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An antisense oligonucleotide against *SOD1* delivered intrathecally for patients with *SOD1* familial amyotrophic lateral sclerosis: a phase 1, randomised, first-in-man study



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Summary

Background Mutations in *SOD1* cause 13% of familial amyotrophic lateral sclerosis. In the *SOD1* Gly93Ala rat model of amyotrophic lateral sclerosis, the antisense oligonucleotide ISIS 333611 delivered to CSF decreased *SOD1* mRNA and protein concentrations in spinal cord tissue and prolonged survival. We aimed to assess the safety, tolerability, and pharmacokinetics of ISIS 333611 after intrathecal administration in patients with *SOD1*-related familial amyotrophic lateral sclerosis.

Methods In this randomised, placebo-controlled, phase 1 trial, we delivered ISIS 333611 by intrathecal infusion using an external pump over 11·5 h at increasing doses (0·15 mg, 0·50 mg, 1·50 mg, 3·00 mg) to four cohorts of eight patients with *SOD1*-positive amyotrophic lateral sclerosis (six patients assigned to ISIS 333611, two to placebo in each cohort). We did the randomisation with a web-based system, assigning patients in blocks of four. Patients and investigators were masked to treatment assignment. Participants were allowed to re-enrol in subsequent cohorts. Our primary objective was to assess the safety and tolerability of ISIS 333611. Assessments were done during infusion and over 28 days after infusion. This study was registered with Clinicaltrials.gov, number NCT01041222.

Findings Seven of eight (88%) patients in the placebo group versus 20 of 24 (83%) in the ISIS 333611 group had adverse events. The most common events were post-lumbar puncture syndrome (3/8 [38%] vs 8/24 [33%]), back pain (4/8 [50%] vs 4/24 [17%]), and nausea (0/8 [0%] vs 3/24 [13%]). We recorded no dose-limiting toxic effects or any safety or tolerability concerns related to ISIS 333611. No serious adverse events occurred in patients given ISIS 333611. Re-enrolment and re-treatment were also well tolerated.

Interpretation This trial is the first clinical study of intrathecal delivery of an antisense oligonucleotide. ISIS 333611 was well tolerated when administered as an intrathecal infusion. Antisense oligonucleotides delivered to the CNS might be a feasible treatment for neurological disorders.

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Introduction

Our knowledge of the genetic basis of many neurodegenerative diseases has progressed greatly in the past 20 years. Causative mutations have been identified for Huntington's disease, spinal muscular atrophy, spinal and bulbar muscular atrophy, Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis.¹ The challenge now is to turn this knowledge into effective treatments. For dominantly inherited disorders in which a mutant protein becomes toxic, reducing the concentration of the protein is a potential approach and antisense oligonucleotides are one means of doing so.² Antisense oligonucleotides are short, synthetic nucleic acids that have been chemically modified to increase their stability in biological fluids and their potency in binding their mRNA target. One mechanism by which antisense oligonucleotides function is by binding to a specific target mRNA through Watson-Crick base-pairing and causing degradation of the mRNA by activation of the nuclear enzyme RNase H.³ Because antisense

oligonucleotides do not cross the blood–brain barrier,⁴ they must be delivered directly to the CNS to treat neurodegenerative diseases. One possible approach is to administer them intrathecally into the CSF, which results in widespread delivery to the CNS.^{4–6}

Genetic changes in more than ten genes are known to cause familial amyotrophic lateral sclerosis, an adult-onset neurodegenerative disease characterised by loss or dysfunction of both upper and lower motor neuron pathways⁷ and in some cases dementia. Mutations in *SOD1* account for roughly 2% of all cases of amyotrophic lateral sclerosis. Although such mutations were identified almost 20 years ago, no treatments exist that substantially slow either the sporadic or *SOD1*-linked forms of amyotrophic lateral sclerosis. Different *SOD1* mutations are associated with different ages of onset and rates of progression, and nearly all are inherited dominantly.⁸ The toxicity of *SOD1* is the result of a gain of toxic function rather than a loss of enzymatic function; thus, reducing concentrations of the mutant protein

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is predicted to slow progression of *SOD1*-linked amyotrophic lateral sclerosis.⁷ Evidence of the usefulness of antisense oligonucleotides for treatment of neurodegenerative diseases includes: (1) widespread distribution of antisense oligonucleotides throughout the CNS after CSF administration;^{4,6} (2) reduction of *SOD1* mRNA and protein in the brain and spinal cord tissues;⁴ and (3) increased survival after direct delivery to the CSF in an *SOD1* Gly93Ala animal model of amyotrophic lateral sclerosis.⁴ The drug used in these studies was ISIS 333611, an antisense oligonucleotide that reduces expression of wild-type and mutant human *SOD1* protein in transgenic rats and in cultured human cells.^{4,9}

We aimed to assess the safety, tolerability, and pharmacokinetics of a single dose of ISIS 333611 in patients with *SOD1* familial amyotrophic lateral sclerosis. This is the first clinical study to test the effects of delivering an antisense oligonucleotide directly into human CSF as a treatment for a widespread disorder of the CNS.

