Trial of Celecoxib in Amyotrophic Lateral Sclerosis

Merit E. Cudkowicz, MD, MSc,1,2 Jeremy M. Shefner, MD, PhD,3 David A. Schoenfeld, PhD,4 Hui Zhang, MSc,5 Katrin I. Andreasson, MD,3 Jeffrey D. Rothstein, MD, PhD,5 Daniel B. Drachman, MD,5 and the Northeast ALS Consortium

Objective: To determine whether chronic treatment with celecoxib, a cyclooxygenase-2 inhibitor that has been shown to be beneficial in preclinical testing, is safe and effective in amyotrophic lateral sclerosis (ALS).

Methods: A double-blind, placebo-controlled, clinical trial was conducted. Three hundred research subjects with ALS were randomized (2:1) to receive celecoxib (800mg/day) or placebo for 12 months. The primary outcome measure was the rate of change in upper extremity motor function measured by the maximum voluntary isometric contraction strength. Secondary end points included safety, survival, change in cerebrospinal fluid prostaglandin E₂ levels, and changes in the rate of decline of leg and grip strength, vital capacity, ALS Functional Rating Scale-Revised, and motor unit number estimates.

Results: Celecoxib did not slow the decline in muscle strength, vital capacity, motor unit number estimates, ALS Functional Rating Scale-Revised, or affect survival. Celecoxib was well tolerated and was not associated with an increased frequency of adverse events. Prostaglandin E₂ levels in cerebrospinal fluid were not elevated at baseline and did not decline with treatment.

Interpretation: At the dosage studied, celecoxib did not have a beneficial effect on research subjects with ALS, and it was safe. A biological effect of celecoxib was not demonstrated in the cerebrospinal fluid. Further studies of celecoxib at a dosage of 800mg/day in ALS are not warranted.

Ann Neurol 2006;60:22–31

Amyotrophic lateral sclerosis is a neurodegenerative disorder characterized by loss of motor neurons in the motor cortex, brainstem, and spinal cord. Median survival is 3 to 5 years and is modestly prolonged by riluzole, an inhibitor of neuronal glutamate release.¹ There are no other known effective pharmacological treatments. The cause of ALS is unknown; however, several lines of evidence suggest that glutamate excitotoxicity, inflammation, and oxidative toxicity may play important roles in the pathogenesis of sporadic and familial ALS.²,³ Recent evidence demonstrates that uptake of neuronally released glutamate by astrocytic transporters is impaired in ALS. In addition to taking up glutamate, astrocytes synthesize and release glutamate. Astrocytic release of glutamate is stimulated by prostaglandins via a calcium-dependent pathway. Released transmitter triggers further astrocytic glutamate release by a positive feedback mechanism and increases neuronal glutamate release.⁴ Prostaglandin synthesis within the central nervous system (CNS) is dependent on the catalytic action of cyclooxygenase-2 (COX-2). Because cyclooxygenase inhibitors markedly reduce astrocytic glutamate release,⁴ they may have a therapeutic effect in ALS. COX-2 activity also results in inflammation and the production of free radicals that may play an important role in the pathogenesis of ALS.⁵

Celecoxib, a 1,5-diaryl-substituted pyrazole derivative containing a sulfonamide substituent, is a US Food and Drug Administration–approved COX-2 inhibitor agent for arthritis that blocks prostaglandin synthesis. Levels of COX-2 messenger RNA and its product prostaglandin E₂ (PGE₂) are increased in transgenic mice with a form of ALS due to mutant superoxide dismutase-1 (SOD1).⁶,⁷ Increased levels of both COX-2 expression⁸ and PGE₂ levels⁶ have been reported in human postmortem spinal cords and in cerebrospinal fluid (CSF) of patients with sporadic ALS.⁶,⁵ We demonstrated that COX-2 inhibition pro-
tects motor neurons in culture from excitotoxic cell death and prolongs survival in the mutant SOD1 mouse model of ALS by 25 to 30%.

Mice treated with celecoxib had reduced levels of spinal cord COX-2 messenger RNA and PGE$_2$ and protection of motor neurons.

