Published in final edited form as:
Lancet Neurol. 2013 May ; 12(5): 435–442. doi:10.1016/S1474-4422(13)70061-9.

A Phase I, Randomised, First-in-Human Study of an Antisense Oligonucleotide Directed Against SOD1 Delivered Intrathecally in SOD1-Familial ALS Patients

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Abstract

Objective—To evaluate the safety, tolerability, and pharmacokinetics of an antisense oligonucleotide designed to inhibit SOD1 expression (ISIS 333611) following intrathecal administration in patients with SOD1-related familial amyotrophic lateral sclerosis (ALS).

Background—Mutations in SOD1 cause 13% of familial ALS. In animal studies, ISIS 333611 delivered to the cerebrospinal fluid (CSF) distributed to the brain and spinal cord, decreased SOD1 mRNA and protein levels in spinal cord tissue, and prolonged survival in the $SOD1^{G93A}$ rat ALS model.

Methods—In a randomized, placebo controlled Phase 1 trial, ISIS 333611 was delivered by intrathecal infusion using an external pump over 11.5 hours at increasing doses to four cohorts of eight SOD1 positive ALS subjects (randomized 6 drug: 2 placebo/cohort). Subjects were allowed

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Conflict of interest statements: KA, DN, KB, CFB are employees of Isis Pharmaceuticals. Isis Pharmaceuticals provided support for this trial and provides antisense oligonucleotides to TMM for animal studies. RS, CFB, Center for Neurologic Study, Isis Pharmaceuticals have filed a patent for use of the antisense oligonucleotide described here.

Author contributions: TMM, MEC obtained funding from ALSA, MDA. TMM, CFB, MEC, KB, RS designed study, AP, DW, JR, ES enrolled subjects, AP, DW, JR, ES, TMM, MEC, LWO, KM, PA, KA, KB, SA participated in study conduct; TM, CFB, MEC, KB, DS, EM, DN analyzed data; LWO, GM, MC analyzed subject tissues; TM, CFB, MEC, KB analyzed data and wrote the manuscript; TM, AP, WD, JR, ES, KM, PA, KB, LWO, EM, DN, GM, MC, RS, CFB, MEC edited manuscript.

to re-enroll in subsequent cohorts. Safety and tolerability assessments were made during the infusion and periodically over 28 days following the infusion. CSF and plasma drug levels were measured.

Findings—No dose-limiting toxicities were identified at doses up to 3.0 mg. No safety or tolerability concerns related to ISIS 333611 were identified. There were no serious adverse events (AEs) in ISIS 333611-treated subjects. Re-enrollment and re-dosing of subjects with ISIS 333611 was also well tolerated. Dose-dependent CSF and plasma concentrations were observed.

Interpretation—In this first clinical study to report intrathecal delivery of an antisense oligonucleotide, ISIS 333611 was well tolerated when administered as an intrathecal infusion in subjects with SOD1 familial ALS. CSF and plasma drug levels were consistent with levels predicted from preclinical studies. These results suggest that antisense oligonucleotide delivery to the central nervous system may be a feasible therapeutic strategy for neurological disorders.

Source of funding—ALS Association, Muscular Dystrophy Association, Isis Pharmaceuticals

INTRODUCTION

In the last 20 years substantial progress has been made in our knowledge regarding the genetic bases of many neurodegenerative diseases. Causative mutations for Huntington's disease, spinal muscular atrophy, spinal and bulbar muscular atrophy, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS) have been identified [1]. A remaining challenge is turning this knowledge of the genetic basis of these diseases into effective therapies. For dominantly inherited disorders where data suggest a toxic gain of function of the mutant protein, lowering levels of the protein is a potential approach and antisense oligonucletoides (ASOs) is one means of doing so.[2] ASOs are short, synthetic nucleic acids that have been chemically modified to increase stability in biologic fluids and increase potency in engaging the mRNA target. One mechanism by which ASOs function is by binding to a specific target mRNA through Watson-Crick base-pairing and causing the destruction of that mRNA by activation of the nuclear enzyme RNase H[3]. Since ASOs do not cross the blood brain barrier,[4] they must be delivered directly to the CNS for the potential treatment of neurodegenerative diseases. One possible approach is to deliver them intrathecally, into the cerebral spinal fluid (CSF), which results in widespread delivery of the ASO to the CNS (Supplemental Table 3, 4) [4-6].

