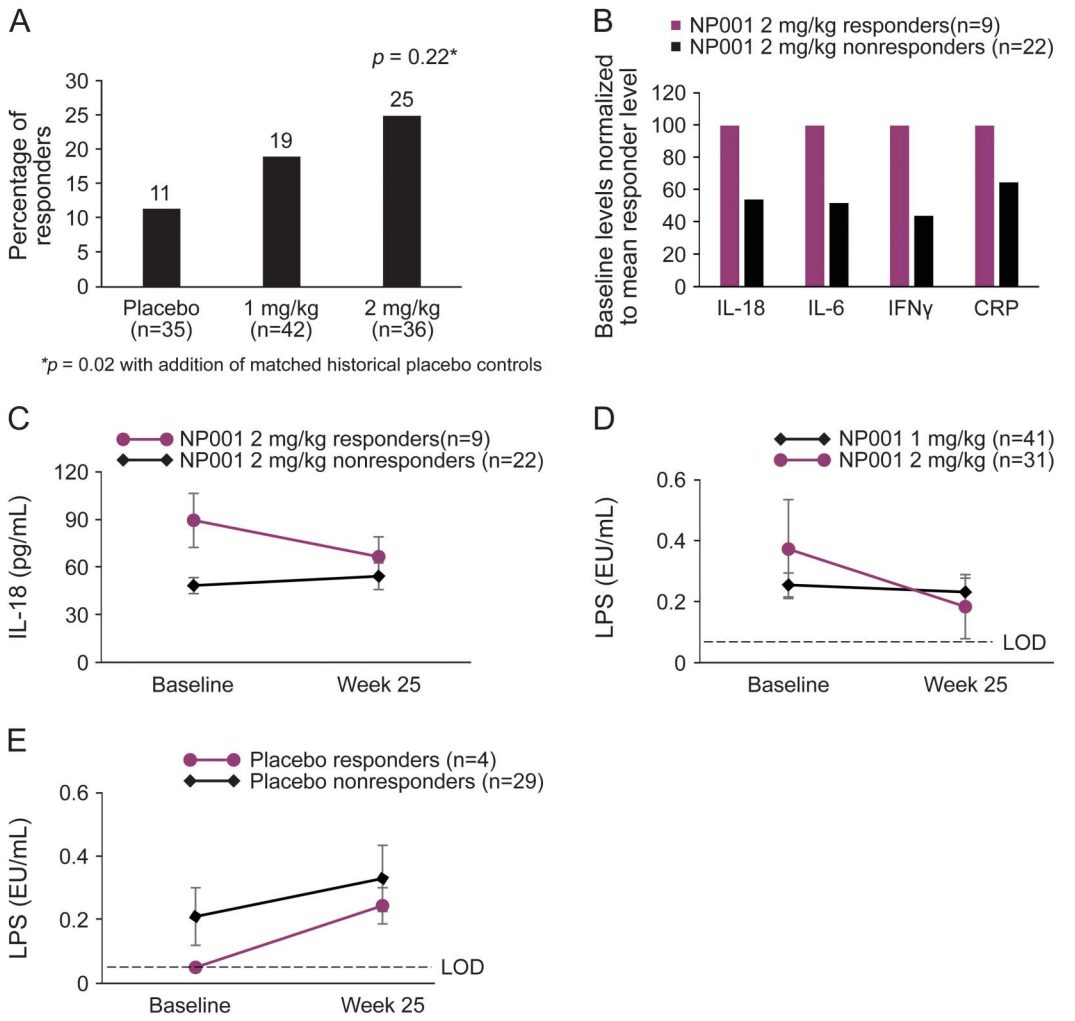
from baseline in the high-dose group, with 21% slowing without ($p = 0.48$) and 17% slowing with ($p = 0.44$) historical controls (figure 2B).

The effect of NP001 did seem to be related to the degree of baseline inflammation. Those patients treated with NP001 whose baseline wr-CRP levels were at or above the median for the entire randomized population had greater slowing of progression than placebo patients whose baseline wr-CRP values were also at or above the median (figure 2C). The estimated slope decline in points per month was −0.55 for the NP001 2 mg/kg group, −0.73 for the NP001 1 mg/kg group, and −0.93 for the placebo group. The slowing in the rate of progression in the 2 mg/kg group represented a 41% improvement compared with placebo ($p = 0.20$). In patients whose baseline wr-CRP levels were below the median, the estimated slopes were −0.87, −1.38, and −0.84 for the 2 mg/kg, 1 mg/kg, and placebo groups, respectively. The only trend for the differences in slope was for the NP001 1 mg/kg group compared with placebo ($p = 0.09$). In contrast to wr-CRP, there was no effect of NP001 with regard to MCP-1 (data not shown).