Based on these preclinical data, we conducted a controlled clinical trial to determine whether celecoxib slows disease progression and whether it is safe and well tolerated in research patients with ALS.

**Subjects and Methods**

### General Study Design

A randomized, double-blind, placebo-controlled trial of celecoxib in 300 patients with ALS treated for 12 months was performed. The institutional review boards of each participating institution approved the protocol and consent forms. Eligible subjects were randomized at a ratio of 2:1 to receive treatment with celecoxib or placebo. A 2:1 randomization plan was chosen to enhance enrollment and to obtain more safety information on the use of celecoxib for 12 months in subjects with ALS. We used a computer-generated randomization method to assign subjects to celecoxib or placebo treatment, and subjects were stratified by clinical site to ensure that the treatment groups were balanced within each site. All site investigators, coordinators, clinical evaluators, and staff of the coordination and data management center were blind to treatment assignment throughout the study. Study medication, consisting of celecoxib or matching placebo, was given at a dosage of 400mg twice a day (800mg/day). Research subjects who completed the study had the option to receive open-label celecoxib for up to 12 months or until data analysis was complete.

The primary outcome measure was change in the rate of decline of maximum voluntary isometric contraction (MVIC) strength of eight arm muscle groups (bilateral shoulder and elbow flexion and extension). MVIC is a standardized, validated measurement tool developed to characterize disease progression in ALS. An outcome measure that captures muscle strength is of high clinical relevance because a main symptom in ALS is muscle weakness. Secondary end points included the rate of decline of MVIC strength in 10 leg muscle groups (bilateral hip and knee flexion and extension, and bilateral ankle dorsiflexion), grip strength, vital capacity (VC), motor unit number estimates (MUNE), ALS functional rating scale-revised (ALSFRS-R), CSF PGE$_2$ levels, and the safety and tolerability of celecoxib in this population. Survival was also evaluated as a secondary outcome measure. Survival was defined as time to death, tracheostomy, or permanent assisted ventilation. Ventilation was defined as permanent when noninvasive or invasive ventilation was used for more than 22 hours in a 24-hour period for 14 consecutive days. The date of permanent assisted ventilation was the first day of the 14-day period. Prior to any site enrolling subjects into this trial, formal training was provided on all outcome measures (details are provided in the online supplementary material). An independent safety monitoring committee reviewed the safety data approximately every 3 months throughout the study. The study was conducted under an investigator investigational new drug (IND) application (IND #62,176).

### Patient Selection Criteria

Eligible subjects, 18 years or older, had a clinical diagnosis of familial or sporadic ALS, a VC greater than or equal to 60% predicted, and disease duration of no more than 5 years. Upper extremity strength had to be sufficient so that at least four of eight muscle groups could be evaluated using MVIC. Subjects were excluded if they had taken other investigational drugs in the preceding 4 weeks. Subjects were permitted to continue to take riluzole if the dose had been stable for at least 30 days before the baseline visit, but were not allowed to change the dosage of riluzole during the trial.

### Study Procedures

The study design and the informed consent procedure were explained to all prospective subjects. Screening procedures included signing of the consent form, assessment of eligibility criteria, a complete medical history, general physical examination, VC determination, medication review, and a screening safety panel (complete blood count, electrolytes, blood urea nitrogen, creatinine, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, bilirubin, Helicobacter pylori antibody test, stool guaiac, urinalysis, and a pregnancy test [for women only]). Subjects who tested positive for $H. pylori$ received standard antibiotic treatment for $H. pylori$ before the baseline visit. Baseline visit and randomization took place within 14 days after the screening visit.

Before initiation of study medication, subjects were evaluated at the baseline visit using measurements of muscle strength and VC, MUNE (163 subjects at 16 sites only), and completion of ALSFRS-R. Participation in the CSF collection was optional. Subjects who consented underwent a lumbar puncture (LP), with removal of 5ml CSF for testing of PGE$_2$. A second LP was performed at the month 2 visit. The CSF samples were coded and frozen in 1ml aliquots at $-80^\circ$C. At the end of the study, CSF samples were analyzed for PGE$_2$ levels.