Methods

Study design and participants

We did this placebo-controlled, double-blind, randomised, dose escalation phase 1 study at four centres in the USA (Washington University in St Louis, St Louis, MO; Massachusetts General Hospital, Boston, MA; Johns Hopkins University, Baltimore, MD; and Methodist Neurological Institute, Houston, TX). Patients were eligible if they were aged 18 years or older, had a documented mutation in *SOD1*, showed clinical signs of weakness attributed to amyotrophic lateral sclerosis, had a forced vital capacity of more than 50% of their predicted value, were not using invasive respiratory support, and were medically able to undergo insertion of a temporary intrathecal catheter. We used a 50% cutoff for forced vital capacity because patients with amyotrophic lateral sclerosis who have a forced vital capacity of more than 50% 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 before the start of the study and had to stay on that dose throughout the study. Patients were ineligible if they had been treated with an investigational drug for amyotrophic lateral sclerosis within 30 days or within a period of five-times the half-life of the drug before screening, had clinically significant abnormalities in laboratory test results (including coagulation measures), or had a medical condition that would interfere with the study.

The institutional review boards of the participating study centres approved the study and we did the trial in accordance with the Declaration of Helsinki and the International Conference on Harmonisation Good Clinical Practice guidelines. All participants provided written informed consent. A data safety and monitoring board monitored the trial.

Randomisation and masking

Participants were enrolled sequentially in four cohorts of escalating single doses of ISIS 333611 (0.15 mg, 0.50 mg, 1.50 mg, 3.00 mg). Within each cohort, patients were randomly assigned to ISIS 333611 or placebo in a ratio of 3:1. An approved protocol amendment made after completion of treatment of the first dose cohort allowed participants to re-enrol in a different cohort if more than 60 days had elapsed since they were treated previously and they still qualified for the study. Re-enrolled patients were re-assigned without regard to previous assignment. After participants were deemed eligible for the study (provided informed consent and met all inclusion criteria), they were sequentially allocated unique patient identification numbers and assigned to a treatment group.

We generated the randomisation sequence with WebEZ, an independent, centralised, web-based randomisation system (Almac Clinical Services, Souderton, PA, USA). We randomly assigned patients in blocks of four sequential participants. Randomisation codes were accessed by the site pharmacist. Study drug or placebo was labelled only with the patient identification number before 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 by the pharmacist at each site, the data safety and monitoring board, and a central biostatistician who provided statistical support to the data safety and monitoring board. No events required premature unmasking of patient allocation or study data.

Procedures

ISIS 333611 (provided in sterile, unpreserved, buffered saline solution at 20 mg/mL, pH=7.4) and the placebo plus diluents (sterile, phosphate-buffered saline) were provided by Isis Pharmaceuticals. ISIS 333611 was diluted to the appropriate concentration for each dose and used within 24 h of preparation. The study drug was given as one intrathecal infusion. ISIS 333611 or placebo (0.25 mL) was infused intrathecally for 11 h 22 min with a CADD-MS 3 syringe-based ambulatory infusion pump (Smiths Medical MD, St Paul, MN, USA) equipped with a 3 mL Medication Cartridge via a Codman FlexTip Plus intraspinal catheter. The infusion time was chosen to slowly deliver the exact volume within the programming features of the pump. We chose this volume and timing to provide roughly half a day of dosing, assuming chronic, continuous infusion of 0.5 mL per day in future clinical trials. The tip of the intrathecal catheter was placed under fluoroscopic guidance near the T8–T10 spinal level by a lumbar puncture using a 17G Tuohy needle inserted into the L3/L4 space, although the protocol allowed placement at a different level if patient anatomy or clinical judgment dictated. We took a CSF sample via the catheter placement needle before infusion

to analyse pharmacokinetics and SOD1 protein concentrations. At the end of the infusion, the catheter was immediately removed and another CSF sample taken by a separate lumbar puncture one segment above or below the catheter placement site. We measured ISIS 333611 concentrations in plasma 13 times from before infusion to 12 h after infusion.

