Antisense oligonucleotide jacifusen for *FUS*-ALS: an investigator-initiated, multicentre, open-label case series

Neil A Shneider, Matthew B Harms, Vlad A Korobeynikov, Olivia M Rifai, Benjamin N Hoover, Elizabeth A Harrington, Sonya Aziz-Zaman, Jessica Singleton, Arish Jamil, Vikram R Madan, Ikjae Lee, Jinsy A Andrews, Richard M Smiley, Mahabub M Alam, Lauren E Black, Minwook Shin, Jonathan K Watts, David Walk, Daniel Newman, Robert M Pascuzzi, Markus Weber, Christoph Neuwirth, Sandrine Da Cruz, Armand Soriano, Roger Lane, Scott Henry, Joel Mathews, Paymaan Jafar-Nejad, Dan Norris, Frank Rigo, Robert H Brown, Stephan Miller, Rebecca Crean, C Frank Bennett

Summary

Background Pathogenic variants of fused in sarcoma (*FUS*) cause amyotrophic lateral sclerosis (*FUS*-ALS), with evidence of gain of function. Jacifusen is an antisense oligonucleotide targeting *FUS* pre-mRNA, previously shown to delay neurodegeneration in a mouse model and potentially slow functional decline in a first-in-human study. Here, we sought to further evaluate use of jacifusen as a treatment for *FUS*-ALS.

Methods This expanded access programme was conducted through a series of single-patient investigational new drug applications at five sites (four hospitals in the USA and one in Switzerland). Participants carried a *FUS* variant and had clinical evidence of motor neuron disease onset or electrophysiological abnormalities, if not a diagnosis of ALS. Participants were ineligible if chronically ventilated with tracheostomy. Enrolled sequentially, participants received serial intrathecal injections of jacifusen over 2·8–33·9 months. Based on multiple ascending doses of jacifusen (from 20 mg to 120 mg), successive protocols were modified as safety and other data were acquired, with the last participants enrolled receiving 120 mg doses monthly from the start of their treatment. Safety was assessed using the Common Terminology Criteria for Adverse Events version 4.0 and standard cerebrospinal fluid (CSF) metrics. Concentration of neurofilament light chain (NfL) in CSF was used as a biomarker of axonal injury and neurodegeneration, and the ALS Functional Rating Scale-Revised (ALSFRS-R) score was used as an overall measure of motor function. Biochemical analysis and immunohistochemical staining were done on post-mortem CNS tissues to quantify FUS protein expression and assess the burden of FUS pathology.

Findings Between June 11, 2019, and June 2, 2023, we recruited 12 participants (median age 26 years [range 16–45]; seven [58%] were female and five [42%] were male) into the expanded access programme. Transient elevations in cell counts or total protein concentration in CSF (six [50%] participants) were unrelated to treatment duration. The most common adverse events were back pain (six [50%]), headache (four [33%]), nausea (three [25%]), and post-lumbar puncture headache (three [25%]). Two participant deaths were recorded during the programme, both thought to be unrelated to the investigational drug. The concentration of NfL in CSF was reduced by up to 82·8% after 6 months of treatment. Although most participants had continued functional decline (as measured by ALSFRS-R) after starting treatment with jacifusen, one showed unprecedented, objective functional recovery after 10 months, and another remained asymptomatic, with documented improvement in electromyographic abnormalities. Biochemical and immunohistochemical analysis of CNS tissue samples from four participants showed reduced FUS protein levels and an apparent decrease in the burden of FUS pathology.

Interpretation The findings suggest the safety and possible efficacy of jacifusen for treating *FUS*-ALS. The efficacy of jacifusen is being further evaluated in an ongoing clinical trial.