### Study Intervention and Follow-up

The study medication consisted of capsules containing either celecoxib or matching placebo supplied by the manufacturer (Pharmacia, Gaithersburg, MD). The celecoxib dosage was 800mg/day, taken orally in two divided doses (capsule size = 200mg). Subjects were instructed to take two capsules in the morning and two in the evening, preferably during or just after a meal. Subjects returned monthly for the first 4 months, then every 2 months for up to 12 months. Assessment of outcome measures was performed every 2 months. At each visit, vital signs, drug accountability, reports of concomitant medications, and reports of adverse events were recorded. Safety laboratory testing was conducted monthly for the first 4 months, then every 2 months throughout the study (complete blood count, electrolytes, blood urea nitrogen, creatinine, serum glutamic-oxaloacetic transaminase, serum glutamic-pyruvic transaminase, bilirubin, and urinalysis). Stool guaiac tests were performed every month. Death, tracheostomy, and permanent assisted ventilation were de-
fined end points for the survival analysis. Serum samples were obtained from all subjects at the screening visit and at the 4- and 8-month visits, and they were stored at $-80^\circ C$ for analysis of celecoxib levels at the end of the study.

**Prostaglandin E$_2$ Analysis**

We assayed PGE$_2$ by a commercial enzyme-linked immunoassay (PGE$_2$ HS correlate-EIA; Assay Designs, Ann Arbor MI). Initial measurements of PGE$_2$ in the CSF samples from ALS patients, using the above method and the Cayman Chemical Prostaglandin E$_2$ method (Cayman Chemical, Ann Arbor, MI), were below the limit of assay detection, requiring a column extraction/concentration step. In other respects, the manufacturer’s methods were followed (details are provided in online supplementary material).

**Sample Size and Statistical Analysis**

The sample size was calculated from the average decline in arm megascore and standard deviation obtained from the recently reported trials of topiramate and creatine in ALS.$^{14,15}$ Power analysis of these data showed that a cohort of 300 subjects (2:1 drug/placebo) studied for 12 months would have an 81% chance of detecting a decrease in the rate of decline of 35% with a 2-sided probability of 0.05. The 2:1 randomization required 12.5% more patients and was factored into the power calculation. In addition, active treatment of 200 subjects for 1 year provides a 95% chance of detecting at least 1 occurrence of any side effect that occurs with a true frequency of 2% or more. Based on the intent-to-treat principle, the data set for analysis included all randomized subjects. Subjects who discontinued therapy were encouraged to complete their remaining study visits until month 12, and results from subsequent visits were used in the analysis. Analysis of the primary and secondary outcome variables used a mixed model analysis of variance. For the analysis of mortality, survival curves were plotted using the method of Kaplan–Meier and tested for a treatment effect using a log-rank test. The survival analysis was adjusted for baseline covariates (Table 1) using a Cox proportional hazards model. Comparison of spinal fluid PGE$_2$ levels at baseline and at treatment month 2 was analyzed with a Wilcoxon test. Further details of the statistical analysis are included in the online supplementary material.

**Results**

**Enrollment, Baseline Characteristics, and Study Compliance**

Between December 2001 and June 2003, 300 subjects with ALS at 27 participating sites were randomized to celecoxib (N = 201) or placebo (N = 99). Ninety (27%) subjects participated in at least one LP. One hundred and sixty-four subjects participated in the open-label follow-up study. Demographic features, clinical variables, and values of primary and secondary outcome variables were comparable between the two groups at baseline (see Table 1). There were no significant differences between the groups. SD = standard deviation; ALS = amyotrophic lateral sclerosis; VC = vital capacity; ALSFRS-R = ALS Functional Rating Scale-Revised; MUNE = motor unit number estimates.