Genetic changes in over 10 different genes are known to cause familial ALS, an adult onset neurodegenerative disease characterized by loss or dysfunction of both upper and lower motor neuron pathways[7] and in some cases dementia. Mutations in superoxide dismutase 1 (SOD1) account for approximately 2% of all ALS cases. Although mutations in SOD1 were identified almost 20 years ago, there are no therapies that substantially slow either the sporadic or SOD1-linked forms of ALS. Individual SOD1 mutations are associated with varying age of onset and progression rates of disease, though all share a high penetrance and nearly all are inherited dominantly[8]. The toxicity of SOD1 is secondary to a gain of toxic function rather than a loss of enzymatic function of the SOD1 enzyme, thus reducing levels of the mutant protein is predicted to slow progression in SOD1-linked ALS[7]. Evidence for the utility of ASOs for the treatment of neurodegenerative diseases such as ALS include: 1) widespread distribution of antisense oligonucleotides throughout the CNS following CSF administration; 2) reduction of SOD1 mRNA and protein in the brain and spinal cord tissues; and 3) prolongation of survival in an $SOD1^{G93A}$ animal model of ALS, following directed delivery to the CSF. The ASO drug used in these studies was ISIS 333611, an ASO that has been previously demonstrated to effectively reduce the expression of wild-type and mutant human SOD1 protein in transgenic rats and human cells. [4, 9]

The initial development of ISIS 333611 intended the drug to be delivered long-term by continuous intrathecal infusion using an implantable pump. The purpose of the present first-in-human study was to assess the safety/tolerability and pharmacokinetics of a 'single dose' (i.e. an 11 hour 22 minute infusion) of ISIS 333611in patients with SOD1 familial ALS. In this report, we document the results of the first clinical study to examine the effects of delivering an ASO directly into human CSF as a treatment for a widespread disorder of the CNS.

METHODS

Study Participants

A placebo controlled, double-blind, randomized, dose escalation Phase 1 study was carried out at four centers in the USA (Washington University in St. Louis, Massachusetts General Hospital, Johns Hopkins University, and Methodist Neurological Institute). A total of 21 participants, studied 32 times, were enrolled between March, 2010 and December, 2011. Patients were eligible if they were 18 years of age or older, had documentation of a genetic mutation in SOD1, exhibited clinical signs of weakness attributed to ALS, had a forced vital capacity (FVC) ≥ 50% of their predicted value, were not using invasive respiratory support, and were medically able to undergo insertion of a temporary intrathecal catheter. An FVC>50% cut off was used because ALS patients with FVC>50% are known to have fewer complications with minor procedures. [10] Participants who were taking riluzole had to have been on a stable dose for at least 30 days prior to Study Day 1 and had to remain on that dose throughout their participation in the study. Patients were ineligible if they were treated with an investigational drug for ALS within 30 days or 5 drug half-lives of screening, had clinically significant abnormalities in laboratory values (including coagulation parameters), or had a medical condition that would interfere with the conduct and assessments of the study.

The study was initiated after approval from the IRBs of the participating study centers and was carried out in accordance with the Declaration of Helsinki and the International Conference on Harmonization Good Clinical Practice guidelines. All study participants provided written informed consent prior to their participation. A data safety monitoring board (DSMB) monitored the trial. This study was registered with Clinicaltrials.gov, number NCT01041222.

Enrollment

Participants were enrolled sequentially in four cohorts of eight patients to receive escalating single doses of ISIS 333611 (0.15 mg, 0.5 mg, 1.5 mg, 3 mg). Within each cohort, patients were randomized 3:1 to ISIS 333611 or placebo. An approved protocol amendment instituted after completion of the first dose level allowed participants to re-enroll in multiple cohorts if >60 days had elapsed since they were dosed previously and they re-qualified for the study. Re-enrolling patients were re-randomized without regard to prior assignment. After the participants were considered eligible for the study (provided informed consent and met all inclusion/exclusion criteria), they were sequentially allocated unique subject identification numbers and randomized to receive ISIS 333611. ISIS 333611 (provided in sterile, unpreserved, buffered saline solution at 20 mg/mL, pH=7.4) and the placebo/diluent (sterile, phosphate-buffered saline) were provided by Isis Pharmaceuticals. ISIS 333611 was diluted to the appropriate concentration to achieve each desired dose and used within 24 hours of preparation.

Randomization and masking

Study group randomization was performed using **WebEZ™**, an independent, centralized web-based randomization system (Almac Clinical Services). Randomization occurred in blocks of 4 sequential subjects. Randomization codes were accessed by the site pharmacist on the day of drug preparation. Blinded, pprepared study drug or placebo was labeled only with the subject ID prior to transport to the treatment room and delivery to the study staff. Patients, caregivers, investigators (including other staff at participating institutions), and Isis Pharmaceuticals personnel were masked to treatment allocation throughout the study. Treatment assignment was known only to the pharmacist at each site, the study DSMB, and a central unblinded biostatistician who provided statistical support to the DSMB. No events required premature unmasking of patient allocation or unblinding of study data.