There were no significant differences for VC, ALSFRS-R subscores, or sex (data not shown).

Of importance, the responder analysis showed a dose-dependent increase in the percentage of responders. In the high-dose group, 25% of patients did not progress over the 6-month treatment period (figure 3A), which is more than 2 times greater than the percentage in the placebo group (11%), although it did not reach statistical significance ($p = 0.22$). However, there was statistical significance ($p = 0.02$) with the

Figure 3 **Responder subset**



(A) Percentage of "responders" (nonprogressing patients) at week 25 after 6 months of treatment; percentage of "responders" in historical controls was 10%. (B) Normalized mean baseline plasma concentrations of inflammatory biomarkers. (C) Mean plasma interleukin (IL)-18 in high-dose "responders" vs "nonresponders" at baseline and after the 6-month treatment period (week 25). (D) Mean plasma lipopolysaccharide (LPS) in all patients treated with 1 mg/kg or 2 mg/kg NP001 at baseline and after the 6-month treatment period (week 25). (E) Mean LPS in placebo "responders" and "nonresponders" at baseline and after the 6-month treatment period (week 25). Error bars represent standard error. Limit of detection (lod) for LPS = 0.05 EU/mL. CRP = C-reactive protein; IFN = interferon.

addition of matched historical placebo controls, of whom 10% did not progress. Consistent with these findings was a dose-dependent smaller decline in VC (mean ± SD) in responders (1 mg: −8.95 ± 10.1; 2 mg: −3.76 ± 5.7) compared with nonresponders (1 mg: −17.7 ± 17.9; 2 mg: −14.5 ± 13.2) after 6 months of treatment. Responders had elevated baseline IL-18, IL-6, IFN-γ, and CRP compared with nonresponders in the high-dose group (figure 3B). It is important to note that all of the high-dose responders were positive for LPS in their plasma, and the majority had elevated baseline plasma IL-18 (figure 3C). After 6 months of treatment, 70% of high-dose responders had decreased LPS and 80% had decreased IL-18 (figure 3, C and D). Similarly, in the low-dose group, 7 of 8 responders were LPS-positive and 75% had baseline IL-18 at or above the baseline median for all patients. After 6 months of treatment, half of the patients had decreased LPS and 3 patients had decreased IL-18. Other biomarkers did not significantly decrease after treatment. All 4 of the placebo responders were LPS-negative at baseline, yet 3 of 4 had elevated IL-18. Notably, LPS levels in all placebo patients (responders and nonresponders) increased over the 6-month treatment period (figure 3E).

Tolerability and safety. TEAEs occurred in 95.6%, 93.9%, and 97.6% of patients in the 2 mg/kg, 1 mg/kg, and placebo groups, respectively. TEAEs considered possibly, probably, or definitely related to study medication occurred in the highest percentage of patients in the 2 mg/kg group (71.1%); the

1 mg/kg and placebo groups were similar (57.1% and 54.8% of patients, respectively).

The most common TEAEs in ≥5% of patients are shown in table 2. Falls, fatigue, and headache were the most common TEAEs and occurred with similar frequency or less often in the treatment groups compared with the placebo group. Both active treatment groups had a higher percentage of patients with infusion site pain (NP001 2 mg/kg, 33.3% and NP001 1 mg/kg, 18.4%, $p = 0.0022$) than the placebo group (4.8%). Dizziness occurred in a higher percentage of patients in the NP001 2 mg/kg group (20.0%) than in the NP001 1 mg/kg (8.2%) and placebo (7.1%) groups, but the difference was not significant ($p = 0.1275$).

Serious TEAEs were most frequent in the NP001 2 mg/kg group (15.6%), followed by the NP001 1 mg/kg group (8.2%) and then the placebo group (4.8%). Serious TEAEs were all considered unlikely or unrelated to study drug, with the exception of 1 patient with increased troponin associated with a pulmonary embolism. There were 8 deaths during the trial: 6 patients had respiratory failure, 1 had respiratory failure and pneumonia, and 1 had an unknown cause of death. All deaths were assessed as unlikely or unrelated to study drug. No clinically relevant changes in vital signs or ECG parameters were noted.