**Table 1. Baseline Characteristics**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Celecoxib (n = 201)</th>
<th>Placebo (n = 99)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age, yr (SD)</td>
<td>54.5 (11.8)</td>
<td>55.0 (12.4)</td>
</tr>
<tr>
<td>Male sex, %</td>
<td>64</td>
<td>67</td>
</tr>
<tr>
<td>Race, % white</td>
<td>90.0</td>
<td>91</td>
</tr>
<tr>
<td>Family history of ALS, %</td>
<td>4.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Mean time since symptom onset, yr (SD)</td>
<td>1.85 (1.16)</td>
<td>1.91 (1.23)</td>
</tr>
<tr>
<td>Mean time since diagnosis, yr (SD)</td>
<td>0.77 (0.78)</td>
<td>0.82 (0.90)</td>
</tr>
<tr>
<td>Mean time from symptom onset to diagnosis, yr (SD)</td>
<td>1.08 (0.87)</td>
<td>1.08 (0.92)</td>
</tr>
<tr>
<td>Limb onset, %</td>
<td>82.1</td>
<td>82.8</td>
</tr>
<tr>
<td>Mean weight, kg (SD)</td>
<td>79.21 (18.16)</td>
<td>80.13 (16.09)</td>
</tr>
<tr>
<td>Mean body mass index (SD)</td>
<td>27.37 (9.71)</td>
<td>26.95 (4.96)</td>
</tr>
<tr>
<td>Taking riluzole, %</td>
<td>67</td>
<td>71</td>
</tr>
<tr>
<td>Mean arm megascore (SD)</td>
<td>0.76 (1.24)</td>
<td>0.83 (1.09)</td>
</tr>
<tr>
<td>Mean leg megascore (SD)</td>
<td>0.62 (1.09)</td>
<td>0.66 (1.04)</td>
</tr>
<tr>
<td>Mean grip megascore (SD)</td>
<td>1.37 (1.45)</td>
<td>1.43 (1.44)</td>
</tr>
<tr>
<td>Mean VC % predicted (SD)</td>
<td>88.39 (16.85)</td>
<td>85.36 (15.13)</td>
</tr>
<tr>
<td>Mean ALSFRS-R score (SD)</td>
<td>42.88 (5.54)</td>
<td>43.24 (5.17)</td>
</tr>
<tr>
<td>Mean MUNE (SD)</td>
<td>55.25 (25.28)</td>
<td>49.93 (28.94)</td>
</tr>
</tbody>
</table>

There were no significant differences between the groups. SD = standard deviation; ALS = amyotrophic lateral sclerosis; VC = vital capacity; ALSFRS-R = ALS Functional Rating Scale-Revised; MUNE = motor unit number estimates.

The mean compliance (assessed by pill counts) was similar in the 2 groups: 94.4 ± 10.9% in the celecoxib group, and 96.4 ± 13.5% in the placebo group ($\rho = 0.20$, t test). After study completion, serum samples from months 4 and 8 visits, taken from a randomly selected cohort of subjects (29 from the placebo group and 49 from the celecoxib group) were analyzed for celecoxib levels. None of the subjects in the placebo group had detectable serum levels of celecoxib at any of the visits. Subjects in the celecoxib group all had measurable drug levels at the months 4 and 8 visits, confirming good compliance with study medication. The average serum levels of celecoxib were 1,286.8 ±
827.5 ng/ml (± standard deviation) at month 4 and 1,211.6 ± 741.1 ng/ml at month 8.

**Efficacy of Celecoxib**

The intent-to-treat analysis showed that arm strength megascores of celecoxib-treated subjects declined at the same rate as megascores of subjects taking placebo (0.09519 ± 0.006 vs 0.09011 ± 0.0084 U/month; \( p = 0.63 \); Table 2; Fig 1A). There was no effect of celecoxib on decline in leg or grip strength, VC percentage predicted, ALSFRS-R score (see Table 2 and Fig 1), MUNE, or tracheostomy-free survival (\( p = 0.59 \), log-rank test). The additional analysis with last observation carried forward imputation of missing data resulted in no significant difference in any outcome measures between the two groups. There was no evidence that the treatment effects varied significantly among the centers or were affected by riluzole use (data not shown).