Procedures

The study drug was given as a single intrathecal infusion on study Day 1. ISIS 333611 or placebo (0.25 mL volume) was infused intrathecally for 11 hours and 22 minutes using a Smiths Medical MD, Inc. CADD-MS™ 3 syringe-based ambulatory infusion pump equipped with a Smiths Medical MD, Inc. 3 mL Medication Cartridge via a Codman FlexTip® Plus intraspinal catheter. 11 hours, 22 minutes was chosen to slowly deliver the exact volume (0.25 mL) within the programming features of the pump. This volume/timing was chosen to approximate ½ day of dosing, assuming chronic, continuous infusion of 0.5 mL/day in future clinical trials. The tip of the intrathecal catheter was placed near the T8-10 spinal level under fluoroscopic guidance via a lumbar puncture (LP) using a 17G Tuohy needle inserted into the L3/L4 space, although the protocol allowed placement at an alternative level if patient anatomy or clinical judgment dictated. A pre-infusion CSF sample was obtained for pharmacokinetic analyses and SOD1 protein levels, via the catheter placement needle. At the end of the infusion, the catheter was immediately removed and a post-infusion CSF sample obtained via a separate lumbar puncture one segment above or below the catheter placement site.

Outcomes

Our primary objective was to assess the safety and tolerability of ISIS 333611 given as a single intrathecal infusion in patients with SOD1 familial ALS. Secondary objectives were to evaluate drug CSF and plasma pharmacokinetics following intrathecal delivery. Autopsy was not included in the protocol, but was obtained through a separate existing protocol, Spinal cord tissue samples were obtained from a $SOD1^{A4V}$ patient who participated in Cohorts 3 and 4 of the study and who died from ALS-related causes approximately three months after their participation in the study. This autopsy sample allowed direct assessment of drug levels and SOD1 protein concentrations in this patient. For comparison, SOD1 protein concentrations were measured in cervical and lumbar spinal cord from six non-trial subjects (three with SOD1 familial ALS and three with sporadic ALS).

Safety assessments included the collection of adverse events, physical and neurological examinations, vital signs, clinical laboratory tests (hematology, clinical chemistry, complement, coagulation), ECGs, ALS functional rating scale-revised (ALSFRS-R) assessments, forced vital capacity (FVC), and recording of use of concomitant medications. Based upon preclinical findings with high exposures to the drug, we carefully monitored for signs of cerebellar dysfunction. Safety assessments were performed on Study Day 1 pre- and post-infusion, Study Day 2, Study Day 8, and Study Day 29. Patients were also monitored on Study Day 15 by a phone call. Safety information was reviewed by the DSMB following completion of Study Day 8 of the last member of each cohort to provide a dose escalation recommendation.

ISIS 333611 levels were measured in plasma at 13 time points from pre-infusion to 12 hours post-infusion and in CSF pre-infusion and immediately post-infusion. ISIS 333611 concentrations were determined using a modification of the hybridization ELISA method[11]. The method was validated for human plasma and CSF in accordance with current standard practice for immunobinding assays by PPD Bioanalytical (Wilmington, NC). The assay is accurate, precise, reproducible, sensitive and selective for ISIS 333611 quantification. Stability of ISIS 333611 in a frozen matrix (up to 3 months) and up to five freeze-thaw cycles was also confirmed.

While the single dose of ISIS 333611 given in this study was not predicted to be efficacious, we collected preliminary data on CSF SOD1 protein concentration as a pharmacodynamic biomarker in all participants, including after re-enrollment.

An autopsy was obtained in one $SOD1^{A4V}$ patient who participated in Cohort 3 (1.5 mg), subsequently participated in Cohort 4 (3 mg) 13 weeks later, and then died 12.5 weeks following the 3 mg dose. Patient spinal cord tissue samples obtained at autopsy from the cervical, thoracic, and lumbar regions were analyzed for ISIS 333611 drug concentrations by ELISA. SOD1 protein levels in CSF and spinal cord tissue, (100-200 mg samples of frozen cervical and lumbar spinal cord) were measured by a validated, commercially available human SOD1 protein ELISA (eBioscience BMS222MST). The SOD1 protein level represents the average of four independent ELISA assays for CSF and three independent assays for spinal cord tissue..

Statistical analysis

The sample size of 6 ISIS 333611 and 2 placebo treated subjects per cohort was selected based upon prior experience with Phase 1 single-dose studies of antisense oligonucleotides to ensure that the safety and tolerability of ISIS 333611 would be adequately assessed while minimizing unnecessary patient exposure. There was no statistically-based rationale for the number of subjects chosen. All patients who received the single dose were included in the safety, tolerability and pharmacokinetic assessments. The main purpose of the placebo cohort was to help evaluate adverse events.

Role of the funding sources

The sponsor was involved in the design of the study, data collection, data analysis, interpretation of data, and writing of the report. All authors had full access to the trial data and are responsible for the data accuracy and interpretation of the results. The corresponding author had final responsibility for submission of the report. The ALS Association and Muscular Dystrophy Association commented on initial trial design, but did not participate in decisions regarding the conduct of the trial.