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Department of Neurology, Columbia University Irving Medical Center, New York, NY, USA (Prof N A Shneider MD, M B Harms MD, V A Korobeynikov MD, O M Rifai PhD, B N Hoover BA, E A Harrington MS, S Aziz-Zaman MS, J Singleton BA, A Jamil BA, V R Madan MPH, I Lee MD, J A Andrews MD, Prof R M Smiley MD, M M Alam MS); **Charles River Laboratories, Wilmington, MA, USA** (L E Black PhD); **College of Pharmacy, Sookmyung Women's University, Seoul, South Korea** (M Shin PhD); **UMass Chan Medical School, University of Massachusetts, Worcester, MA, USA** (Prof J K Watts PhD); **University of Minnesota Medical School, Minneapolis, MN, USA** (Prof D Walk MD); **Henry Ford Hospital, Detroit, MI, USA** (D Newman MD); **Indiana University School of Medicine, Indianapolis, IN, USA** (Prof R M Pascuzzi MD); **Kantonsspital St Gallen, St Gallen, Switzerland** (Prof M Weber MD, C Neuwirth MD); **VIB-KU Leuven Center for Brain and Disease Research, Department of Neurosciences, KU Leuven and Leuven Brain Institute, Leuven, Belgium** (Prof S Da Cruz PhD); **Ionis Pharmaceuticals, Carlsbad, CA, USA** (A Soriano BS, R Lane MD, S Henry PhD, J Mathews PhD, P Jafar-Nejad MD, D Norris PhD, F Rigo PhD, Prof R H Brown MD, S Miller PhD, R Crean PhD, C F Bennett PhD)

Introduction

Pathogenic variants of fused in sarcoma (*FUS*) are associated with the neurodegenerative disease amyotrophic lateral sclerosis (ALS), including early-onset and rapidly progressive forms.[1–5] Over 40 causative *FUS* variants have been identified, accounting for 3% of cases of familial ALS in European populations and 6–10% of cases in Asian populations, as well as 1% of cases without a family history of this disease.[6,7] The clinical presentation of *FUS*-associated ALS (*FUS*-ALS)

Correspondence to:
Prof Neil A Shneider, Department of Neurology, Columbia University Irving Medical Center, New York, NY 10032, USA
ns327@cumc.columbia.edu

Research in context

Evidence before this study

We searched PubMed from database inception to Dec 31, 2024, with the search string "("antisense oligonucleotide") AND (FUS OR "Fused in sarcoma") AND (ALS OR "motor neuron disease" OR neurodegenerative OR neurodegeneration)", with no language restrictions. Cellular and animal models, as well as human pathological data, show gain-of-function toxicity for fused in sarcoma (FUS) protein and, thus, support reduction of FUS expression as a therapeutic approach for *FUS*-associated amyotrophic lateral sclerosis (*FUS*-ALS). Our first-in-human study evaluating serial intrathecal injections of jacifusen, a *FUS* antisense oligonucleotide, indicated that FUS pathology was reduced and functional decline possibly slowed in a single patient with *FUS*-ALS. We did not identify any other clinical studies evaluating the safety, tolerability, or efficacy of jacifusen or any related *FUS* antisense therapeutic in patients with *FUS*-ALS or any related neurodegenerative disorder.

Added value of this study

To our knowledge, this case series is the first expanded access programme involving serial intrathecal administration of jacifusen. It involved 11 participants in addition to the patient in our previous study. Minimal drug-related adverse events were observed. The data show substantial reduction of neurofilament light chain, a biomarker of neurodegeneration, in cerebrospinal fluid in participants with pathogenic *FUS* variants and possible clinical benefit in two participants. Preclinical studies and post-mortem analyses further characterised jacifusen pharmacokinetics in animal models and in four study participants, including changes in the amount and aggregation of FUS protein.

Implications of all the available evidence

There are currently no approved treatments for *FUS*-ALS, a uniformly fatal disease often associated with early onset and rapid progression. The evidence in this report suggests that serial intrathecal administration of jacifusen to reduce FUS protein production is a safe and possibly effective intervention. Favourable clinical outcomes were associated with earlier intervention, a long and consistent treatment course, or both. The results of the ongoing global, phase 1–3, double-blind, placebo-controlled trial of jacifusen will be needed to definitively establish clinical efficacy.