Baseline VC percentage predicted, ALSFRS, subject age, and time from symptom onset to screening were important predictors of survival (Table 3). Higher baseline VC percentage predicted values, greater baseline ALSFRS-R scores, younger age, and longer time from symptom onset were predictors of longer survival.

**Tolerability and Safety**

Celecoxib was as well tolerated as the placebo. Treatment assignment did not affect the likelihood of study completion (\( p = 0.17 \), log-rank test; hazard ratio, 1.36). There were no differences in the number of dose reductions or dose suspensions in the two treatment groups. The flow of subjects is shown in Figure 2. Two hundred subjects completed the 12-month study. The probability of continuing to take the study medication was similar for the treatment groups (\( p = 0.55 \), log-rank test; hazard ratio, 1.13). A total of 109 subjects from the celecoxib group and 55 from the placebo group elected to enroll in the open-label study. The
open-label study was discontinued on October 19, 2004, when results of the blinded study were analyzed.

There were no differences in frequency of adverse events (serious or nonserious) in the treatment groups. Eighty-five percent of subjects had at least one adverse event (85.6% in celecoxib group and 84.8% in placebo group; \( p = 0.86 \)). Twenty-eight percent of subjects had at least one serious adverse event (28.4% in celecoxib group and 27.3% in placebo group; \( p = 0.89 \)). The most commonly reported adverse experiences in both treatment groups included infection, headache, diarrhea, constipation, depression, nausea, and dyspepsia (see Supplementary Table 1). Of these events, nausea (14.4 vs 7.1%; \( p = 0.09 \)) and dyspepsia (23.9 vs
6.1%; p = 0.08) were slightly more common in the celecoxib-treated group. These adverse events are expected side effects of celecoxib. The most common serious adverse events were respiratory failure, dyspnea, pneumonia, and hospitalization for percutaneous endoscopic gastrostomy. Forty-seven deaths occurred during the study; 33 deaths were in the celecoxib group and 14 were in the placebo group (16.4 vs 14.1%).

During the course of the study, there were no differences between groups in the events of tracheostomy, feeding tube placement, or noninvasive positive pressure ventilation use. Three subjects underwent tracheostomy (1 subject receiving celecoxib and 2 receiving placebo), 60 (20%) underwent feeding tube placement (18% receiving celecoxib and 23% receiving placebo; p = 0.36), and 76 (25%) began treatment with noninvasive positive pressure ventilation (22% receiving celecoxib and 31% receiving placebo; p = 0.12). Vascular events were infrequent and occurred in both treatment groups (2% of celecoxib group and 2% of placebo group). All but one of these subjects had preexisting stable cardiovascular disease or relevant risk factors. The percentage of subjects who developed abnormalities in laboratory safety studies was similar in the two groups for all tests. The most common adverse events in the open-label component of the study were respiratory failure, depression, constipation, dysphagia, and insomnia (see Supplementary Table 2).

**Prostaglandin E2 Levels**

Ninety subjects from 16 sites had a LP at baseline visit. Of these, 63 agreed to a second LP at month 2. Subject choice was the most common reason for not having a second LP. For the PGE2 comparison from baseline to month 2, only CSF levels from subjects receiving study medication who had given two CSF samples (baseline and month 2) were included in the analysis. There was no difference in the PGE2 levels at baseline between subjects receiving celecoxib or placebo. The mean PGE2 level was 12.4 ± 8.9 pg/ml (range, 0.10–64.2 pg/ml) in 62 subjects assigned to celecoxib, and 11.2 ± 5.8 pg/ml (range, 4.13–31.7 pg/ml) in 28 subjects assigned to placebo (p = 0.44). The overall mean PGE2 level in 90 subjects with ALS was 12.0 ± 8.0 pg/ml. There was no difference in the PGE2 levels at baseline between subjects with limb or bulbar disease onset.