RESULTS

A total of 21 individual patients enrolled, with seven patients enrolling two times, and two patients enrolling three times (Fig. 1). All dosed participants completed the study. Participants had a spectrum of SOD1 mutations the most common being $SOD1^{A4V}$) with associated variability in age at disease onset and time since diagnosis (Table 1). Demographics and background disease characteristics were similar comparing ISIS 333611 and placebo treated groups, except for a greater proportion of males in the ISIS 333611 group (Table 1B).

Overall, 84 % of participants reported adverse events (Table 2A). The most common adverse events were post-LP syndrome and back pain, which in all cases were judged to be related to the intrathecal infusion procedure and not to ISIS 333611. The frequency of the

most commonly reported AEs did not differ between ISIS 333611 and placebo treated groups, and there was no increase in the number of AEs with increasing dose of ISIS 333611. Two SAEs (lacunar infarction and pneumonia, both requiring hospitalization) were reported during the study, both occurring in the same placebo treated patient. In the participants that received ISIS 333611 in more than one cohort, the type and frequency of reported AEs did not increase based on the number of times a subject participated, and AEs decreased with re-enrollment (Table 2B).

No ISIS 333611 related changes in vital signs, neurological or physical exams, hematological assessments, clinical chemistry, coagulation parameters, complement, urinalysis, or ECGs were reported. All participants exhibited a slight, not clinically significant decrease in hematological laboratory values on Day 2 that recovered by Day 29, likely reflecting mild hemodilution secondary to increased hydration in an attempt to prevent post-LP syndromes. Consistent with recent reports of nystagmus in some ALS patients [12, 13], 5 participants (2/8 placebo participants; 3/24 ISIS 333611 participants) exhibited abnormal nystagmus and/or eye movements during the study. One of the 3 ISIS 333611 participants exhibited these findings at baseline pre-dose. In general, the nystagmus/eye movement abnormalities were intermittent, evident upon neurological exam only, not clinically significant, and were not clearly related to ISIS 333611 or the LP procedure.

ALSFRS-R and FVC were generally stable during the study and did not differ between placebo and ISIS 333611 treated participants (ALSFRS-R median total score absolute change: placebo = −1.5, ISIS 333611 = −0.6; FVC median absolute change: placebo = −3.0%, ISIS 333611 = −4.8%). Post-dosing, ISIS 333611 was detected in the CSF of all ISIS 333611 treated participants. CSF levels of ISIS 333611 increased with increasing doses (Fig.2A). Predicted CSF levels of ISIS 333611 were estimated based on CSF volume scaling and observed CSF drug levels from preclinical Rhesus monkey studies. The observed concentrations were close to the predicted concentrations (within 2- to 3-fold). Although ASOs do not cross the blood brain barrier when administered systemically, they are cleared from CSF into the plasma consistent with CSF turnover following intrathecal delivery. [4] In cohorts 1 and 2, plasma drug concentrations were generally below the lower limit of quantification of the assay (1 ng/mL). In cohorts 3 and 4, plasma drug levels increased during the 12-hour infusion period and then rapidly decreased over the next 12 hours. Observed ISIS 3336111 exposure levels in plasma (AUC0-24hrs) were near predicted levels (within 2-fold) based on body weight scaling and pre-clinical exposure (AUCss24hrs) data from Rhesus monkeys (Table 3, Fig. 2B, Supplemental table 2). Analysis of spinal cord tissue samples obtained at autopsy from the $SOD1^{A4V}$ patient who participated in Cohort 3 (1 mg), subsequently participated in Cohort 4 (3 mg) 13 weeks later, and then died 12.5 weeks following the 3 mg dose provided an opportunity to evaluate ISIS 333611 concentrations in human tissue using the ELISA methodology. ISIS 333611 concentrations were 218 ng/g, 122 ng/g and 39 ng/g in lumbar, thoracic, and cervical spinal cord, respectively. These observed human tissue levels and the lumbar to cervical gradient are consistent with expected tissue levels based on Rhesus monkey pre-clinical studies (Supplemental Table 3). The CSF concentrations at the end of the infusion for this patient during cohort 3 and 4 were 3.5 μg/mL and 6.3 μg/mL, respectively. SOD1 protein concentrations were measured in cervical and lumbar spinal cord from the trial subject and several non-trial subjects (N=6, three SOD1 mutations and three sporadic ALS). In the non-trial subjects SOD1 protein ranged from 2141-4543 ng/mL in the cervical cord and from 1494-3478 ng/mL in the lumbar cord. The treated subject SOD1 level was 2259 ng/mL in the cervical and 1867 ng/mL in the lumbar regions. The cervical:lumbar ratio of SOD1 concentrations was 1.2 in the subject in the study and ranged from 0.9 to 1.7 in subjects not in this study.