is heterogeneous, but onset generally occurs at a younger age in patients with *FUS*-ALS (mean age 37 years; range 11–80) than in patients in the overall ALS population.[8] *FUS* variants are the most common cause of rare, early-onset forms of ALS,[6,8–10] accounting for more than 40% of juvenile cases (ie, onset between the ages of 18 years and 25 years) and more than 50% of paediatric ALS cases (ie, onset before age 18 years).[10] In particular, the pPro525Leu variant and truncating frameshift variants are associated with the earliest onset and most rapid progression.[8,9,11] In-vitro and in-vivo studies support a toxic gain-of-function mechanism for mutant FUS proteins,[12,13] suggesting that reducing production of FUS might be of therapeutic benefit for patients with *FUS*-ALS.[13] Indeed, reducing *FUS* expression with the antisense oligonucleotide jacifusen (also referred to as ulefnersen or ION363) in a *FUS*-ALS mouse model delayed the onset of motor neuron degeneration and provided the rationale for a first-in-human trial.[14]

See Online for appendix

The *FUS* antisense oligonucleotide jacifusen was designed to reduce translation of FUS protein by targeting a highly conserved sequence in intron 6 of human *FUS* pre-mRNA, triggering RNase H-mediated degradation. We provided preliminary evidence that, in a patient with early-onset ALS and a pPro525Leu variant, repeated intrathecal injections of jacifusen effectively decreased the amount of FUS protein in the brain and spinal cord and markedly reduced FUS neuropathology.[14] Through a series of individual-use, expanded access investigational new drug applications, we have now treated 11 additional participants (ie, 12 in total) with *FUS* variants on a compassionate-use basis. Here, we describe the results of this investigator-initiated treatment programme, providing initial evaluation of the safety, pharmacokinetics, and pharmacodynamics of jacifusen, as well as biomarker and clinical data and pathological findings in participants with *FUS*-ALS.

Methods

Study design

The open-label, expanded access programme was approved by the Human Research Protection Office of Columbia University Irving Medical Center. The programme was done at five sites (university-affiliated hospitals; four in the USA and one in Switzerland). Investigators, sites and institutional review board (IRB) protocol numbers are listed in the appendix (p 4). Through a reliance agreement with all other sites, Columbia University served as the IRB of record for all treatments, with data analysed under IRB-AAAU8004. This investigator-initiated, multicentre case series was conducted through a series of single-participant investigational new drug applications, with modifications to treatment protocols based on knowledge gained through the treatment of each successive participant. All participants or their legal representatives provided written informed consent to participate and for publication of detailed, de-identified information. We evaluated the safety and tolerability of jacifusen, as well as the clinical benefit of chronic administration of jacifusen via clinical assessments and analysis of neurofilament light chain (NfL) in cerebrospinal fluid (CSF).

Participants

Participants underwent genetic testing in a Clinical Laboratory Improvement Amendments-certified laboratory to confirm *FUS* mutations. Although there were no predefined inclusion criteria for the expanded access programme, all participants carried a *FUS* variant and either met El Escorial[15] and Gold Coast criteria[16] for a diagnosis of ALS, had clinical evidence of disease onset (participant 4), or had electrophysiological abnormalities as detected by electromyography (appendix p 9) and a family history of *FUS*-ALS (participant 11). Symptom onset was defined by the first motor manifestations of disease (ie, bulbar, spinal, or respiratory). At treatment initiation, most participants had a clinical diagnosis of probable or definite ALS; time of diagnosis was established when criteria for probable or definite ALS were met. Those who were chronically ventilated with a tracheostomy were ineligible to participate, but if mechanical ventilation was required during treatment, participation was allowed to continue.

Procedures

The methods for preclinical studies in human cells, wild-type and transgenic mice, and cynomolgus monkeys (including quantification of *FUS* mRNA after administration of jacifusen in all models, analysis of tissue half-life and duration of action of jacifusen in wild-type mice, and analysis of jacifusen concentration in cynomolgus monkeys) are described in the appendix (pp 5–8).