Forty-one subjects assigned to celecoxib had repeat LP at month 2; all were taking study drug. Twenty-two subjects assigned to placebo had a second LP, but two of the subjects had discontinued treatment before the second LP. At 2 months, PGE2 levels did not decrease more in subjects assigned to the celecoxib group than in subjects assigned to the placebo group. The mean change in PGE2 level was -1.4 ± 9.0 pg/ml (range, -47.4 to +17.5 pg/ml) in the celecoxib-treated group and -0.7 ± 6.1 pg/ml (range, -22.7 to +9.2 pg/ml) in the placebo-treated group (Fig 3). There was no difference in distribution of change in PGE2 levels (p = 0.46, Wilcoxon test). There were no differences in baseline characteristics between subjects taking celecoxib or placebo who participated in the CSF study. A subset analysis of subjects taking celecoxib who had a decrease in PGE2 levels with treatment (n = 22) compared with those taking celecoxib without a decrease in PGE2 levels (n = 19) did not demonstrate any significant differences in the outcome measures. There was no difference in the rate of decline in the outcome measures between subjects with serum celecoxib levels greater than or equal to 1,110 ng/ml and those with serum celecoxib levels less than 1,110 ng/ml.

**Discussion**

The main outcome of this carefully designed and executed controlled trial is that treatment of ALS patients receiving celecoxib at the dosage given did not result in a beneficial effect on any of the disease parameters measured. Neither the primary outcome measure, a change in the rate of decline of arm strength over time, nor any of the other parameters measured, including leg and grip strength, VC, ALSFRS-R, MUNE, and survival, differed in the celecoxib-treated group compared with the placebo control group. Moreover, the treated patients had no more adverse effects than the control subjects. Thus, celecoxib neither helped nor hurt patients with ALS.

![Fig 3. The prostaglandin E2 (PGE2) levels at baseline and month 2 are shown. There was no treatment effect on this measurement. The regression line for the celecoxib (Celebrex) treatment group (plus signs) is shown as a solid line; the placebo group (open diamonds) regression line is a dashed line. CSF = cerebrospinal fluid.](image-url)
It is worth analyzing this disappointing result to consider possible explanations why it may have failed, and what can be done in future trials. First, it should be emphasized that the underlying rationale, as well as the experimental evidence from preclinical studies, strongly supported testing celecoxib treatment in patients with ALS. Celecoxib is a potent and selective inhibitor of the enzyme COX-2. Inhibition of COX-2 interferes with two processes thought to play important roles in the pathogenesis of neuronal damage in ALS: glutamate-induced excitotoxicity and inflammation. COX-2 has been shown to be present in the CNS in neurons and astrocytes, as well as in macrophages and microglia. COX-2 catalyzes the synthesis of prostaglandins from arachidonic acid and also plays a pivotal role in inflammatory processes in the CNS. Prostaglandins, especially PGE2, stimulate the release of glutamate by astrocytes, via a calcium-dependent pathway. Inhibitors of COX have been shown to reduce astrocytic glutamate release markedly and should therefore counteract the role of this major source of the excitotoxic neurotransmitter glutamate. Another mechanism by which COX-2 inhibitors may be protective in ALS is by interruption of inflammatory processes that cause the production of reactive oxygen species, free radicals, proinflammatory cytokines, and other toxic molecules. Importantly, reported studies of postmortem spinal cords of 29 patients who died of ALS, and of mice with the mutant SOD1 G93A transgenic form of ALS, showed markedly increased levels of messenger RNA for COX-2, COX-2 protein, and/or increased levels of PGE2.

Experimental studies showed that inhibition of COX-2 protected motor neurons from excitotoxic damage in an organotypic spinal cord culture model of ALS. In this model, threohydroxyaspartate added to the spinal cord cultures inhibits uptake of glutamate by astrocytes, resulting in excitotoxicity and progressive loss of motor neurons. Inhibition of COX-2 in this system prevented motor neuron loss. Although this beneficial effect may be due to prevention of prostaglandin synthesis, there is evidence that prostaglandins may be “double agents” and may also exert a protective role in this model system (see later).

