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NP001 regulation of macrophage activation markers in ALS: A phase I clinical and biomarker study

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Abstract

This is a phase I, placebo-controlled, single ascending dose safety and tolerability study of NP001 in patients with ALS. NP001 is a novel regulator of inflammatory macrophages and monocytes. As ALS progression is thought to be related to neuroinflammation, an additional objective of the study was to assess the effects of NP001 administration on monocyte activation markers. Thirty-two ALS patients were enrolled and received either placebo (eight) or one of four (six at each dose) ascending single i.v. doses (0.2, 0.8, 1.6 and 3.2 mg/kg NP001). Patients were monitored for safety, and blood monocyte immune activation markers CD16 and HLA-DR were assessed pre- and 24 h post-dosing. Changes from baseline were calculated. Results showed that NP001 was generally safe and well tolerated. Importantly, a single dose of NP001 caused a dose-dependent reduction in expression of monocyte CD16, a marker of monocyte activation/inflammation. Additionally, monocyte HLA-DR expression was also decreased in those patients with elevated values at baseline. In conclusion, these data indicate that NP001 has an acute effect on inflammatory monocytes in ALS patient blood. The potential for modulation of inflammation in the context of ALS disease progression will require further study with long-term follow-up.

Keywords

NP001; ALS; inflammation; monocyte; macrophage

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Supplementary material available online

Supplementary Figure 1 to be found online at http://informahealthcare.com/doi/abs/10.3109/21678421.2014.951940.

Introduction

Amyotrophic lateral sclerosis (ALS) is a debilitating neurological disorder in which neurodegeneration occurs in concert with an ongoing inflammatory process (1–4). The loss of motor neurons in ALS manifests as muscle weakness leading to death. The only approved therapy, riluzole, prolongs survival by two to three months (5). Recent animal model studies implicate inflammatory mechanisms and inappropriate immune activation in the pathogenesis of ALS (6–10). More recently, the involvement of inappropriate NF-κB activation in motor neuron degeneration has garnered attention in ALS (11,12). Although the critical events involved in disease induction remain unknown, there is an emerging consensus that ALS disease progression is associated with inappropriate systemic inflammation and abnormal inflammatory macrophage activation within affected spinal cord and brain regions resulting in neuronal loss (13–19).

Monocytes are bone marrow derived precursors of tissue macrophages that are critical effectors of wound healing, clearance of bacteria and cellular debris and induction and resolution of inflammation. Macrophages that are associated with classical inflammation are termed M1 and those cells produce factors such as TNF-α, IL-1 and other proinflammatory factors. Macrophages that are associated with reversal of inflammation and suppression of immune responses are termed M2. In the context of ALS pathogenesis, the M2 macrophage phenotype within the spinal cord is associated with normal function, whereas the appearance of new M1 type macrophages within the spinal cord is associated with disease progression (15).

Abnormal secretion of macrophage activation products and proinflammatory cytokines, such as monocyte chemoattractant protein-1 (MCP-1) (13,20–23), and interleukin-6 (IL-6) (24,25) have been reported in cerebral spinal fluid (CSF) and sera in patients with ALS. Enhanced levels of tumor necrosis factor-α (TNF-α) also have been seen in the blood of ALS patients (26–28). The study from Keizman et al. showed that a heightened systemic inflammatory state was associated with a negative prognosis in patients with ALS (17). Recent laboratory studies implicate inappropriate systemic macrophage activation (29,30) and alteration of blood monocyte populations with disease progression in patients with ALS (23,31). Blood from patients with ALS exhibit elevated levels of CD16 + monocytes and these CD16 + monocytes are characterized by high expression of the monocyte activation marker HLA-DR and low expression of the MCP-1 receptor, CCR2. A previous study also found significantly increased levels of plasma endotoxin/lipopolysaccharide (LPS), a systemic macrophage activator in ALS patient plasma that correlated with the level of inflammatory monocytes in peripheral blood (32). In addition, a recent study in an ALS animal model (33) attempting to interfere with an activator of chronic immune activation (antibody to CD40 ligand) showed the best survival benefit of any approach tested in this model to date. These data suggest that systemic macrophage associated inflammation may play a significant role in ALS disease progression.

NP001, a highly purified, pH-adjusted stabilized form of sodium chlorite, is a novel effector molecule that represents a new class of drug for targeting inflammatory macrophages and regulating macrophage function both in vitro and in vivo (34). Chlorite mediates its anti-

inflammatory effect in macrophages by the creation of high intracellular levels of taurine chloramine, a factor known to down-regulate NF-κB induced inflammatory pathways (35,36). In previous clinical studies with another form of chlorite, this regulation has been shown to reverse inflammation and cause systemic macrophages to revert to a normal wound healing phagocytic state (34). Recent studies have shown that disease progression in the G93A strain of ALS mice is directly associated with migration of inflammatory monocytes into the spinal cord (16). Preliminary studies of NP001 in the G93A-SOD1 congenic strain of mice showed a significant survival improvement in treated compared to control mice (37). If NP001 could regulate monocyte/macrophage activation in ALS patients, inflammation associated disease progression might be affected in a manner similar to that seen in the ALS mouse model.

The present study explores the effects of NP001 on monocyte/macrophage activation in blood specimens obtained during a single-dose phase I trial of NP001 in patients with ALS.