CSF SOD1 protein concentration could be a pharmacodynamic biomarker for ISIS 333611. Although the single-doses given in this study are not predicted to provide drug levels that are high enough to reduce CSF SOD1 concentrations, we analyzed CSF SOD1 levels in all participants, including analysis of re-enrollments. Overall, as expected[9], there were no substantial changes in CSF SOD1 in participants dosed in more than one cohort, with most SOD1 CSF concentrations within 12% of the pre-dose or previous cohort value (Fig. 3).

DISCUSSION

The ability to directly modulate disease causing gene expression in the brain and spinal cord affords many novel therapeutic opportunities for neurological diseases, in particular neurodegenerative disorders. Our first-in-human clinical study makes an important step towards realizing this goal by demonstrating the feasibility of ASO delivery into the CNS via intrathecal delivery. Given the lack of experience with ASO administration into the CNS, this trial proceeded cautiously with single, escalating, low-dose infusions. ISIS 333611 was well tolerated with no dose-limiting toxicities or safety concerns identified at the dose levels tested. We conclude that intrathecal delivery of this antisense drug appears well tolerated at the doses studied. Although the small numbers of subjects (32 doses in 21 individual subjects) and relatively low doses limits broader conclusions about intrathecal ASO tolerability, the data from this study provide encouragement for further development of ASOs for the treatment of neurodegenerative diseases. Direct infusion of an ASO into tumor tissue by convection enhanced delivery appeared to be similarly well tolerated. [14]

Analysis of drug concentrations in CSF and plasma demonstrated a clear dose-dependent relationship. Observed CSF and plasma concentrations were consistent with predicted values (within 2- to 3-fold) based on preclinical (monkey to human) scaling. The agreement between observed pharmacokinetic data and the predicted values support the use of CSF volume and body weight scaling for human CSF concentration and plasma exposure predictions, respectively, and better enable the selection of doses and dose levels in future clinical studies.

Autopsy material from a patient who died from ALS about 3 months after receiving ISIS 333611 provided an opportunity to examine drug concentrations in spinal cord tissues. Based on an estimated tissue half-life of approximately 28 days in preclinical studies, the autopsy material was collected approximately 3 half-lives from when the last dose was given. Drug concentrations in spinal cord tissue were easily measured with concentrations well above the limit of quantification (5 ng/g). Concentrations in the lumbar cord, near the site of infusion, were approximately five-fold -higher than in the cervical cord (. By scaling data from Rhesus monkey tissue to humans by CSF volume and by allowing for elimination consistent with a 28-day half-life, estimates of the expected human tissue concentrations were made for this patient at the time of autopsy. The observed human tissue values are within 2-fold of the concentrations calculated from the monkey data suggesting the human elimination half-life may be in the same range as that observed in the monkey studies (~30 days).

In the subset of subjects who participated in more than one cohort and therefore for whom repeat CSF samples were available (N=9), SOD1 CSF concentrations were on average within 12% of the original value (Fig. 3). This relative lack of change of CSF SOD1 protein values is consistent with the low dose of ASO given in the trial and also consistent with prior measurements of repeat SOD1 CSF measurements showing, on average, 7% variation in SOD1 CSF protein levels when measured months apart[9]. The stability of CSF SOD1 measurements in these data, coupled with rat data showing that reduction of SOD1 in brain

correlates with reduced SOD1 in the CSF[9], strongly support using CSF SOD1 protein levels as a pharmacodynamic marker for ASO target reduction in future SOD1 ALS clinical trials.

Our conclusion that intrathecal delivery of ISIS 333611 was well tolerated with no safety concerns identified at the dose levels testedis limited by the doses given and the small number of subjects studied. The doses given were intended to reflect a single dose of a drug that would be given as a continuous infusion and thus were low. We predict that the highest dose given here would need to be given continuously for 4 days to achieve reduction of SOD1 mRNA and protein levels in the human spinal cord. The small number of subjects also limits conclusions regarding safety since very rare events may not be revealed in a small number. Lastly, because of the small number of subjects enrolled the ISI 333611 group had a greater number of males compared with the placebo group. Nevertheless, we consider this study an important first step in using ASOs to treat neurologic disease. For SOD ALS, patients with A4V mutations typically progress from symptom onset to death very rapidly (i.e. in less than 12 months) and thus only shorter term lowering of SOD1 may be needed to provide a benefit.. Before considering longer term dosing in more slowly progressive SOD1-related ALS or treating patients presymptomatically, it will be important to more fully understand the effects of chronic, long-term reductions of SOD1 since knockout of SOD1 has been linked to liver cancer[15] and late lifemotor neuropathy [16]. The fact that our approach will lead to only a partial reduction of SOD1 and there is very limited exposure to peripheral tissues following intrathecal delivery also mitigates these concerns. The ASO used here is designed to activate RNase H mediated destruction of all SOD1, rather than a particular mutation, which allows application of this approach broadly to almost all of the >100 different mutations in SOD1 known to cause ALS. [7] Experiments in mice demonstrate that wild type SOD1 may enhance the toxicity of mutant SOD1[17] and thus there is an additional theoretical benefit to decreasing both mutant and wild-type forms of SOD1.