Autopsy was not included in the protocol, but through a separate existing protocol we obtained spinal cord tissue samples from a SOD1 Ala4Val patient who was enrolled in cohorts 3 and 4 and who died from amyotrophic lateral sclerosis-related causes 3 months after the study. These samples enabled us to directly assess drug and SOD1 concentrations in this patient. We analysed ISIS 333611 concentration in spinal cord tissue samples taken at autopsy from the cervical, thoracic, and lumbar regions. We measured SOD1 concentration in CSF and spinal cord tissue (100–200 mg samples of frozen cervical and lumbar spinal cord) by a validated, commercially available human SOD1 ELISA (eBioscience BMS222MST). The SOD1 protein concentration is the mean of four independent ELISA assays for CSF and three independent assays for spinal cord tissue. For comparison, we measured SOD1 concentrations in cervical and lumbar spinal cord from autopsy tissue collected at Washington University and Johns Hopkins University (USA) from six patients not included in this trial (three with SOD1 familial amyotrophic lateral sclerosis and three with sporadic amyotrophic lateral sclerosis).

Safety assessments were: recording adverse events, physical and neurological examinations, recording vital signs, clinical laboratory tests (haematology, clinical chemistry, complement, coagulation), electrocardiograms (ECGs), assessment of amyotrophic lateral sclerosis functional rating scale revised score, forced vital capacity, and recording use of concomitant medications. Based on preclinical findings of high exposure to the drug, we monitored for signs of cerebellar dysfunction. Safety assessments were done on day 1 before and after infusion, day 2, day 8, and day 29. Patients were also assessed by a telephone call on day 15. Safety information was reviewed by the data safety and monitoring board after day 8 for the last member of each cohort, to provide a dose escalation recommendation.

We collected data on CSF SOD1 concentration as a pharmacodynamic biomarker in all participants, including after re-enrolment. We estimated ISIS 333611 concentrations by a modification of the hybridisation 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, USA). Stability of ISIS 333611 in a frozen matrix (up to 3 months) and up to five freeze-thaw cycles was also confirmed.

Our primary objective was to assess the safety and tolerability of ISIS 333611 given as a single intrathecal

infusion in patients with SOD1 familial amyotrophic lateral sclerosis. Secondary objectives were to assess CSF and plasma pharmacokinetics after intrathecal delivery.

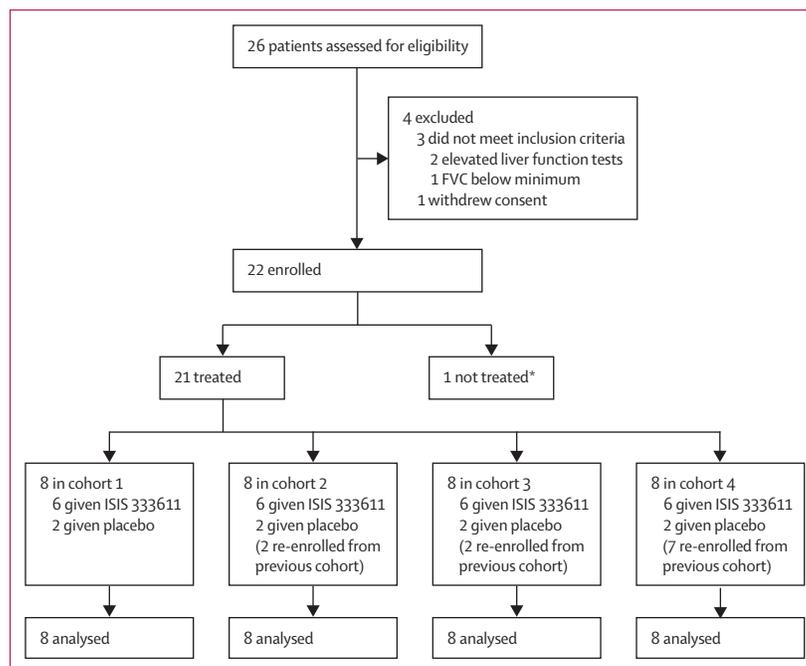


Figure 1: Trial profile

FVC=forced vital capacity. *Not treated because of failed placement of intrathecal catheter.