Transgenic mice with human mutant SOD1 exhibit progressive weakness and death due to loss of motor neurons. Histopathological study of their spinal cords demonstrates astrocytic and microglial activation typical of ALS. Treatment of these mice with celecoxib delayed the onset of weakness and weight loss, prolonged survival by greater than 25%, and diminished astrogliosis and microglial activation. Celecoxib treatment also inhibited the production of PGE2 in the spinal cords of these mice. The observation that COX-2 inhibitors have beneficial effects in SOD mutant transgenic mouse models of ALS has been confirmed in several laboratories using celecoxib, rofecoxib, or nimesulide. Of interest, when rofecoxib was administered only three times per week, the beneficial effect was less pronounced than in studies where it was fed continuously, and the spinal cord PGE2 levels were not reduced.

Given this rather extensive body of positive information, we anticipated at least some beneficial effect in the human trial; the negative results led to reexamination of a number of the theoretical and practical factors in the trial.

First, dosage was reexamined. We wondered whether the dosage of celecoxib used to treat the subjects was comparable with the effective dose in the mouse model. The average dosage of celecoxib ingested by 20gm mice, eating 3 to 4gm chow per day containing 1,500 parts per million of celecoxib, was approximately 4.5 to 6mg/day or 225 to 300mg/kg body weight per day. Although this dosage is more than 20-fold greater than the 800mg/day dosage given to a patient of average weight, there is evidence that the metabolism of celecoxib is much more rapid in mice than in humans (Peter Isakson and Walter Smith, personal communications). Plasma levels of celecoxib in mice fed the chow ad libitum were 0.68 to 1.35µg/ml, which is similar to the serum levels in our celecoxib-treated subjects at 4 (1.28µg/ml) and 8 months (1.21µg/ml). Thus, the dosage used in this study produced circulating levels of celecoxib comparable with those in the mouse studies. From a practical point of view, we used the maximum dosage of celecoxib (800mg/day) that was approved by the US Food and Drug Administration for various indications, whereas higher doses would have posed problems in undertaking the treatment trial.

Second, pharmacological action in the CNS was reexamined. Celecoxib is lipophilic, and it has been shown to penetrate the CNS in rodents and humans. Its penetration into the CNS of transgenic SOD mutant mice was sufficient to inhibit PGE2 production in the spinal cord. To determine whether celecoxib treatment was effective in inhibiting COX-2 in the CNS of subjects with ALS in this study, we sought to use CSF levels of PGE2, a main product of the enzymatic activity of COX-2, as a surrogate measure of the pharmacological effect of celecoxib treatment. Based on two previous studies, we expected that the PGE2 levels would be elevated in the CSF of ALS subjects and anticipated that effective inhibition of COX-2 in the CNS should result in reduction of PGE2 in the CSF. However, there was no reduction in the PGE2 levels in patients treated with celecoxib, and an additional set of 10 CSF samples from ALS patients and 10 disease control patients did not show significant differences in levels of PGE2 (data not shown). The discrepancy in results between our subject group...
and those previously reported remains unexplained, but may, in part, be due to a change in the assay kit currently available, or to the larger sample size in our study (n = 90) compared with those of the two previous studies (n = 17 and 9, respectively). Our measurements (performed in K.I.A.’s prostaglandin laboratory) indicated that the PGE₂ levels in CSF from ALS and control patients were below the limit of detection in both commercially available assay platforms that are used for this purpose (Prostaglandin E₂-monoclonal EIA [Cayman Chemical] and PGE₂ HS correlate-EIA [Assay Designs]). This necessitated concentration of our CSF samples for measurement. Moreover, there was no reduction of PGE₂ levels in subjects treated with celecoxib for 2 months compared with their pretreatment levels, or with placebo control subjects. Because the pretreatment PGE₂ levels were not elevated in our large sample of patients, COX-2 inhibition would not be expected to result in further lowering of the levels. This leaves open whether celecoxib treatment actually exerted its pharmacological effect of inhibiting COX-2 in the CNS, which is the most problematic aspect of this study. That we did not find elevated levels of PGE₂ in the CSF samples from our groups of patients with ALS may also suggest that the degree of inflammation at the stage of the disease studied here may not be dramatically increased, or that the CSF levels of PGE₂ do not necessarily reflect COX-2 activity in the CNS.