Individualised treatment protocols were developed for repeated, intrathecal bolus administration of jacifusen via lumbar puncture (see appendix p 17 for detailed administration schedules). Briefly, successive protocols were modified as safety and other data were acquired. For participant 1, the safety of biweekly (ie, every 2 weeks) ascending doses (20 mg, 40 mg, 60 mg, and 80 mg) of jacifusen was assessed over a 10-week period, after which a monthly (ie, 4-weekly) administration schedule was initiated, starting with a 100 mg dose and followed by 120 mg doses thereafter. Participant 2 received biweekly ascending doses (40 mg, 80 mg, and 120 mg) of jacifusen, followed by monthly administration of 120 mg. Participants 3, 4, 5, 6, and 10 received an initial 60 mg dose of jacifusen, followed by one 120 mg dose 2 weeks later, then monthly 120 mg doses. Subsequently enrolled participants 7, 8, 9, 11, and 12 received monthly doses of 120 mg jacifusen throughout their treatment. In some cases, at the discretion of the principal investigator, 120 mg of jacifusen were administered every 2 months following at least three monthly doses. Prespecified reasons for which treatment would have been stopped or interrupted are described in the appendix (p 9).

Before drug administration on each day of treatment, motor, neurological, and respiratory function were assessed by standard motor exam and the ALS Functional Rating Scale-Revised (ALSFRS-R),[17] standard neurological exam, and slow vital capacity, respectively. Participants underwent up to 24 h of inpatient observation after the initial administration of jacifusen and after each dose escalation. For subsequent administrations, observation was shortened to 6 h at the investigator's discretion. In-person or telephone follow-ups were conducted 3–12 days after each administration, with a final telephone follow-up 28 days after the last treatment. Blood samples and CSF samples (used for both standard metrics and NfL measurements) were collected before each treatment administration; blood and urine samples were collected approximately 1 h after each treatment administration, for safety monitoring. Concentration of NfL in CSF (a biomarker of axonal injury and neurodegeneration) was assessed with an antibody assay (appendix p 9). Jacifusen concentration in post-mortem motor cortex tissue samples was measured with a hybridisation enzyme-linked immunosorbent assay (appendix p 11)

Methods for western blotting and immunohistochemistry (to evaluate FUS protein levels in post-mortem motor cortex and the burden of FUS pathology in post-mortem motor cortex and spinal cord, respectively) are in the appendix (pp 10–11). For immunohistochemical staining of FUS, 3,3□-diaminobenzidine (brown) was used for detection, with a haematoxylin counterstain (blue) to visualise nuclei. Semiquantitative assessment of the abundance of pathological features on de-identified slides of tissue sections (stained with anti-FUS antibody; appendix pp 11, 36) was done by a pathologist (VAK) with the following scale: absent (–); extremely rare (+/–; ie, one feature in the entire examined section); rare (+; ie, one feature in several ×20 fields); occasional (++; ie, features easily found but not in every ×20 field); and common (+++; ie, features very easily found, up to several in most ×20 fields). Details related to all tissues, including untreated *FUS*-ALS and non-neurological control tissues, are in the appendix (pp 34–35).

Outcomes

The main outcome was safety. Safety monitoring included evaluation of vital signs, assessment of adverse events using the Common Terminology Criteria for Adverse Events version 4.0, and evaluation of standard CSF metrics—ie, total protein concentration and cell count in CSF. Headache, seizure, transient deficits in lower spinal cord reflexes, platelet decline, and proteinuria were considered expected adverse events.

The functional status of participants was assessed via the ALSFRS-R (which measures bulbar function, gross and fine motor skills, and respiratory function on a 0–48 point scale, with lower scores indicating greater impairment).[17] For some participants, respiratory muscle function was quantified as percentage of predicted slow vital capacity; collection of this dataset was limited by