Materials and methods

Study design

This was a phase I safety and tolerability study of NP001 in patients with ALS. A double-blind, placebo-controlled, single ascending dose safety and tolerability study of NP001 was conducted by Neuraltus Pharmaceuticals, Inc. (Palo Alto, CA), and the Western ALS Study Group (Clinicaltrials. org NCT01091142), in 32 male and female patients with probable or definite ALS according to modified El Escorial criteria (38). Patients were included if age < 75 years, stable riluzole dose for 30 days, and able to provide informed consent. Patients with tracheostomy, other active diseases besides ALS, or taking immunosuppressant therapy were excluded. Clinical features of the patients are listed in Table I. Patients receiving either placebo or ascending doses of NP001 were monitored for the primary endpoints of: safety, changes in clinical status; and secondary endpoints of: blood monocyte immune activation markers CD16 and HLA-DR responses to NP001 among blood monocytes before dosing and 24 h post-dosing. Changes from baseline in each monocyte marker were included in the statistical plan and those values were obtained by an independent flow cytometry laboratory at UCSF using validated procedures for the determinations. Statistical analysis was performed by an independent statistician for the CD16 values and by Neuraltus scientists for the HLA-DR values.

Informed consent and ethics approval

The study was conducted at three clinical sites in the United States: California Pacific Medical Center, San Francisco, California; University of Kansas Medical Center Research Institute, Kansas City, Kansas; University of Kentucky ALS Center, Lexington, Kentucky. Patients with ALS provided informed consent in accordance with guidelines established by California Pacific Medical Center and University of California San Francisco (UCSF) committees on human research, coordinated by the AIDS and Cancer Specimen Resource (ACSR). Similar approvals were obtained at the other two clinical sites. All research was conducted according to Declaration of Helsinki principles. Each participant was identified

by number and not by name. Both patients and evaluators were blinded as to treatment assignment.

Blood monocyte activation/inflammation assays

The secondary objective of this study was to explore the effects of single doses of NP001 on macrophage inflammatory activation markers potentially relevant to the pathogenesis and progression of ALS. The Revised ALS Functional Rating Scale (ALSFRS-R), scored 0–48, was used to evaluate overall patient functional status (39). Estimated disease progression rate was calculated as follows:

$$\text{Mean monthly decline rate} = (48 - \text{ALSFRS-R score at baseline}) / \text{disease duration}$$

The monocyte activation markers measured were the levels of CD16 and HLA-DR expression on CD14 + monocytes from stained whole blood. CD16 and HLA-DR expression on CD14 + monocytes are measures of monocyte/macrophage inflammatory activation at the cellular level (31,40–43). Blood specimens for exploratory monocyte activation marker analysis were collected from patients before dosing and 24 h post-dosing. Specimens were transported from the clinical site to a designated laboratory for same day sample preparation. Stained and fixed samples were then transported to the UCSF Core immunology laboratory (UCSF, San Francisco, CA) for flow cytometer measurement by LSR II flow cytometer (Becton Dickinson) using FACSDiva software (BD Biosciences, San Jose, CA). Data were compensated and analyzed by FlowJo software (TreeStar Inc., Ashland, OR). The results from flow cytometry analysis were expressed as the geometric mean fluorescence intensity (Geo MFI) of monocyte activation markers.

A typical gating strategy used to identify HLA-DR and CD16 expression on CD14 + monocytes by flow cytometry included: CD3 and CD16 used to exclude the CD3 + lymphocytes and CD16 granulocytes that contaminate in the mononuclear cell gate. CD14 + HLA-DR+ cells are then gated from a HLA-DR vs. CD14 dot plot, which excludes remaining lymphocytes including B-cells and NK cells. Total monocytes are then gated on a CD14 vs. side scatter plot (CD14+). From the CD14 + gate the geometric mean fluorescence intensity of HLA-DR (Geo MFI CD14/HLA-DR) is measured. The proportions of CD16 + and CD16 bright cells are also gated from the CD14 + cells on a CD14 vs. CD16 dot plot. CD16 bright gate (in general 10x brighter than standard CD16 intensity) captures all the dim CD14 + CD16+ bright cells (Supplementary Figure 1 – which is only available in the online version of the journal. Please find this material with the following direct link to the article: http://informahealthcare.com/doi/abs/10.3109/21678421.2014.951940).

From the CD16 bright gate the geometric mean fluorescence intensity of CD16 bright (Geo MFI CD16 on CD14/CD16 bright) is measured.

Safety and clinical status variables

After NP001 treatment, patients were monitored for a variety of safety and clinical status variables during and for 8 h after infusions and at one, four and seven days after dosing. This included physical examinations, including inspection of the infusion site for reactions, and

clinical laboratory tests involving blood counts, a multi-channel chemistry panel, urinalysis, electrocardiograms and vital capacity. Safety data from the full cohort of eight patients from each dose level were reviewed by the safety monitoring committee before escalating to the next higher dose. Flow cytometer assessment of NP001 treatment in blood monocyte was performed before dosing and 24 h post-dosing.

Statistical analysis

Statistical analysis was performed by GraphPad Prism 6.0 program (GraphPad Software, San Diego, California, USA). Flow cytometer results are expressed as the mean ± SED unless otherwise stated. Statistical significance was assessed using one-way ANOVA, and linear regression, as indicated in the tables and figures legends. For all analysis, a value of $p < 0.05$ was considered statistically significant.