	Sex	Age (years)	Family history of amyotrophic lateral sclerosis	SOD1 mutation	Age at onset (years)	Site of onset
1	Female	49	Yes	Glu49Lys	47	Limb
2	Male	59	Yes	Ala4Val	59	Limb
3	Female	36	Yes	Gly37Arg	23	Limb
4	Male	41	Yes	Ala4Thr	41	Limb
5	Male	47	Yes	Leu38Val	45	Limb
6	Male	51	Yes	Ile113Thr	47	Limb
7	Female	50	Yes	Ala4Val	50	Limb
8	Female	58	Yes	Ala4Val	58	Limb
9	Male	63	Yes	Gly85Arg	63	Limb
10	Male	52	Yes	Ala4Val	51	Limb
11	Male	48	Yes	Asn139Lys	45	Limb
12	Male	54	Yes	Ile113Thr	48	Limb
13	Male	44	No	Ala89Val	42	Limb
14	Female	56	Yes	Ile113Thr	43	Limb
15	Male	55	Yes	Gly93Ser	45	Limb
16	Male	46	Yes	Ala4Val	46	Bulbar
17	Male	22	Yes	Gly41Ser	22	Limb
18	Male	56	Yes	Asp90Ala	55	Limb
19	Male	51	Yes	Leu8Val	43	Limb
20	Female	38	Yes	Gly93Ala	37	Limb
21	Female	49	Yes	Gln22Leu	45	Limb

Table 1: Demographic and clinical characteristics of each participant

Statistical analysis

We estimated a sample size of six patients treated with study drug and two treated with placebo per cohort on the basis of previous phase 1 single-dose studies of antisense oligonucleotides to ensure that the safety and tolerability of ISIS 333611 would be adequately assessed while minimising unnecessary patient exposure. We did not do a statistical test of the number of patients needed. We predicted CSF concentrations of ISIS 333611 on the basis of CSF volume scaling and CSF drug concentrations measured in preclinical studies of Rhesus monkeys. We did not do a statistical test to compare predicted and measured CSF concentrations of ISIS 333611. All patients who received treatment were included in the safety, tolerability, and pharmacokinetic assessments. All data summaries and pharmacokinetic

analyses were done with Phoenix WinNonlin (version 6.2). This study was registered with Clinicaltrials.gov, number NCT01041222.

Role of the funding source

The sponsors were involved in the design of the study, data collection, data analysis, data interpretation, and writing of the report. All authors had full access to the trial data and are responsible for the accuracy of the data and interpretation of the results. The corresponding author had final responsibility for the decision to submit for publication.

Results

We enrolled 22 participants between March, 2010, and December, 2011, 21 of whom received treatment (figure 1). Seven patients enrolled twice, and two patients enrolled three times. All treated participants completed the study. Participants had various SOD1 mutations (the most common was SOD1 Ala4Val) with associated variability in age at disease onset and time since diagnosis (table 1). Demographic and disease characteristics were much the same between groups, except for the proportion of men (table 2).

27 patients (84%) reported adverse events (table 3). The most common adverse events were post-lumbar puncture syndrome and back pain, which in all cases were judged to be related to the infusion procedure and not to the study drug. No adverse events were deemed related to treatment. The most common adverse events did not differ between ISIS 333611 and placebo groups, and the number of adverse events did not increase with increasing dose of ISIS 333611. Two serious adverse events (lacunar infarction and pneumonia, both requiring admission to hospital) were reported, both in the same placebo-treated patient. In the participants that received ISIS 333611 in more than one cohort, the number of adverse events decreased with re-enrolment (table 4).

We recorded no changes related to study drug for vital signs, neurological or physical examinations, haematological assessments, clinical chemistry, coagulation measures, complement, urinalysis, or ECGs (data not shown). All participants had a slight, not clinically significant decrease in haematological test results on day 2 that recovered by day 29, probably caused by mild haemodilution secondary to increased hydration in an attempt to prevent post-lumbar puncture syndromes (data not shown). Consistent with reports^{12,13} of nystagmus in some patients with amyotrophic lateral sclerosis, five participants (two in the placebo group and three in the ISIS 333611 group) had nystagmus or abnormal eye movements during the study. One of the participants treated with ISIS 333611 had these signs before treatment. Generally, these abnormalities were intermittent, only evident on neurological examination, not clinically significant, and not clearly related to treatment with ISIS 333611 or lumbar puncture.

	Placebo group (n=8)	ISIS 333611 group (n=24)
Mean age (range; years)	52 (38–63)	50 (22–64)
Men (%)	3 (38%)	19 (79%)
White (%)	8 (100%)	18 (75%)
Mean amyotrophic lateral sclerosis functional rating scale, revised score (range)	33 (22–41)	36 (24–44)
Mean forced vital capacity (range)	83% (53–110)	84% (54–113)