DISCUSSION This phase 2 trial assessed the potential utility of NP001 as a treatment for ALS. This trial was designed as an exploratory safety, tolerability, and preliminary efficacy study that was underpowered to detect even a large slowing of progression (>30%). No statistically significant benefit was seen in ALS progression over a 6-month treatment period. However, there was a tendency to more slowing of ALSFRS-R decline in the NP001 treatment groups in patients with more systemic inflammation at baseline (i.e., ≥ the baseline median wr-CRP for the randomized population). In this subset of patients, there was a 41% reduction in progression with high-dose NP001 vs placebo, compared with a 13% decrease for the group as a whole. In addition, the 1 mg/kg subgroup also showed a greater improvement in

Table 2 Common clinical treatment-emergent adverse events (>5% in any treatment group)

Preferred term	Placebo (N = 42)	NP001 1 mg/kg (N = 49)	NP001 2 mg/kg (N = 45)	Fisher exact p value
Fall	18 (42.9)	16 (32.7)	17 (37.8)	0.5985
Fatigue	14 (33.3)	8 (16.3)	16 (35.6)	0.0741
Infusion site pain	2 (4.8)	9 (18.4)	15 (33.3)	0.0022
Infusion site extravasation	6 (14.3)	9 (18.4)	10 (22.2)	0.6727
Headache	11 (26.2)	11 (22.4)	9 (20.0)	0.7896
Dizziness	3 (7.1)	4 (8.2)	9 (20.0)	0.1275
Nausea	6 (14.3)	6 (12.2)	7 (15.6)	0.9516
Cough	4 (9.5)	7 (14.3)	7 (15.6)	0.6995
Infusion site erythema	5 (11.9)	6 (12.2)	6 (13.3)	0.9999
Nasopharyngitis	2 (4.8)	6 (12.2)	6 (13.3)	0.3790
Involuntary muscle contractions	2 (4.8)	3 (6.1)	6 (13.3)	0.3234
Back pain	3 (7.1)	1 (2.0)	5 (11.1)	0.1898
Muscular weakness	3 (7.1)	1 (2.0)	5 (11.1)	0.1898
Dysphagia	5 (11.9)	2 (4.1)	4 (8.9)	0.3540
Constipation	0 (0.0)	5 (10.2)	4 (8.9)	0.0957
Diarrhea	1 (2.4)	5 (10.2)	4 (8.9)	0.3504
Rash	0 (0.0)	4 (8.2)	4 (8.9)	0.1312
Contusion	5 (11.9)	3 (6.1)	3 (6.7)	0.5805
Pain in extremity	4 (9.5)	3 (6.1)	3 (6.7)	0.8447
Peripheral edema	3 (7.1)	3 (6.1)	3 (6.7)	0.9999
Muscle spasms	1 (2.4)	5 (10.2)	2 (4.4)	0.3135
Anxiety	2 (4.8)	3 (6.1)	2 (4.4)	0.9999
Infusion site swelling	3 (7.1)	2 (4.1)	2 (4.4)	0.7939
Nasal congestion	3 (7.1)	2 (4.1)	2 (4.4)	0.7939

All values are n (%).

slope change when stratified by median wr-CRP. Although wr-CRP is a crude marker of systemic inflammation, published data suggested that rate of disease progression was related to wr-CRP.[17] Disease progression was unrelated to baseline median plasma MCP-1 values, perhaps reflecting the greater importance of CSF MCP-1 in driving inflammatory monocyte migration into the CNS.[4]

The most striking finding of this clinical trial was the halting of symptom progression in a dose-dependent fashion in a subset of patients: 25% vs 19% vs 11% in the 2 mg/kg, 1 mg/kg, and placebo groups, respectively. The percentage of patients who met the definition of responder in the matched historical placebo control database over a comparable 6-month period, 10%, was similar to the percentage of responders in the placebo group (11%) in this trial. This consistency between placebo group responder rates lends credence to the potential effects seen with NP001. This observation of possibly arresting progression has not been reported in any other ALS clinical trial. Since the clinical features of these responders were not different from nonresponders, it is unclear what baseline factors might allow identification of patients who would be more likely to respond to drug. It is also unknown whether longer treatment duration or a higher dose of NP001 would result in a greater proportion of responders.

Although the possibility of random bias cannot be ruled out, this is highly unlikely, as the majority of responders to NP001 were LPS-positive and had elevated baseline levels of other inflammatory biomarkers such as IL-18. Elevated IL-18 observed in these patients is consistent with another study in which serum IL-18, compared with other IL-1 family cytokines, was elevated in ALS.[19] Their hypothesis that IL-18 plays a pathologic role in ALS is in accord with our findings, as is the decreased IL-18 in responders after treatment with high-dose NP001. In addition, LPS levels, which may be a signaling pathway for macrophage activation in ALS,[8] decreased in most patients treated with NP001 regardless of responder status, which is consistent with improved macrophage function. In contrast, placebo patients, both responders and nonresponders, became LPS-positive or had increasing LPS levels consistent with worsening immune status, suggesting that NP001 may have a beneficial effect on neuroinflammation in ALS.