	Participant characteristics				ALS characteristics				Symptom onset		Baseline clinical characteristics		Treatment duration	Timing of percutaneous endoscopic gastrostomy placement and non-invasive ventilation			Disposition at study cutoff		
	Sex	Age, years	Race or ethnicity	Twin	Possible, probable, or definite	Inheritance	Variant	Variant classification	Age, years	Symptoms	ALSFRS-R total score	Slow vital capacity, % predicted	Treatment duration, months	Percutaneous endoscopic gastrostomy	Non-invasive ventilation	Riluzole use	Alive	Trache-ostomy	Entered FUSION trial
1	F	25	White	Yes	Definite	Sporadic	pPro525Leu	Pathogenic	25	Limb	17	9·1	9·0	None	Baseline	No	No	NA	No
2	M	20	White	No	Definite	Sporadic	pPro525Leu	Pathogenic	19	Limb	32	41·3	21·9	During treatment	Baseline	Yes	Yes	Yes	No
3	M	22	White	Yes	Definite	Familial	pGly515_Glu516insAsp	Variant of uncertain significance	21	Bulbar	40	39·3	10·2	During treatment	During treatment	Yes	Yes	Yes	No
4	M	22	White	Yes	Definite*	Familial	pGly515_Glu516insAsp	Variant of uncertain significance	22	Bulbar	48	84·8	10·6	During treatment	During treatment	No	Yes	Yes	Yes
5	F	38	White	No	Definite	Familial	pArg521Leu	Pathogenic	38	Limb	39	··†	9·7	During treatment	Baseline	Yes	No	NA	Yes
6	F	20	White	No	Definite	Sporadic	pGln519IlefsTer9	Pathogenic	20	Bulbar	35	··†	2·8	Baseline	During treatment	No	No	NA	No
7	F	45	White	No	Definite	Familial	pArg521Gly	Pathogenic	43	Limb	7	28·0	9·5	Baseline	Baseline	No	No	NA	No
8	F	37	White	No	Definite	Familial	pArg521Leu	Pathogenic	36	Limb	46	91·0	5·6	None	None	No	Yes	No	Yes
9	M	45	White	No	Probable	Sporadic	pGly191Ser	Variant of uncertain significance	39	Limb	11	··‡	3·7	Baseline	Baseline	Yes	No	NA	No
10	F	16	White	No	Definite	Sporadic	pPro525Leu	Pathogenic	15	Respiratory	28	··‡	33·9	Baseline	Baseline	No	Yes	Yes	No
11	M	36	White, non-Hispanic or Latinx	No	Presympt-omatic	Familial	pArg521Leu	Pathogenic	36	NA	48	88·5	7·9	None	None	No	Yes	No	Yes
12	F	26	Unknown, non-Hispanic or Latinx	No	Definite	Sporadic	pPro525Leu	Pathogenic	25	Limb	28	··‡	6·3	Baseline	Baseline	Yes	Yes	No	No

Sex and race or ethnicity were self-reported; Hispanic or Latinx specified if information was available. No participants were receiving edaravone, except for participant 10 in the last 2 months of treatment (appendix pp 30–31). No participants had a tracheostomy at baseline; tracheostomy data are listed as yes or no if the participant was alive at study cutoff and NA otherwise. ALS=amyotrophic lateral sclerosis. ALSFRS-R=ALS Functional Rating Scale-Revised. NA=not applicable. *Patient was symptomatic at baseline, later diagnosed with definite ALS. †Protocol modified due to COVID-19. ‡Participant not willing to perform task.

***Table 1*: Demographics, baseline disease characteristics, and outcomes for participants in the expanded access programme**

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restrictions during the COVID-19 pandemic. CSF NfL concentration was used as a biomarker of axonal injury and neurodegeneration. Baseline nerve conduction studies and electromyography studies for participant 11 were conducted in line with the protocol for the ALS Families Project at Columbia University (appendix pp 9–10); further electromyography assessments were done after 1 year. Reduction of FUS protein expression and pathological burden were assessed with western blotting and immunohistochemistry methods, respectively. Safety metrics, NfL measurements, and ALSFRS-R assessments were collected for all participants to the extent to which they were able. Sex and race or ethnicity data were reported as listed in electronic health records.

Statistical analysis

Clinical data were reported using descriptive statistics, with a data cutoff of Dec 15, 2023. Pre-treatment ALSFRS-R rates of decline were approximated on the basis of the assumption of a maximal score of 48 at symptom onset. NfL concentrations and ALSFRS-R data were plotted with the ggplot2 package, R version 4.3.2.[18,19] For analyses of pathological data, p values were calculated with one-way ANOVA and post-hoc Tukey's multiple comparisons test and visualised with GraphPad Prism version 10.2.3.