Table 2: Patient characteristics by treatment group

	Placebo group (n=8)	ISIS 333611 group (n=24)	Events in the ISIS 333611 group (n)			
			Cohort 1	Cohort 2	Cohort 3	Cohort 4
Any serious adverse event	1 (13%; 2)	0 (0%; 0)	0	0	0	0
Any adverse event	7 (88%; 23)	20 (83%; 50)	23	9	7	11
Post-lumbar puncture syndrome	3 (38%; 5)	8 (33%; 8)	4	2	1	1
Back pain	4 (50%; 4)	4 (17%; 4)	2	1	1	0
Nausea	0 (0%; 0)	3 (13%; 3)	2	0	1	0
Vomiting	0 (0%; 0)	2 (8%; 2)	2	0	0	0
Headache	1 (13%; 1)	2 (8%; 2)	0	2	0	0
Fall	0 (0%; 0)	2 (8%; 2)	1	1	0	0
Dizziness	0 (0%; 0)	2 (8%; 2)	1	0	0	1

Data are number of patients (%; number of events) unless otherwise stated. Events listed are those that occurred in >5% of ISIS 333611 treated patients (ie, occurred in >1 patient).

Table 3: Adverse events by group and cohort

	First enrolment (n=21)	Second enrolment (n=9)	Third enrolment (n=2)
Post-lumbar puncture syndrome	10 (48%; 12)	1 (11%; 1)	0 (0%; 0)
Back pain	6 (29%; 6)	2 (22%; 2)	0 (0%; 0)
Nausea	3 (14%; 3)	0 (0%; 0)	0 (0%; 0)
Headache	2 (10%; 2)	1 (11%; 1)	0 (0%; 0)

Data are number of patients (%; number of events). Only events that occurred in >7% of patients are included (ie, >2 enrolments).

Table 4: Adverse events by enrolment

The amyotrophic lateral sclerosis functional rating scale revised score and forced vital capacity were generally stable during the study and did not differ substantially between placebo and ISIS 333611 groups (median amyotrophic lateral sclerosis functional rating scale revised score absolute change -1.5 vs -0.6 ; median forced vital capacity absolute change -3.0% vs -4.8%). After treatment, we detected ISIS 333611 in CSF of all participants in the ISIS 333611 group. CSF concentrations of ISIS 333611 increased with increasing doses (figure 2A). The measured concentrations were within two to three times of the predicted concentrations. Although antisense oligonucleotides do not cross the blood–brain barrier when administered systemically, they are cleared from CSF into the plasma consistent with CSF turnover after 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 concentrations increased during infusion and then rapidly decreased over the next 12 h (figure 2B). Table 5 and the appendix show ISIS 333611 concentrations in plasma (area under the curve_{0–24}) compared with predicted concentrations.

We analysed spinal cord tissue samples obtained at autopsy from a patient with a SOD1 Ala4Val mutation. ISIS 333611 concentrations were 218 ng/g in a lumbar spinal cord sample, 122 ng/g in a thoracic spinal cord sample, and 39 ng/g in a cervical spinal cord sample. These results and the gradient between lumbar and cervical samples are consistent with expected tissue concentrations based on preclinical studies of Rhesus monkeys (predicted concentration vs measured concentration: 344 ng/g vs 218 ng/g in the lumbar sample, 282 ng/g vs 122 ng/g in the thoracic sample, 36 ng/g vs 39 in the cervical sample; appendix). The CSF concentration of ISIS 333611 at the end of infusion for this patient was 3.5 µg/mL during cohort 3 and 6.3 µg/mL during cohort 4.

We measured SOD1 protein concentrations in cervical and lumbar spinal cord samples from one trial participant and six patients who did not take part in the trial. In the non-trial patients, SOD1 protein concentration ranged from 2141 ng/mL to 4543 ng/mL in the cervical cord and from 1494 ng/mL to 3478 ng/mL in the lumbar cord. For the treated patient, SOD1 concentration was 2259 ng/mL in the cervical sample and 1867 ng/mL in the lumbar sample. The cervical:lumbar ratio of SOD1 concentrations was 1.2:1 in the trial participant and ranged from 0.9:1 to 1.7:1 in patients not treated in the study. In our analysis of CSF SOD1 concentrations in all participants, as expected,⁹ CSF SOD1 concentration did not change substantially in participants enrolled in more than one cohort, with most SOD1 CSF concentrations within 12% of the pretreatment or previous cohort value (figure 3).