Results

Safety results

This phase I safety and tolerability study of NP001 in subjects with ALS was completed by the Western ALS Study Group and Neuraltus Pharmaceuticals, Inc. in 2010. In this trial, 32 patients (clinical features in Table I) were enrolled and four cohorts of patients received a single dose of NP001 (0.2, 0.8, 1.6 or 3.2 mg/kg NP001 chlorite, $n = 6$ per cohort, total 24 NP001 patients) or placebo (saline, $n = 2$ per cohort, total eight placebo patients) as a 30-min infusion on day 1. All doses of NP001 were generally safe and well tolerated and there were no treatment-related serious adverse events (Table II) or clinically relevant changes in safety associated laboratory parameters. In addition, blood monocyte activation markers, CD16 and HLA-DR, were quantitated at baseline and 24 h after a single dose of the drug or placebo infusion.

Baseline monocyte/macrophage activation-related inflammatory cell surface markers are increased in ALS patients in relation to rate of ALS disease progression

In a previous report, the degree of systemic monocyte/macrophage activation (monocyte overexpression of both HLA-DR and CD16) was found to be associated with the rate of ALS disease progression (31): the higher the level of activation the more rapid the ALS disease progression. ALS patient blood monocytes obtained at baseline in the NP001 phase I study showed evidence for monocyte activation as defined by CD14 cell coexpression of HLA-DR, levels of which were related to the estimated rate of ALS disease progression (ALSFRS-R score loss per month based on evaluation of patient symptom duration) ($r = 0.4310$, $p = 0.0138$; $n = 32$) (Figure 1A). A positive correlation was also observed between the ALS disease progression rate and levels of CD16 on CD16 bright monocytes, the most activated subset of proinflammatory monocytes that act as differentiated monocytes or tissue macrophages (40,42,44–46) ($r = 0.4499$, $p = 0.0098$; $n = 32$) (Figure 1B). Moreover, a multiple regression analysis revealed that the two monocyte activation markers were independent of each other in relationship to ALS disease progression rate, and when combined showed an enhanced association with rate of ALS disease progression (Multiple R = 0.5734, $p = 0.0031$). No relationship was found between baseline ALSFRS-R score and levels of either monocyte HLA-DR or monocyte CD16 bright subset coexpression.

NP001 decreases level of monocyte HLA-DR in patients with elevated HLA-DR values at baseline

Following NP001 treatment, changes in monocyte levels of HLA-DR did not demonstrate a dose-dependent effect; however, HLA-DR expression was down-regulated at all doses of NP001 in patients with the high baseline levels of monocyte HLA-DR. Figure 2 shows the scatter plot of change in NP001-induced monocyte HLA-DR expression levels as a function of monocyte HLA-DR baseline levels for the 32 subjects dosed in the NP001 phase I study. The placebo group showed relatively stable monocyte HLA-DR after treatment ($r = -0.07721$, $p = 0.8558$; $n = 8$). The changes of HLA-DR expression on monocytes in the NP001 treatment response were linearly related to the degree of baseline monocyte HLA-D expression 24 h after treatment ($r = -0.4967$, $p = 0.0135$; $n = 24$). The greater the starting monocyte HLA-DR levels at baseline the greater the HLA-DR response to NP001. A representation of the data based on starting monocyte HLA-DR levels at baseline is shown in Figure 3. In the group of 12 patients with elevated baseline monocyte HLA-DR, the average percent change from baseline 24 h after NP001 was more than 10%, whereas those patients with lower range monocyte HLA-DR showed no change from baseline ($p = 0.0153$).

NP001 associated change in monocyte HLA-DR expression is associated with the estimated rate of ALS disease progression

A post hoc analysis to evaluate the effect of ALS estimated disease progression rate on these results was conducted. Figure 4 shows the results of monocyte HLA-DR expression change after NP001 treatment (pooled data) as a function of each patient's historical rate of ALS disease progression since onset of symptoms (based on review of clinical charts at the participating institutions). The average ALS patient declines at a rate of approximately 1 unit/month using the ALSFRS-R scoring scale. Patients who were slow progressors (defined as estimated rates of progression < 0.5 unit per month) showed no change in HLA-DR regardless of whether the patient received NP001 or placebo. In contrast, patients with estimated rates of progression ≥ 1 unit per month showed the greatest change in HLA-DR expression following NP001 dosing ($R^2 = 0.2310$, $p = 0.0058$ for the linear trend comparison).

NP001 induces a dose-dependent decreased level of CD16 expression on the bright CD16 subset of CD14 monocytes in vivo

Dose-dependent changes in NP001 treated patients compared to placebo were observed in the level of CD16 expression on the CD16 bright subset of monocytes. The degree of monocyte CD16 modulation was not correlated with baseline CD16 expression or the estimated rate of decline as assessed by the change in ALSFRS-R since disease onset. Figure 5 shows the dose-dependent relationship trend between change in monocyte CD16 expression from baseline and the dose of NP001 administered ($R^2 = 0.1958$, $p = 0.0085$ for the linear trend comparison; placebo, $n = 8$; NP001, $n = 6$ for each dose). Note that there was no significant change in the level of monocyte CD16 expression in the placebo group.