The major limitations of this study are the small sample size and underpowering for slope change, the limited duration of treatment, and the post hoc nature of the responder analysis. Strengths of the study include the identification of potential biomarkers of inflammation relevant to ALS progression and the use of historical placebo controls. Given the mechanism of action of NP001 and the findings related to plasma LPS and IL-18, these as well as additional inflammatory biomarkers or microRNAs in plasma and/or peripheral monocytes may aid in better preselection of potential responders to NP001 and possible stratification of patients in future trials. We conclude that the uniqueness of the responder and inflammatory biomarker findings, coupled with a good safety and tolerability profile, justify continued clinical development to fully characterize the potential disease-modifying effects of NP001.

AUTHOR CONTRIBUTIONS

R.G. Miller: revising the manuscript and final approval, study concept and design, interpretation of the data, and chairing of the Western ALS Study Group with sites that played a key role in the study. G. Block: drafting/revising the manuscript and final approval, study concept and design, interpretation of the data. J.S. Katz: revising the manuscript and final approval, interpretation of the data. R.J. Barohn: revising the manuscript and final approval, interpretation of the data. V. Gopalakrishnan: revising the manuscript and final approval, study design, interpretation of the data. M. Cudkowicz: revising the manuscript and final approval, interpretation of the data. R. Zhang: selection of biomarkers and performing the biomarker statistical analyses. M.S. McGrath: revising the manuscript and final approval, interpretation of the data. E. Ludington: conducting statistical analyses and revising the manuscript. S.H. Appel: study design, revising the manuscript and final approval, interpretation of the data. A. Azhir: study design and concept, revising the manuscript and final approval.

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DISCLOSURE

R.G. Miller is on the scientific advisory board for Cytokinetics and Mitsubishi Tanabe, is on the editorial advisory board for *Lancet Neurology*, and received research support from the Muscular Dystrophy Association. G. Block is the Interim Chief Medical Officer of Neuraltus Pharmaceuticals, is the head of the Scientific and Clinical Advisory Board for the company, and received stock options from Neuraltus. J.S. Katz reports no disclosures. R.J. Barohn is on the scientific advisory board for the diaphragm stem study; has received travel funding and/or speaker honoraria from Grifols, NuFactor, KU CME courses, and Walgreens; is on the editorial board for *Journal of Clinical Neuromuscular Disease*; is on the speakers' bureau for Grifols, Baxter, and Sanofi/Genzyme; and received research support from Cytokinetics, GSK, CSL-Behring, Alexion, Sanofi/Genzyme, Biomarin, PTC, Eli Lilly, NIH/NCATS Heartland Institute for Clinical and Translational Work, FDA OPD, PCORI, and NIH/National Institute of Neurological Disorders and Stroke. V. Gopalakrishnan has been employed by Quark Pharmaceuticals and Neuraltus Pharmaceuticals. M. Cudkowicz is on the scientific advisory boards for Coyote Pharmaceuticals, Biogen, Cytokinetics, Genetech, and AstraZeneca; is on the editorial board for *Neurotherapeutics* and *JAMA Neurology*; holds a patent for metabolomics in ALS; receives royalty payments for UptoDate MND chapter; has consulted for Teva, Cytokinetics, Biogen-Idec, Shire, and Voyager; and received research support from National Institute of Neurological Disorders and Stroke, Muscular Dystrophy Association, and ALS Association. J.R. Zhang has consulted for Neuraltus Pharmaceuticals, Inc. M.S. McGrath is on the scientific advisory board for Neuraltus, has consulted for and holds stock options in Neuraltus, and UCSF has a patent licensed to Neuraltus for chlorite regulation of

neurodegenerative disease. E. Ludington has been employed by and consulted for various biopharmaceutical companies through Agility Clinical, Inc. S.H. Appel is on the scientific advisory board for Neuraltus; received speaker honorarium from Avanir, Inc; and received research support from NIH, Muscular Dystrophy Association, and ALS Therapy Development Institute. A. Azhir holds a composition and formulation patent of NP001, has consulted for T1 Translational Catalyst Advisory Board, founded Neurocea, and holds stock options in Neuraltus Inc. Go to Neurology.org/nn for full disclosure forms.

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