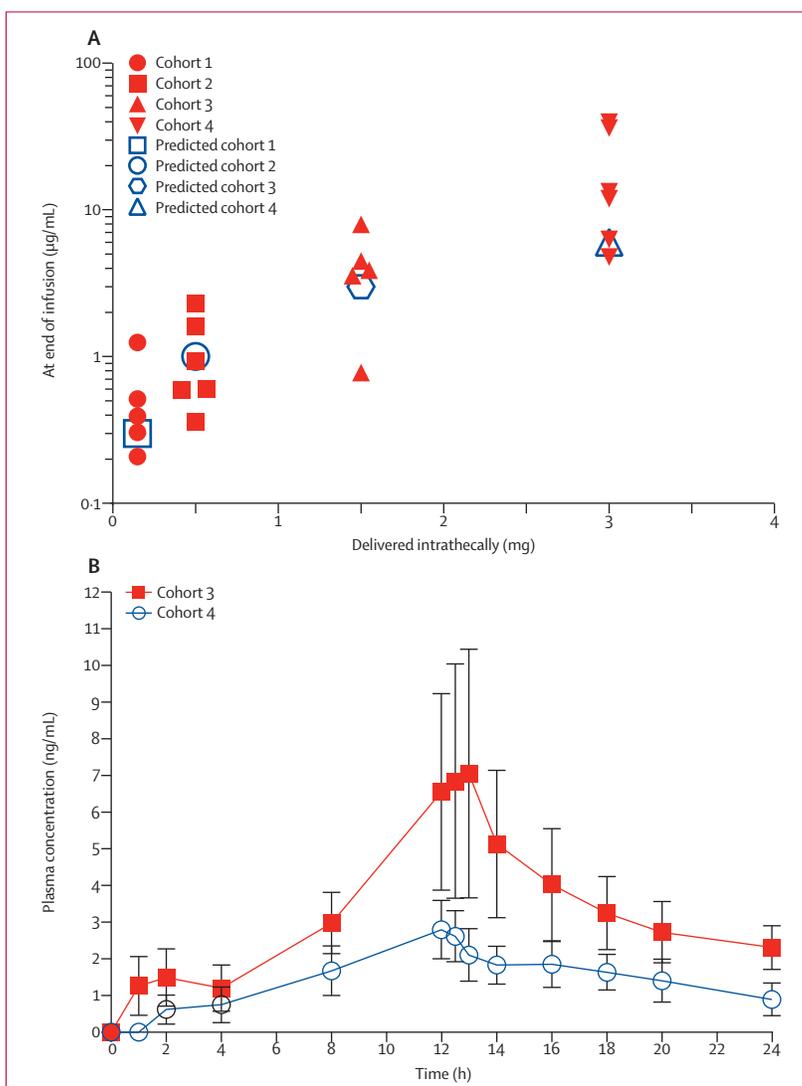


Figure 2: CSF and plasma concentrations of ISIS 333611

Delivered and measured concentrations in the CSF (A) and plasma concentrations over time (B). Plasma concentrations for cohorts 1 and 2 were below the limit of detection of the assay. The CSF draw was unsuccessful for one patient in cohort 1 and one patient in cohort 3.

Dose	n	Area under the curve (0–24 h; h×ng/mL)			
		Mean (SE)	Median (range)	Predicted	
1	0.15 mg	6	4.2 (2.9)	0.0 (0.0–19.9)	..
2	0.50 mg	6	0.0 (0.0)	0.0 (0.0–0.0)	..
3	1.50 mg	6	34.9 (10.3)	25.3 (12.3–77.6)	60.9
4	3.00 mg	6	77.6 (26.2)	62.1 (24.8–199.8)	121.7

Area under the curve was calculated from individual measurements of plasma from start of treatment to 12 h after treatment. Predicted area under the curve = monkey area under the curve at steady state 24 h/(human bodyweight [70 kg]/monkey bodyweight [4 kg]) × (human dose [total mg]/monkey dose [mg/day]). The appendix shows area under the curve values in Rhesus monkeys. The area under the curve value at 0.1 mg per day was used to estimate human values.

Table 5: Predicted and measured human plasma concentration of ISIS 333611 by cohort