Figure 6 shows the absolute level of CD16 in the monocyte CD16 bright subset from patients who received the 1.6 mg/kg dose of NP001 as defined by quantitative flow cytometry. Twenty-four hours after one dose of NP001, the difference between the ALS and

normal control level of monocyte CD16 expression was reduced by approximately 50% toward the normal value compared with baseline pretreatment levels in the ALS patients.

Discussion

Abnormal systemic inflammation and macrophage activation plays a critical role in the pathogenesis of ALS. Many studies have confirmed the presence of systemic inflammation in patients with ALS based on observations of elevated levels of serum and plasma biomarkers related to chronic immune activation/inflammation (29). In contrast to the previously reported plasma biomarkers, which were poorly related to rate of ALS disease progression (29), alteration of blood inflammatory monocyte activation markers appears to be related to estimated rates of disease progression as seen in the current and our previous studies (30,31).

Blood from ALS patients contains abnormally activated monocytes characterized by elevated levels of cell surface inflammatory activation markers CD16 and HLA-DR. This type of macrophage activation is associated with the alteration of ALS blood monocyte gene expression profiles wherein ALS blood monocytes spontaneously express genes associated with LPS/endotoxin activation (30). Evidence accumulated over the past two decades indicates that CD16 + monocytes with higher level expression of HLA-DR appear to be more mature, resemble tissue macrophages, express proinflammatory cytokines such as MCP-1 and TNF-α and show higher potency in antigen presentation assays (40,42,47). These activated cells produce elevated levels of proinflammatory cytokines and monocyte activation-related products. Inflammatory monocytes are also the cells that migrate from the blood into the spinal cord and brain, releasing factors associated with neurodegeneration (48–52). Recent studies on ALS animal models confirmed this process and appear to parallel results observed in humans (15,53). ALS pathogenesis is associated with the appearance of activated macrophages expressing the CD16 gene. Cells bearing FcγRIII (human equivalent CD16) accumulate within spinal cords in SOD mice as ALS disease progresses (53). There is a switch from a normal spinal cord microglial/macrophage phenotype to an inflammatory phenotype (CD16 + macrophage) between presymptomatic and symptomatic status. CD16 is a marker of this disease associated macrophage (15).

NP001, purified sodium chlorite, is hypothesized to exert its clinical action by targeting inflammatory monocytes and macrophages in vivo (34). In the current NP001 phase I safety and tolerability study in ALS patients, a single NP001 treatment changed blood monocyte markers of inflammation 24 h after a 30-min infusion. Elevation in both monocyte CD16 and HLA-DR has been observed in at least half of patients with ALS and, in the current study, patients with elevated baseline levels or highest rates of disease progression showed the largest change toward normal levels for both markers. These inflammatory macrophage activation markers potentially are relevant to the pathogenesis and progression of ALS (31). Therefore, attenuating the elevated expression of HLA-DR and CD16 on ALS monocytes, might block migration of inflammatory macrophages into diseased areas of the CNS. Consistent with previous studies, the baseline monocyte expression of CD16 and HLA-DR results from the NP001 phase I trial showed that the degree of abnormal blood monocyte activation is related to the estimated rate of ALS disease progression. Monocyte expression

of HLA-DR most likely reflects the level of systemic inflammation. Thus, the NP001 monocyte HLA-DR response represents a generalized response to a systemic inflammation signal. In contrast, NP001 dose-dependently inhibited CD16 expression on inflammatory monocytes that differentiate into macrophages capable of migrating into tissues (42,48,51,52). This response, different from the HLA-DR response, suggests that NP001 exerts both a global anti-inflammatory effect (change in monocyte HLA-DR) and may directly affect monocyte migration from blood into tissues (changes in CD16 bright subset).

In the current study, NP001 appeared to have a general anti-inflammatory effect as measured by reversal of abnormal HLA-DR expression. In subsequent phase II studies a prediction of this single dose study would be that patients with the most elevated levels of inflammation might have the best outcome if inflammation is in any way causing ALS disease progression. Baseline inflammatory markers with post study follow-up evaluations might test this theory. The results of the CD16 analyses are also useful in considering the design of a subsequent phase II study. Figure 5 shows that the best effect on CD16 expression occurred at the 1.6-mg/kg dose, very near the dose utilized in earlier studies of chlorite for treatment of post radiation syndrome and AIDS (34) of 2 mg/kg chlorite. As previously shown for WF10 chlorite, 1 mg/kg chlorite appeared to be a minimally effective dose for regulating the immune system (34), similar to the results shown for the 0.8-mg/kg dose of NP001 chlorite in the current study. Given these comparisons, a subsequent phase II study could utilize an apparently immune-regulatory dose of NP001 chlorite (2 mg/kg) compared with a minimally effective dose (1 mg/kg) vs. placebo in a study extended long enough to determine whether regulation of inflammation might affect the rate of ALS disease progression. Therefore, this study has provided the foundation for using defined doses of NP001 targeting markers of inflammation in patients with ALS to test the theory that inflammation may be a significant contributor to ALS disease pathogenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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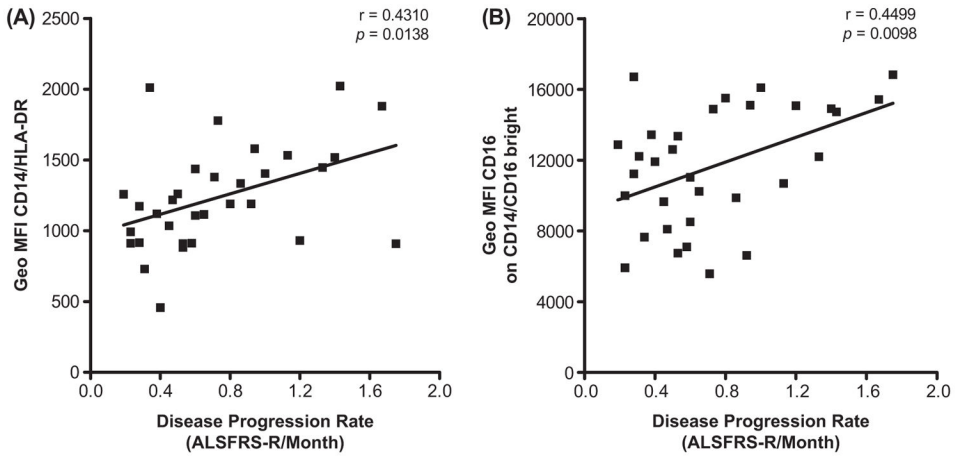