Some recent studies suggest that SOD1 may be involved in sporadic ALS. Gruzman and colleagues found an SOD1 reactive protein (after chemical crosslinking) in a small number of ALS subjects but not in controls [18]. Antibodies that specifically recognize misfolded SOD1 revealed misfolded SOD1 in vulnerable spinal cord neurons of some ALS patients, but not controls[19, 20] while other well-performed, larger studies do not find this pathology in sporadic ALS[21]. Most interestingly, lowering SOD1 levels in astrocytes derived from sporadic ALS subjects reversed the toxicity of these same astrocytes when co-cultured with motor neurons, again implying that SOD1 may contribute to the pathogenesis of sporadic ALS [22]. Though the rationale remains strongest for treatment of familial SOD1-related ALS with the antisense oligonucleotides described here, it is possible, should sufficient and strong data emerge, that this therapeutic approach may be considered for treating sporadic ALS with these same compounds.

Overall, this first human clinical study of an antisense drug delivered intrathecally demonstrated excellent tolerability and predictable pharmacokinetics. This study will facilitate future studies of similar antisense drugs in familial SOD1 ALS, other genetic forms of ALS, as well as other neurodegenerative diseases.

Research in Context Panel

Systematic review—We searched PubMed with the terms antisense oligonucleotide and clinical trial and neurologic. We found no prior human clinical trials using antisense oligonucleotides to the central nervous system. Further searching PubMed for antisense oligonucleotide and glioma revealed one clinical trial using convection enhanced delivery of an antisense oligonucleotide directly to a central nervous system tumor. This type of

delivery was also well tolerated. Our experience and our literature search suggest that our study is the first report of antisense oligonucleotide delivery to the CSF.

Interpretation—The study reported here is the first clinical study delivering an antisense oligonucleotide to the CSF with the goal of delivering it broadly to the CNS. Results of this clinical study suggest that ISIS 333611 was well tolerated when administered as an intrathecal infusion in subjects with SOD1 familial ALS. CSF and plasma drug levels were consistent with levels predicted from preclinical studies. These conclusions are limited by the small doses given and the relatively small numbers of patients studied in this Phase I clinical study.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We would like to sincerely thank the patients who participated in this study, as well as their supportive family members/caretakers who made their participation possible. This trial was funded by the Muscular Dystrophy Association, ALS Association, and Isis Pharmaceuticals. TMM was supported by NIH/NINDS K08NS074194 and NIH/NINDS R01NS078398. Clinical studies at Washington University were supported by the Washington University Institute of Clinical and Translational Sciences grant UL1 TR000448, NIH/NCATS. We appreciate the hard work and dedication of many people who contributed to this study: MGH Coordinating Center (Kate Jackson, Alex Sherman, Marianne Kearney, Hong Yu, Lauren Schnupp, Bryan Sweet, Jason Walker, Igor Katsovskiy, and Ervin Sinani), MGH clinical site (Swati Aggarwal, Melanie Majkut, Jennifer Berndt, Darlene Sawicki), Johns Hopkins (Richard Kimball, Lora Clawson, Kristen Riley), Methodist Neurological Institute (Sharon Halton, Luis Lay), Washington University in St. Louis (Julaine Florence, Catherine Siener, Brian Sommerville, Robert Swarm, Leo Wang, Charlie Wulf), Isis Pharmaceuticals (Viola Kam, Jesse Kwoh, Katherine Kwoh, John Madson, Gina McMullin, Dan Schultz). We also thank Robert Brown, Benjamin Brooks, and Don Cleveland for input regarding clinical study design, the Data Safety and Monitoring Board for this study (Walter Bradley, Karl Kieburtz, Carl Leventhal, Michael McDermott, Stephen Reingold) and the many investigators of the Northeast ALS Trials Consortium (NEALS) who referred patients to the study and for Patients Like Me organization for referrals to Johns Hopkins University.