See Online for appendix

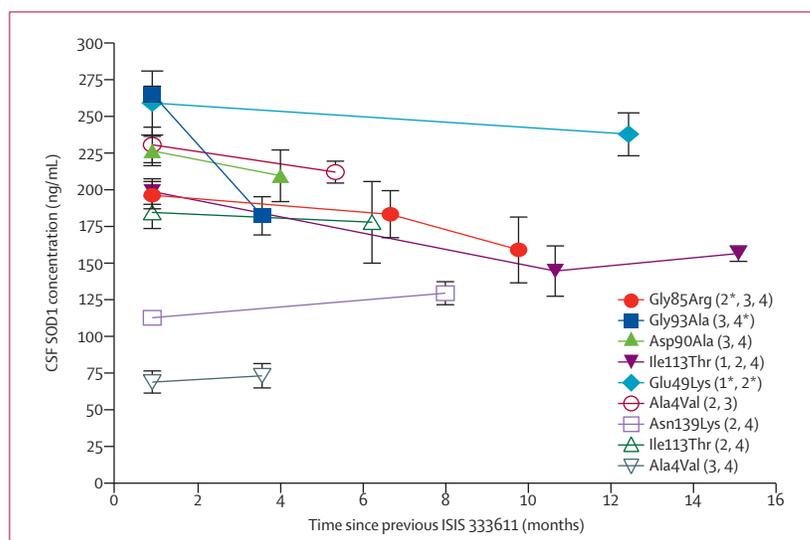


Figure 3: SOD1 protein concentrations in CSF of patients enrolled in more than one cohort. Measured by ELISA. SOD1 mutation and cohort number are shown for each patient. *Placebo group for that cohort.

Discussion

The ability to directly modulate gene expression in the brain and spinal cord affords new treatment opportunities for neurological diseases, especially neurodegenerative disorders. Our first-in-human clinical study shows the feasibility of intrathecal delivery of antisense oligonucleotides into the CNS. Because this approach is new, we used single, escalating, low-dose infusions. ISIS 333611 was well tolerated with no dose-limiting toxic effects or safety concerns. The data from this study provide encouragement for further development of antisense oligonucleotides for the treatment of neurodegenerative diseases, although the small numbers of participants and low doses limit broader conclusions about the tolerability of intrathecal antisense oligonucleotides. Direct infusion of an antisense oligonucleotide into tumour tissue by convection-enhanced delivery also seems to be similarly well tolerated.¹⁴

We report a clear dose-dependent relation between drug concentrations in CSF and plasma. The agreement between measured pharmacokinetic data and the predicted values supports the use of CSF volume and bodyweight scaling for prediction of human CSF concentration and plasma exposure. These findings should help the selection of doses in future clinical studies.

Based on an estimated tissue half-life of roughly 28 days in preclinical studies, the autopsy samples from a patient who died from amyotrophic lateral sclerosis were taken roughly three half-lives from when the last dose was given. Drug concentrations in spinal cord tissue were easily measured and were much higher than the limit of quantification (5 ng/g). Our results suggest that

the human elimination half-life might be in the same range as that in monkeys (roughly 30 days).

In patients who participated in more than one cohort and therefore for whom repeat CSF samples were available, SOD1 CSF concentrations were on average within 12% of the original value. This finding is consistent with the low dose of antisense oligonucleotides used and is also consistent with previous repeated measurements of SOD1 CSF concentration,⁹ showing an average 7% variation in SOD1 CSF protein concentration when measured 1–12 months apart. The stability of CSF SOD1 concentrations shown by these data, coupled with data from studies of rats,⁹ showing that reduction of SOD1 concentrations in brain tissue correlates with reduced SOD1 in the CSF, strongly support use of CSF SOD1 protein concentrations as a pharmacodynamic marker for antisense oligonucleotide activity in future clinical trials of SOD1 amyotrophic lateral sclerosis.

Our study has several limitations. The doses we used were intended to represent a single dose that would be given as a continuous infusion and thus were low. We predict that the highest dose in our study would need to be given continuously for 4 days to reduce SOD1 mRNA and protein concentrations in human spinal cord. The small number of participants also limits conclusions about safety because very rare events might not occur in a small sample. Lastly, the ISIS 333611 group had more men than did the placebo group. Nevertheless, our study is an important first step in using antisense oligonucleotides to treat neurological disease (panel). Patients with Ala4Val mutations in SOD1 typically progress from symptom onset to death very rapidly (ie, in less than 12 months) and thus even short-term reduction of SOD1 concentrations might be beneficial. Before testing long-term treatment in more slowly progressive SOD1-related amyotrophic lateral sclerosis or treating patients before they develop symptoms, we should more fully understand the effects of chronic, long-term reductions of SOD1 concentrations, because knockout of SOD1 has been linked to liver cancer¹⁵ and late life motor neuropathy.¹⁶ Our approach mitigates these concerns because of the only partial reduction of SOD1 concentration and little exposure to peripheral tissues after intrathecal delivery. The antisense oligonucleotide we used is designed to activate RNase H mediated degradation of all SOD1, rather than a particular SOD1 mutant, which enables this approach to be applied broadly to almost all of the more than 100 different mutations in SOD1 known to cause amyotrophic lateral sclerosis.⁷ Experiments in mice show that wild-type SOD1 can increase the toxicity of mutant SOD1,¹⁷ thus decreasing both mutant and wild-type SOD1 has an additional theoretical benefit.