Role of the funding source

With the exception of Ionis Pharmaceuticals, the funding sources of this expanded access programme had no role in programme design, data collection, data analysis, data interpretation, writing of the report, or the decision to submit the Article for publication. Ionis Pharmaceuticals was not involved in decisions related to programme design or collection of clinical data but did contribute to the collection of preclinical data, analysis and interpretation of all data, writing of the report, and the decision to submit the paper for publication.

Results

The ability of jacifusen to reduce the amount of *FUS* mRNA in the CNS was assessed across multiple preclinical models. Jacifusen produced dose-dependent reductions in *FUS* mRNA in human cells, CNS tissues in mice expressing the human *FUS* gene, and CNS tissues from cynomolgus monkeys, which correlated with tissue exposure (appendix pp 18–19). The tissue half-life for jacifusen was 48 days (95% CI 43–55) in mouse spinal cord tissue and 55 days (48–66) in mouse cortical tissue (appendix pp 18). The long half-life in CNS tissue translated to durable pharmacological activity in mice, with the reduction in *FUS* mRNA lasting several months following a single intracerebroventricular dose (appendix p 18).

From June 11, 2019, to June 2, 2023, 12 participants were enrolled into the expanded access programme. Table 1 summarises the demographics and baseline characteristics of the programme participants; medical histories and concomitant medications are in the appendix (pp 30–31). Median participant age was 26 years (range 16–45), seven (58%) participants were female and five (42%) were male, and six (50%) had familial ALS. All participants had a confirmed *FUS* variant. The 12 participants carried six different variants, of which four were classified as pathogenic and two as variants of uncertain significance according to American College of Medical Genetics and Genomics standards. In ten participants, treatment was initiated after ALS symptoms were well established and the criteria for probable or definite ALS had been met. Participant 4 was first treated when their symptoms were mild but went on to meet criteria for definite ALS. For these 11 participants, the median time from symptom onset to treatment initiation was 8 months (range 6–67 months). The final participant had abnormal electromyography findings but no weakness or other signs of clinically manifest disease and remained asymptomatic throughout treatment. For this participant, the time from abnormal electromyography findings to treatment initiation was 3·0 months. For participants with an ALS diagnosis at treatment initiation, the median time from diagnosis to treatment was 3·0 months (1·4–49·8). Four participants transferred to the open-label arm of the FUSION randomised controlled trial (NCT04768972) after 10·9 months (range 7·4–12·3).

Median treatment duration was 9·3 months (range 2·8–33·9). The median total dose of jacifusen administered to patients was 930 mg (range 200–2940), delivered across a median of 9·5 administrations (range 4–25). During the programme, 80 treatment-emergent adverse events occurred in 11 (92%) participants (appendix p 32); the most common of these adverse events were back pain (six [50%] participants), headache (four [33%]), nausea (three [25%]), and post-lumbar puncture headache (three [25%]). Treatment-emergent adverse events were assessed as mild (51 [64%]), moderate (23 [29%]), severe (one [1%]), life-threatening (three [4%]), and fatal (two [3%]). Overall, nine serious adverse events occurred in five (42%) participants; these events were aspiration pneumonitis or pneumonia, aspiration, pain following percutaneous endoscopic gastrotomy tube placement, skull fracture and concussion due to a fall, respiratory distress due to an aspiration event, pleural effusion, atelectasis, and respiratory failure. No serious adverse event was considered possibly or definitely related to treatment (appendix p 33). 12 treatment-emergent adverse events in seven (58%) participants were considered possibly related to treatment; of these, headache was the most common adverse event, assessed as possibly related in two (17%) participants (appendix p 32). No treatment-emergent adverse events were assessed as definitely related to treatment. No cases of meningitis, myelitis, radiculitis, or papilloedema occurred.

Asymptomatic abnormalities in CSF parameters were seen in six (50%) of 12 participants (appendix p 20). Three participants (25%) had at least one cell count of more than five cells per µL after treatment initiation. At least one instance of protein concentration higher than 45 mg/dL in CSF was noted in six (50%) participants, of whom one had had an abnormally high concentration of protein at baseline. The maximum observed cell count was 23 cells per µL and maximum observed protein concentration was 98 mg/dL, in the same participant. These findings did not lead to treatment discontinuation, nor was there a clear relationship between abnormal CSF findings and treatment duration.