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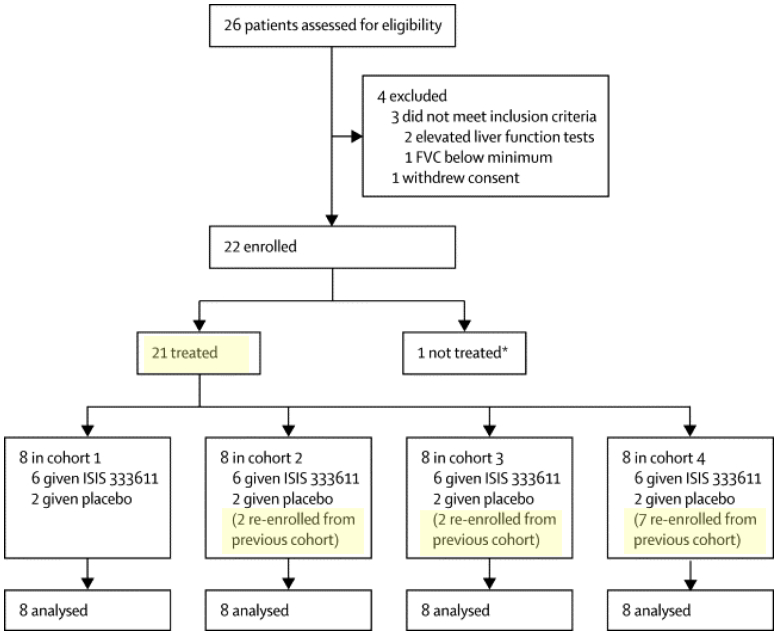

Fig. 1. Subject Disposition

The subject disposition among the 26 subjects assessed is detailed above. One enrolled subject was not treated because of failed intrathecal catheter placement. Some subjects were enrolled in more than cohort.

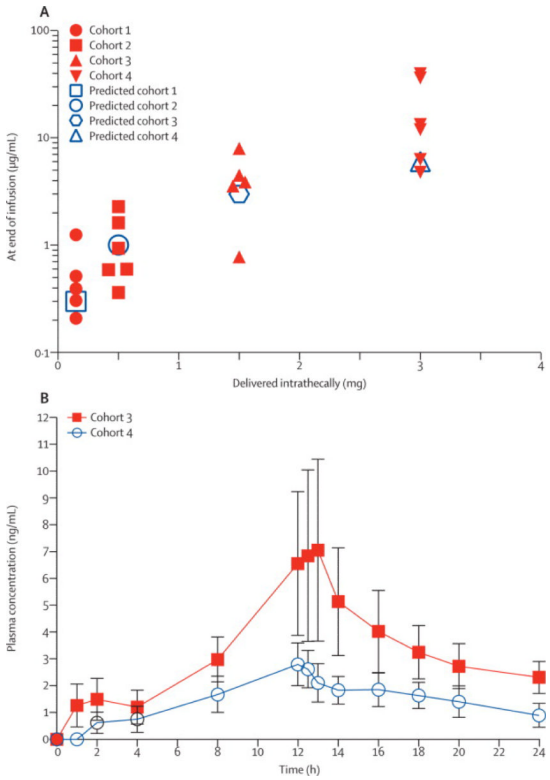

Fig. 2. CSF and Plasma Concentrations of ISIS 3336111

A. CSF was drawn immediately after the end of the intrathecal infusion (11hours, 22 minutes) one level above or below the infusion site. Measured ISIS333611 concentrations and predicted values are shown.

B. Plasma was drawn at each of the indicated time points for all 4 cohorts and ISIS333611 measured by ELISA. As anticipated, plasma levels for cohorts 1 and 2 were below the limit of detection of the assay. (N=6 +/− SE)

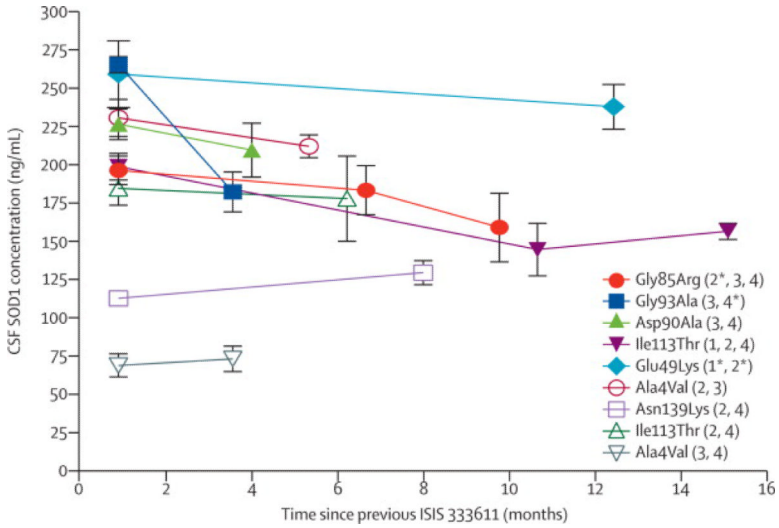

Fig. 3. SOD1 Protein Levels in CSF

SOD1 protein levels in the CSF were measured by ELISA assay in those subjects that were enrolled in more than one cohort and plotted vs. months since previous cohort. SOD1 Mutation and cohort number are indicated for individual subjects; * denotes placebo for that cohort.