Studies suggest that SOD1 could also be involved in sporadic amyotrophic lateral sclerosis. First, Gruzman and colleagues¹⁸ reported an SOD1-reactive protein (after chemical crosslinking of homogenates of spinal

Panel: Research in context**Systematic review**

We searched PubMed for reports in English between Jan 1, 1966, and March 20, 2013. We used the terms “antisense oligonucleotide”, “clinical trial”, and “neurologic”. We found no reports of human clinical trials using antisense oligonucleotides to the CNS. A search for “antisense oligonucleotide” and “glioma” returned one clinical trial using convection-enhanced delivery of an antisense oligonucleotide directly to a CNS tumour. This type of delivery was well tolerated.

Interpretation

Our study is the first clinical report of delivery of antisense oligonucleotides to the CSF. ISIS 333611 was well tolerated when administered as an intrathecal infusion in patients with SOD1 familial amyotrophic lateral sclerosis. CSF and plasma drug concentrations were consistent with those predicted from preclinical studies. These conclusions are limited by the small doses given and the small numbers of patients studied.

cord tissues) in a small number of patients with sporadic amyotrophic lateral sclerosis but not controls. Second, antibodies that target misfolded SOD1 showed misfolded SOD1 in vulnerable spinal cord neurons of some patients with amyotrophic lateral sclerosis, but not control patients^{19,20} while other well designed, larger studies²¹ did not find this pathology in sporadic amyotrophic lateral sclerosis. Third, reduction of SOD1 concentrations in astrocytes derived from patients with sporadic amyotrophic lateral sclerosis reversed the toxic effects of these astrocytes when cocultured with motor neurons,²² again implying that SOD1 might contribute to the pathogenesis of sporadic amyotrophic lateral sclerosis. Although the rationale for treatment with antisense oligonucleotides is strongest for familial SOD1-related amyotrophic lateral sclerosis, this therapeutic approach could be considered for treatment of sporadic amyotrophic lateral sclerosis, should sufficient data emerge. Our study will enable future studies of similar antisense drugs for familial SOD1 amyotrophic lateral sclerosis, other genetic forms of amyotrophic lateral sclerosis, and other neurodegenerative diseases.

Contributors

TMM, CFB, MEC, KMB, and RS designed the study, AP, DW, JR, and ES enrolled patients. AP, DW, JR, ES, TMM, MEC, LWO, KM, PA, KA, KMB, and SA coordinated and managed the study. TM, CFB, MEC, KMB, DS, EM, and DN analysed the data. LWO, GM, and MC analysed tissue samples. TM, CFB, MEC, and KMB wrote the report. TM, AP, WD, JR, ES, KM, PA, KB, LWO, EM, DN, GM, MC, RS, CFB, and MEC edited the report.

Conflicts of interest

KA, DN, KMB, and CFB are employees of Isis Pharmaceuticals. Isis Pharmaceuticals provided support for this trial and provides antisense oligonucleotides to TMM for animal studies. RS, CFB, the Center for Neurologic Study, and Isis Pharmaceuticals have filed a patent for use of the ISIS 333611. ES is a paid speaker for Grifols for Gamunex and served

on an advisory board for CSL Behring. AP receives revenue related to antibody patent licenses and speaker honoraria from Athena; owns stock in Johnson & Johnson; directs the Washington University Neuromuscular Clinical Laboratory, which does antibody testing; and receives research support from the NIH, Muscular Dystrophy Association, Neuromuscular Research Fund, Insmmed, Knopp, Cytokinetics, Biogen Idec, ISIS, Genzyme, GlaxoSmithKline, Ultragenyx, and Sanofi. RS is a consultant for Isis Pharmaceuticals. JR receives grant support from NIH, ALSA, MDA, Cytokinetics, Biogen, and Psyadon Pharmaceuticals. EAM serves on the data and safety monitoring boards for Lantheus Medical Imaging and Shire Human Genetic Therapies. PLA is named as an inventor for patent 7493812. MEC receives grant support from Isis Pharmaceuticals, Biogen, and Knopp and is a consultant for Teva, Cytokinetics, and Biogen. TMM has received grant support from Isis Pharmaceuticals, NIH, ALSA, MDA, Tau consortium, Mallinckrodt Foundation, Cure PSP, Washington University Hope Center, Project 5 for ALS, Johns Hopkins Packard Center, and Washington University ICTS. DS does extensive consulting for pharmaceutical companies including Aptiv Solutions, Agenix, Asubio Pharmaceuticals, Alexion Pharmaceuticals, Cytokinetics, Edison Pharmaceuticals, GlaxoSmith Kline, Merck, NeuroNova, and Pfizer. The other authors declare that they have no conflicts of interest.

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