Figure 1.

Relationship between baseline monocyte inflammatory activation related markers and the historic rate of ALS disease progression. Levels of activation marker at baseline (y-axis) are plotted against disease progression rate (ALS/FRS-R score lost/month) (X-axis). Figure 1A. Levels of baseline monocyte activation defined by CD14 coexpression of HLA-DR was directly related to the rate of ALS disease progression ($r = 0.4310$, $p = 0.0138$; $n = 32$). Figure 1B. Positive correlation was observed between baseline levels of CD16 expression on the CD16 bright subset of monocytes and disease progression rate in ALS ($r = 0.4499$, $p = 0.0098$; $n = 32$).

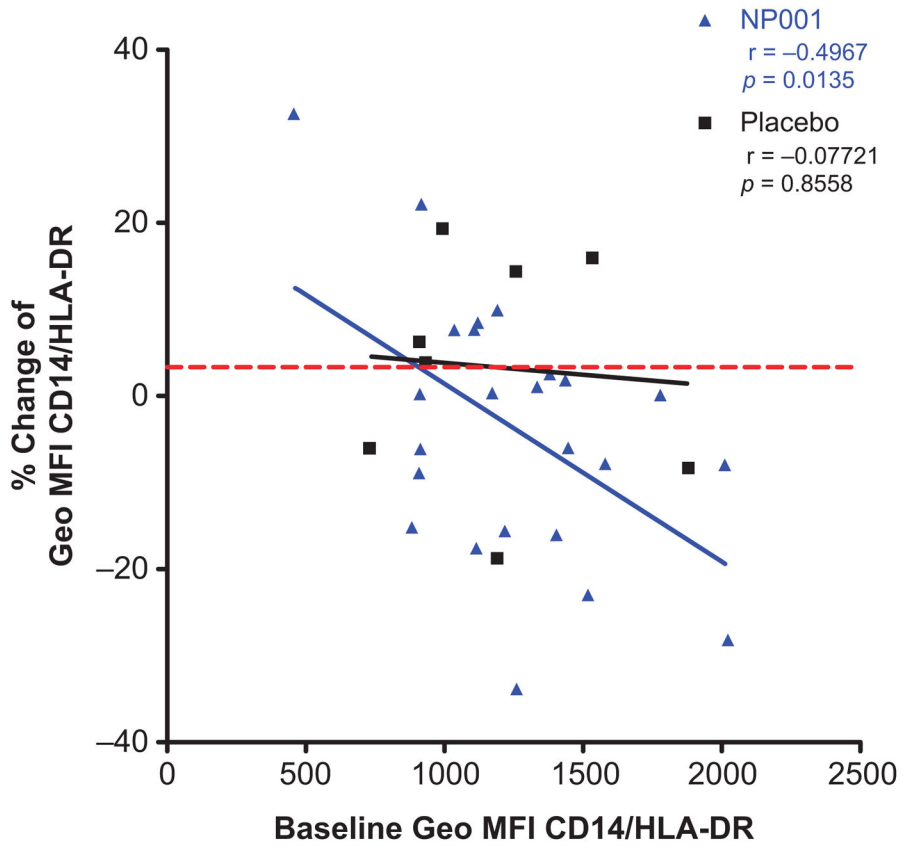

Figure 2.

NP001 treatment changes CD14 monocyte expression of HLA-DR as a function of the degree of monocyte HLA-DR expression at baseline. The x-axis represents the baseline values of the geometric mean fluorescence intensity of monocyte HLA-DR expression (Geo MFI CD14/HLA-DR). The y-axis represents the percent change from baseline in total monocyte HLA-DR expression. The red line represents the mean percentage change of HLA-DR expression on monocytes from eight placebo patients; the black boxes and line represent the actual individual change from placebo group ($r = -0.07721$, $p = 0.8558$; $n = 8$). The blue triangles and line represent the change in monocyte HLA-DR expression after NP001 independent of dose ($r = -0.4967$, $p = 0.0135$; $n = 24$).