Table 1
Demographic and Clinical Characteristics at Baseline

A. Characteristics of Individual Subjects*

Subject #	Gender	Age	ALS Family History?	SOD1 Mutation	Age of Onset	Site of Onset
1	Female	49	Yes	E49K	47	Limb
2	Male	59	Yes	A4V	59	Limb
3	Female	36	Yes	G37R	23	Limb
4	Male	41	Yes	A4T	41	Limb
5	Male	47	Yes	L38V	45	Limb
6	Male	51	Yes	I113T	47	Limb
7	Female	50	Yes	A4V	50	Limb
8	Female	58	Yes	A4V	58	Limb
9	Male	63	Yes	G85R	63	Limb
10	Male	52	Yes	A4V	51	Limb
11	Male	48	Yes	N139K	45	Limb
12	Male	54	Yes	I113T	48	Limb
13	Male	44	No	A89V	42	Limb
14	Female	56	Yes	I113T	43	Limb
15	Male	55	Yes	G93S	45	Limb
16	Male	46	Yes	A4V	46	Bulbar
17	Male	22	Yes	G41S	22	Limb
18	Male	56	Yes	D90A	55	Limb
19	Male	51	Yes	L8V	43	Limb
20	Female	38	Yes	G93A	37	Limb
21	Female	49	Yes	Q22L	45	Limb

* Each unique subject listed only once

B. Characteristics by Treatment Group*

	Placebo	ISIS 333611
No.	8	24
Age (min-max)	52 (38 - 63)	50 (22 - 64)
Male (%)	38	79
Caucasian (%)	100	75
ALSFRS-R (min-max)	33 (22 - 41)	36 (24 - 44)
FVC, % (min-max)	83 (53 -110)	84 (54 - 113)

* Includes re-enrollment of individual subjects ALSFRS-R: ALS Functional Rating Scale, Revised FVC: Forced Vital Capacity

Table 2

Adverse Events

A. All adverse events*

Adverse Event Term	Placebo Subjects N=8 %, n (# events)	ISIS 333611 Subjects N=24 %, n (# events)	Cohort Frequency # events in Cohorts (1, 2, 3, 4)
Any Serious Adverse Event	13%, 1 (2)	0%, 0 (0)	na
Any Adverse Event	88%, 7 (23)	83%, 20 (50)	(23, 9, 7, 11)
Post-LP Syndrome	38%, 3 (5)	33%, 8 (8)	(4, 2, 1, 1)
Back Pain	50%, 4 (4)	17%, 4 (4)	(2, 1, 1, 0)
Nausea	0%, 0 (0)	13%, 3 (3)	(2, 0, 1, 0)
Vomiting	0%, 0 (0)	8%, 2 (2)	(2, 0, 0, 0)
Headache	13%, 1 (1)	8%, 2 (2)	(0, 2, 0, 0)
Fall	0%, 0 (0)	8%, 2 (2)	(1, 1, 0, 0)
Dizziness	0%, 0 (0)	8%, 2 (2)	(1, 0, 0, 1)

* adverse events listed are those that occurred with a frequency >5% in ISIS 333611 treated subjects (i.e. occurring in >1 subject).

B. Adverse events in Re-Enrolled Subjects*

Adverse Event Term	1st Enrollment N=21 %, n (# events)	2st Enrollment N= 9 %, n (# events)	3rd Enrollment N=2 % (# events)
Post-LP Syndrome	48%, 10 (12)	11%, 1 (1)	0%, 0 (0)
Back Pain	29%, 6 (6)	22%, 2 (2)	0%, 0 (0)
Nausea	14%, 3 (3)	0%, 0 (0)	0%, 0 (0)
Headache	10%, 2 (2)	11%, 1 (1)	0%, 0 (0)

* adverse events listed are those that occurred with a frequency >7% (i.e. occurring in >2 enrollments).

Table 3

Comparison of Predicted and Observed Human Plasma Exposure after a Single 11-hour Infusion of ISIS 333611

Cohort	Dose (per 12 hr)	N	AUC (0-24hr) (hr*ng/mL)		
			Mean (SE)	Median (range)	Predicted
1	0.15 mg	6	4.15 (2.93)	0 (0 - 19.9)	--
2	0.5 mg	6	0 (0)	0 (0 - 0)	--
3	1.5 mg	6	34.9 (10.3)	25.3 (12.3 - 77.6)	60.9
4	3.0 mg	6	77.6 (26.2)	62.1 (24.8 - 200)	122

AUC: Area under the curve calculated from individual measurements ofplasma from start of dosing through 12 hours after dosing.

Predicted A UC = Monkey A UCss24hr / (human body weight [70 kg]/monkey body weight [4 kg]) x (human dose [total mg]/monkey dose [mg/day]). Monkey AUC values are listed in Supplemental Table 2. Monkey A UC value at 0.1 mg/day was used for human calculations.