Third, the relevance of preclinical models was reexamined. Although both the “excitotoxicity” model using an organotypic spinal cord culture system and the transgenic mutant SOD1 mouse model showed marked beneficial responses to COX-2 blockade, it is not clear how well these models predict the effect of treatment in humans with ALS. As is the case with celecoxib, another intervention, creatine, which significantly slowed the disease course in the transgenic mouse model, failed to alter the course of disease in human patients with sporadic ALS when administered at 5 and 10gm/day. In the mouse studies, treatment was begun before the onset of weakness, whereas patients were of necessity treated after onset, when the disease is established. Moreover, the course of the genetic disease in transgenic mice is reasonably predictable, whereas that of humans with sporadic ALS is much more variable, and indeed, the clinically defined disease may encompass a variety of different disorders. Currently, the decision to test a treatment in ALS remains based on a rational hypothesis and supportive preclinical data. New developments in high-throughput models for testing potential therapeutic agents are in progress. A number of promising therapeutic agents being studied in high-throughput screens have already been studied in the ALS transgenic mice. The value of high-throughput screening may also lie in establishing more potent drugs that inhibit already established pathways of neurodegeneration.

Fourth, the role of PGE₂ in pathogenesis of ALS was reexamined. The rationale for a pathogenic role of PGE₂ in ALS is outlined earlier. However, prostaglandins (PGE₂ and prostaglandin D₂) may also have a paradoxical neuroprotective function, which has been demonstrated in the in vitro “excitotoxic” model of ALS and in models of stroke. It is therefore theoretically possible that PGE₂ production could actually be protective in ALS, whereas inhibition might be harmful. However, the celecoxib-treated patients in our study did not have a more rapidly progressive course than the control patients, largely discounting this possibility.

Fifth, trial design and execution was reexamined. The design of this study used the measures of disease progression that have been shown to be most reliable in previous studies of ALS, and they were conducted rigorously and accurately. This study was powered to detect a 35% decrease in the rate of decline of arm strength with a probability of 81%. In the mouse model, celecoxib treatment resulted in approximately a 25% prolongation of survival, and a smaller delay in onset of reduced rotarod function. However, that neither the primary outcome measure nor any of the secondary measures showed even a trend toward benefit makes it highly improbable that an important therapeutic effect was missed.

Currently, we cannot definitively explain the failure of celecoxib to benefit patients with ALS. The most important gap in our understanding of this negative result is the lack of evidence whether the experimental treatment actually inhibited COX-2 in the CNS. In future studies of therapy, it will be important to have a more robust measure of the pharmacological action of the therapeutic agent. The negative results of this trial, as well as the trial of creatine in ALS, raise questions concerning the reliability of the transgenic SOD1 mutant mouse as a test model for therapy of ALS. Metabolism of the therapeutic agent, time of administration in the course of the disease process, and penetration into the relevant part of the nervous system may differ in mice and humans. Although many of the pathological processes that occur in sporadic human ALS are replicated in the transgenic mouse model, differences clearly make it difficult to generalize results from mouse to human. Indeed, the problem of access of therapeutic agents to motor neurons and the surrounding CNS poses an important hurdle in the treatment of ALS. Finally, the magnitude of the therapeutic effect presents a challenge in powering a human trial. Based on the available data, we calculated that a change in the rate of decline of just 35% required a major trial involving 300 patients in 27 institutions treated for 1 year. This means that a modest pilot trial would have
to be remarkably effective to show a definite benefit. The design of future trials of potential therapeutic agents in ALS should consider these concerns.

Disclosure
M.E.C., D.B.D., and K.I.A. have received grant support in excess of $10,000 from Pfizer.

This research was supported by Pharmacia and Pfizer, (M.E.C., D.B.D.) the Muscular Dystrophy Association, (M.E.C., D.B.D.) and General Clinical Research Centers (M01 RR01032, M01-RR-01066, M01 RR01346, M01RR06192, M01RR02602, MO1RR00109, MO1-RR07122, MO1RR00036).

We thank Drs G. McKhann, C. Leventhal, and M. McDermott for participation on the Safety Monitoring Committee.

References