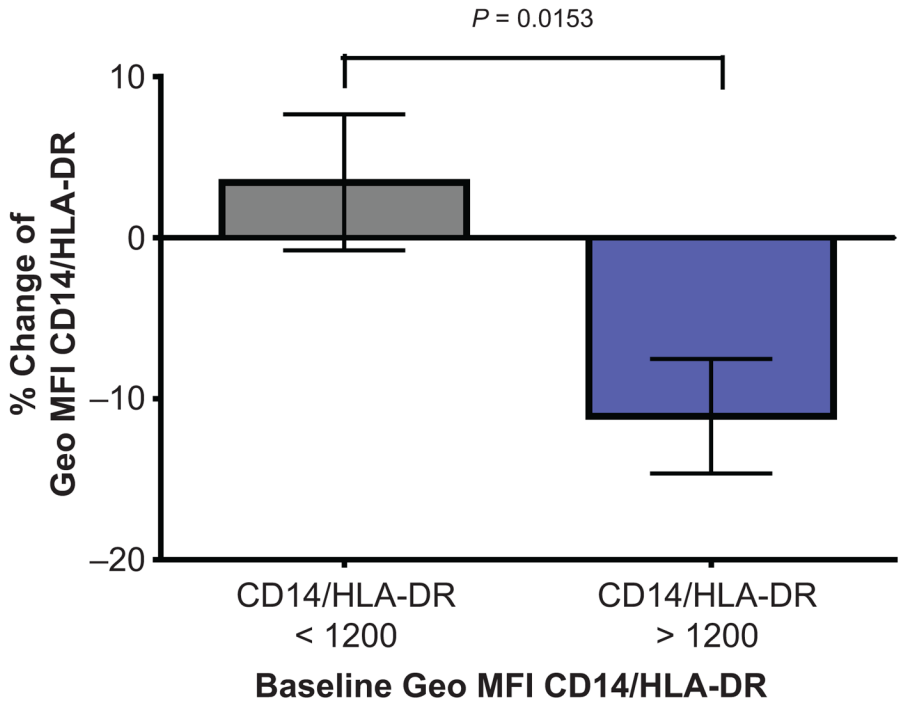

Figure 3.

Comparison of NP001 treatment response between ALS patients with elevated levels of baseline monocyte HLA-DR and those with lower range of baseline monocyte HLA-DR. Patients treated with NP001 were divided into two groups based on the median value of baseline monocyte HLA-DR (Geo MFI CD14/HLA-DR = 1200) from the entire group of all 32 patients enrolled in the phase I clinical study. Baseline Geo MFI CD14/HLA-DR was clustered into two groups as shown on the x-axis (Geo MFI CD14/HLA-DR > 1200, $n = 12$; Geo MFI CD14/HLA-DR < 1200, N = 12). The y-axis represents the percent change in monocyte geometric mean levels of HLA-DR at 24 h compared to baseline. Positive values show an increase in HLA-DR expression and negative values show a relative decrease in HLA-DR expression.

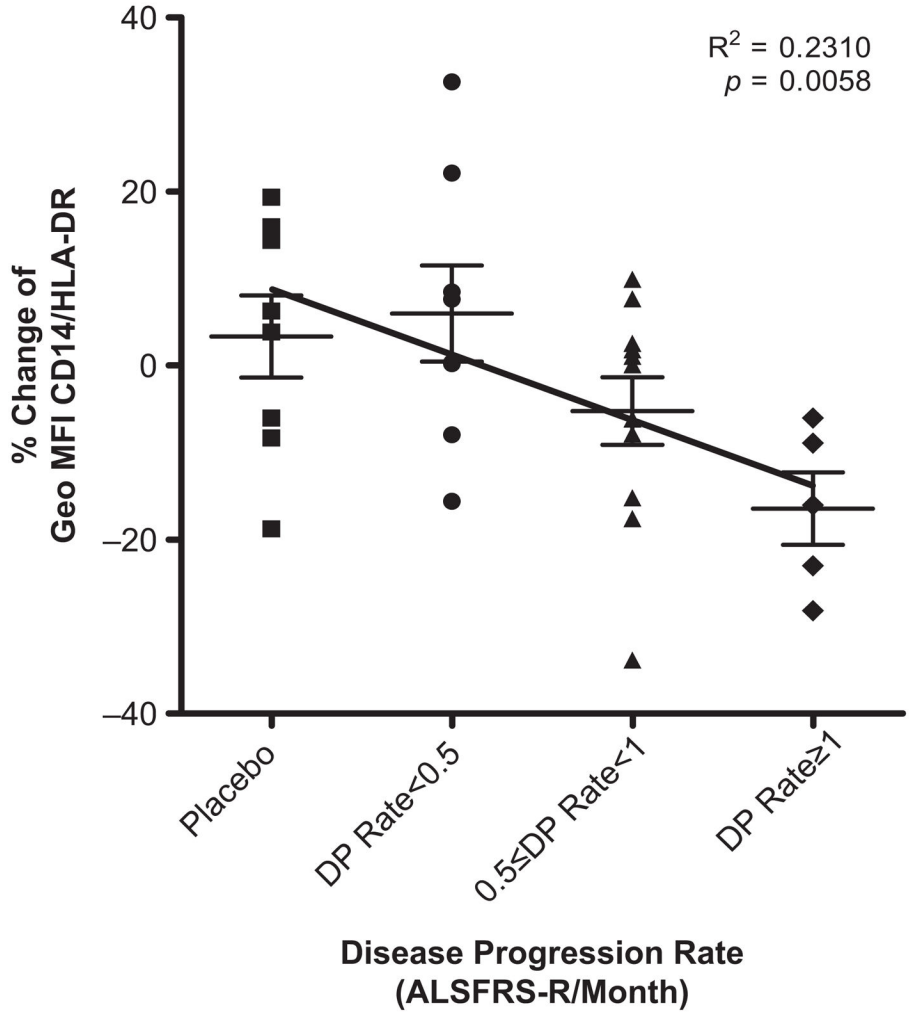

Figure 4.