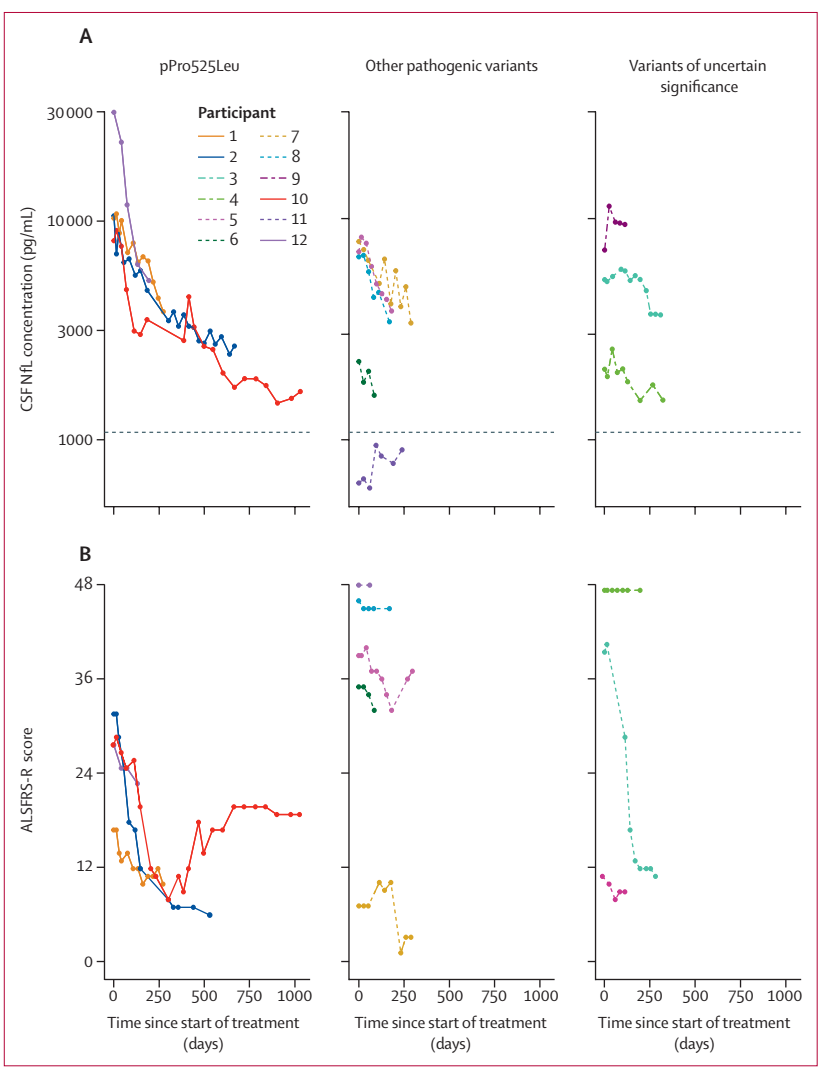


Figure 1: **Concentrations of NfL in CSF and ALSFRS-R scores**
(A) Concentration of NfL in CSF stratified by variant type (ie, pPro525Leu, other pathogenic, and variant of uncertain significance). Values are shown on a log scale. The dashed reference line corresponds to the 90th percentile NfL concentration in age-matched (mean 30 years) healthy controls (1080 pg/mL).[20,21] (B) ALSFRS-R scores stratified by variant type (ie, pPro525Leu, other pathogenic, and variant of uncertain significance). ALSFRS-R measures bulbar function, gross and fine motor skills, and respiratory function on a 0–48 point scale, with lower scores indicating greater impairment. ALSFRS-R=Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised. CSF=cerebrospinal fluid. NfL=neurofilament light chain.