Greatest change in monocyte levels of HLA-DR in ALS patients with the highest rate of disease progression. Patients in the phase I trial were clustered into subgroups based on their historic rate of ALS disease progression, assessed by average monthly change on ALSFRS-R (DP Rate < disease progression rate) and compared to placebo group ($n = 8$). DP rates were clustered into three groups as shown on the x-axis (DP Rate < 0.5, $n = 8$; DP Rate between 0.5 and 1, $n = 11$; DP Rate ≥ 1, $n = 5$). The y-axis represents the percent change in monocyte geometric mean levels of HLA-DR at 24 h compared to baseline. Positive values show an increase in HLA-DR expression and negative values show a relative decrease in HLA-DR expression. $R^2 = 0.2310$, $p = 0.0058$, one-way ANOVA followed by post-test for linear trend.

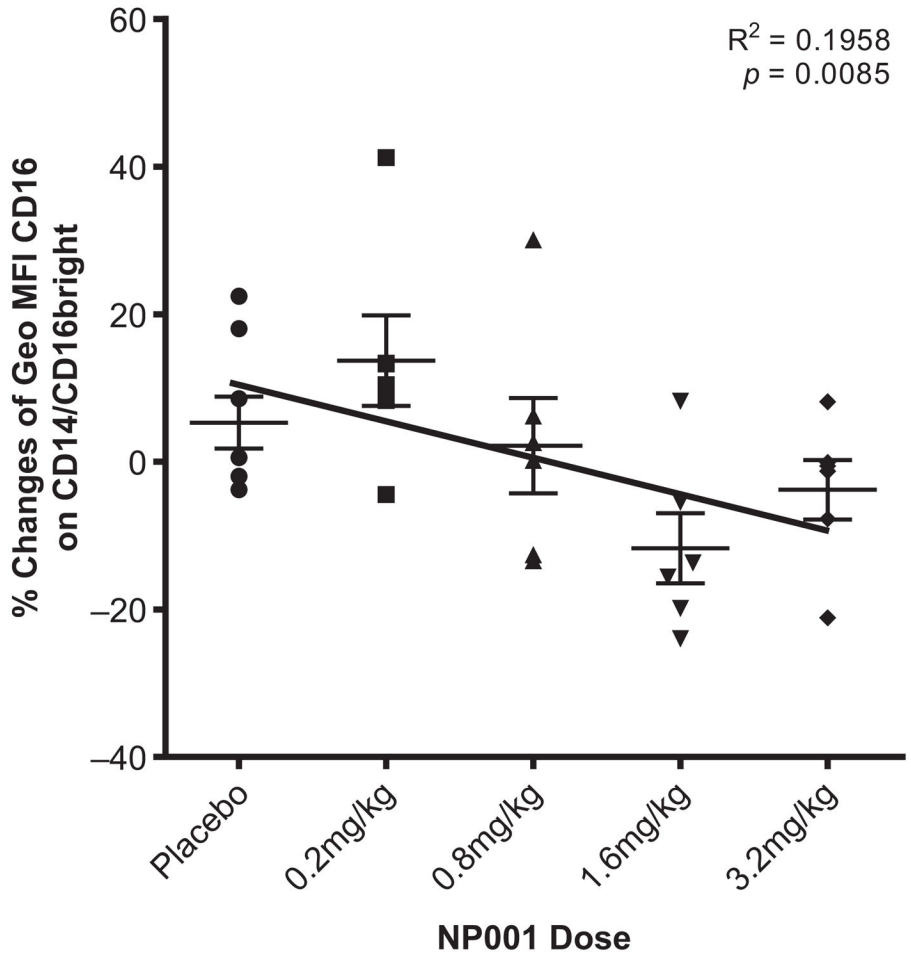

Figure 5.

NP001 induced changes from baseline on CD16 levels expressed on a CD16 bright subset of monocytes in a dose-dependent manner. ALS patients treated with a single dose of NP001 or placebo had baseline values of monocyte CD16 expression compared with the same measurement obtained 24 h after dosing. The percent change in CD16 level expressed on a CD16 bright subset of monocytes 24 h after dosing is plotted on the y-axis. Placebo ($n = 8$) and dose levels ($n = 6$ for each dose) are plotted on the x-axis. $R^2 = 0.1958$, $p = 0.0085$, one-way ANOVA followed by post-test for linear trend.

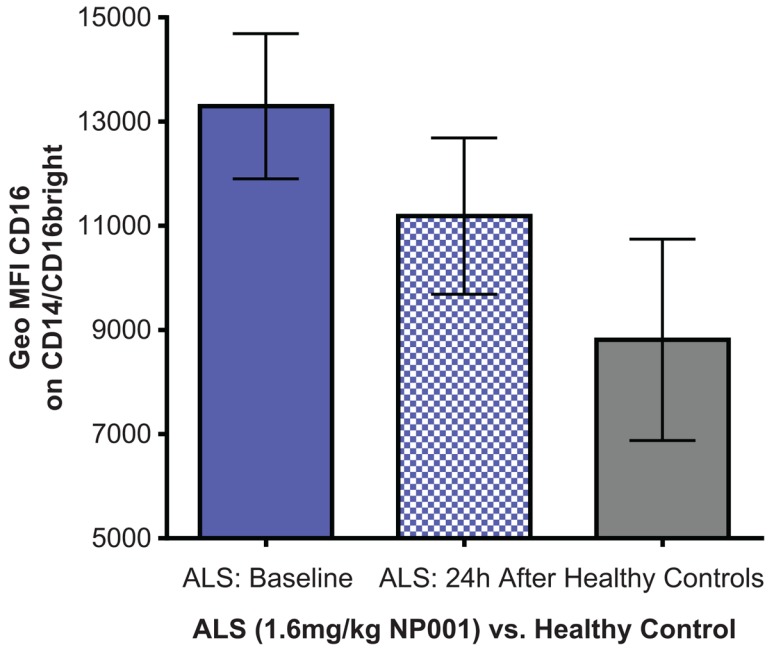

Figure 6.

Comparison of CD16 expression on monocyte CD16 bright subset in patients receiving 1.6-mg/kg dose NP001 relative to healthy controls. The left and middle bars represent mean levels of CD16 expression on ALS patient CD16 bright monocytes at baseline (left) and 24 h after NP001 infusion (middle) ($n = 6$). The bar on the right represents mean level of CD16 expression typically seen in healthy controls ($n = 7$).

Table I

Baseline ALS characteristics (safety analysis population).

Variable	Placebo (*n* = 8)	NP001 0.2 mg/kg (*n* = 6)	NP001 0.8 mg/kg (*n* = 6)	NP001 1.6 mg/kg (*n* = 6)	NP001 3.2 mg/kg (*n* = 6)	All NP001 doses (*n* = 24)
Duration of ALS Symptoms (months); *n* =	8	6	5	6	5	22
Mean (STD)	24.7 (15.7)	22.4 (24.1)	32.5 (21.3)	21.3 (9.5)	21.0 (10.5)	24.1 (17.0)
Median	19.1	14.5	28.9	18.2	24.8	19.4
Type of ALS (*n* (%))						
Familial	0 (0.0)	1 (16.7)	1 (16.7)	0 (0.0)	0 (0.0)	2 (8.3)
Sporadic	8 (100.0)	5 (83.3)	5 (83.3)	6 (100.0)	6 (100.0)	22 (91.7)
Site of ALS onset (*n* (%))						
Bulbar	2 (25.0)	3 (50.0)	2 (33.3)	0 (0.0)	3 (50.0)	8 (33.3)
Bulbar and Limb	0 (0.0)	0 (0.0)	0 (0.0)	2 (33.3)	0 (0.0)	2 (8.3)
Limb	6 (75.0)	3 (50.0)	4 (66.7)	4 (66.7)	3 (50.0)	14 (58.3)
El Escorial ALS criteria (*n* (%))						
Definite	3 (37.5)	2 (33.3)	2 (33.3)	3 (50.0)	1 (16.7)	8 (33.3)
Probable	5 (62.5)	4 (66.7)	4 (66.7)	3 (50.0)	5 (83.3)	16 (66.7)
Riluzole use (*n* (%))						
No	3 (37.5)	2 (33.3)	1 (16.7)	2 (33.3)	3 (50.0)	8 (33.3)
Yes	5 (62.5)	4 (66.7)	5 (83.3)	4 (66.7)	3 (50.0)	16 (66.7)
ALSFRS-R score at baseline; *n* =	8	6	6	6	6	24
Mean (STD)	34.8 (5.2)	34.5 (7.0)	31.0 (7.8)	30.2 (7.7)	38.0 (5.2)	33.4 (7.3)
Median	34.0	37.0	30.5	31.0	39.0	34.5
Vital capacity (l) at baseline; *n* =	8	6	6	6	6	24
Mean (STD)	3.6 (1.6)	2.1 (1.6)	2.6 (1.5)	3.3 (0.8)	3.2 (0.8)	2.8 (1.3)
Median	3.9	2.4	2.8	3.1	3.1	3.1

Table II

Summary of treatment-emergent adverse events that occurred in ≥ 2 subjects in the All NP001 doses or Placebo groups (safety analysis population).

System Organ Class preferred term	Placebo (*n* = 8)	NP001 0.2 mg/kg (*n* = 6)	NP001 0.8 mg/kg (*n* = 6)	NP001 1.6 mg/kg (*n* = 6)	NP001 3.2 mg/kg (*n* = 6)	All NP001 doses (*n* = 24)
Subjects with a TEAE that occurred in ≥ 2 subjects in the All NP001 doses or Placebo groups	1	2	1	2	2	7
Fall	0	1	1	0	2	4
Contusion	0	1	0	1	0	2
Facial pain	0	1	0	0	1	2
Fatigue	1	1	0	1	0	2