Here, we present disease history and biomarker and clinical assessments for participants 1, 2, 10, and 12, who carried the most aggressive *FUS* variant (pPro525Leu). Case vignettes for the remaining eight participants are in the appendix (pp 12–16). Participant 1 was a 25-year-old woman with a de-novo pPro525Leu variant, whose identical twin sister had died of *FUS*-ALS. Participant 1's treatment and outcomes were reported previously.[14] She was diagnosed with ALS 2 months after developing lower extremity weakness and gait difficulty. While jacifusen was being developed and tested, her ALSFRS-R score declined rapidly, from approximately 48 at symptom onset to 17 at treatment initiation (approximately 4·9 points per month), about 6 months after symptom onset. She received 12 administrations of jacifusen over 9 months, with dose starting at 20 mg and increasing to 120 mg monthly. In the first 5 months of treatment, her ALSFRS-R score declined by a mean of 1·3 points per month, to 10 (dose 8), and then remained between 10 and 12 (doses 9–12; figure 1B). NfL concentration in CSF decreased from 10 127 pg/mL at treatment initiation to 3807 pg/mL (dose 12; 62·4% reduction; figure 1A). Participant 1 subsequently died of respiratory failure secondary to ALS progression, 11 months after treatment initiation and 17 months after symptom onset.

Participant 2 had a de-novo pPro525Leu variant, with limb-onset ALS at the age of 19 years. Earlier in life, he had had delayed motor and speech development and learning disabilities. Between symptom onset and treatment initiation, his ALSFRS-R score declined from approximately 48 to 32 (approximately 1·4 points per month). He began jacifusen treatment after approximately 11 months of symptoms and received 21 administrations in total over 22 months, starting at 40 mg and with dose increasing by 40 mg every 2 weeks to a maximum of 120 mg. Treatment was paused for 4 months between doses 8 and 9 during the COVID-19 pandemic. His ALSFRS-R score declined by a mean 2·2 points per month from treatment initiation, to 7 at dose 10, then remained at 6–7 for the subsequent timepoints assessed (doses 10–17; figure 1B). Slow vital capacity decreased from 41·3% of predicted at treatment initiation to 6·7% of predicted at dose 7 and then increased to 15·9% of predicted at dose 13. NfL concentration in CSF decreased from 10 384 pg/mL to 3465 pg/mL at dose 9 (66·6% reduction) and remained at 2400–3900 pg/mL thereafter (doses 10–21; figure 1A). Participant 2 voluntarily withdrew from the programme 22 months after treatment initiation and 33 months after symptom onset. He died as a result of ALS progression 3·4 years after withdrawal from the study, 5·2 years after treatment initiation, and 6·2 years after symptom onset.

Participant 10 has a de-novo pPro525Leu variant. She first presented with tremor at the age of 3 years but met developmental milestones in childhood and early

adolescence. At the age of 15 years, she developed respiratory symptoms (ie, dysarthria). She was diagnosed with ALS at the age of 16 years—12 months after symptom onset. Further details on the process of her diagnosis are in the appendix (p 16). Between symptom onset and treatment initiation, her ALSFRS-R score declined from approximately 48 to 28 (approximately 1·5 points per month). Beginning approximately 13 months after symptom onset, she received an initial 60 mg dose of jacifusen, followed by one dose of 120 mg 2 weeks later and then monthly doses of 120 mg. She received 16 monthly administrations of jacifusen before switching to a bimonthly schedule. Her ALSFRS-R score declined by a mean 2·0 points per month, from 28 at treatment initiation to 8 at dose 10 (figure 1B), at which point she underwent tracheostomy. Subsequently, she had objective functional recovery, reflected by a mean increase of 1·0 points per month in her ALSFRS-R score over 12 months. Her ALSFRS-R score then remained at 19–20 for the final year before data cutoff. Increases were observed in ALSFRS-R items on handwriting, cutting food, dressing and hygiene, turning in bed, walking, dyspnoea, and respiratory insufficiency (appendix p 22). During this time, she also transitioned from continuous to intermittent ventilation, predominantly nocturnal. NfL concentration in CSF decreased from 8907 pg/mL (dose 2) to 1648 pg/mL (dose 25; 81·5% reduction; figure 1A). She continues to receive bimonthly administrations of jacifusen.

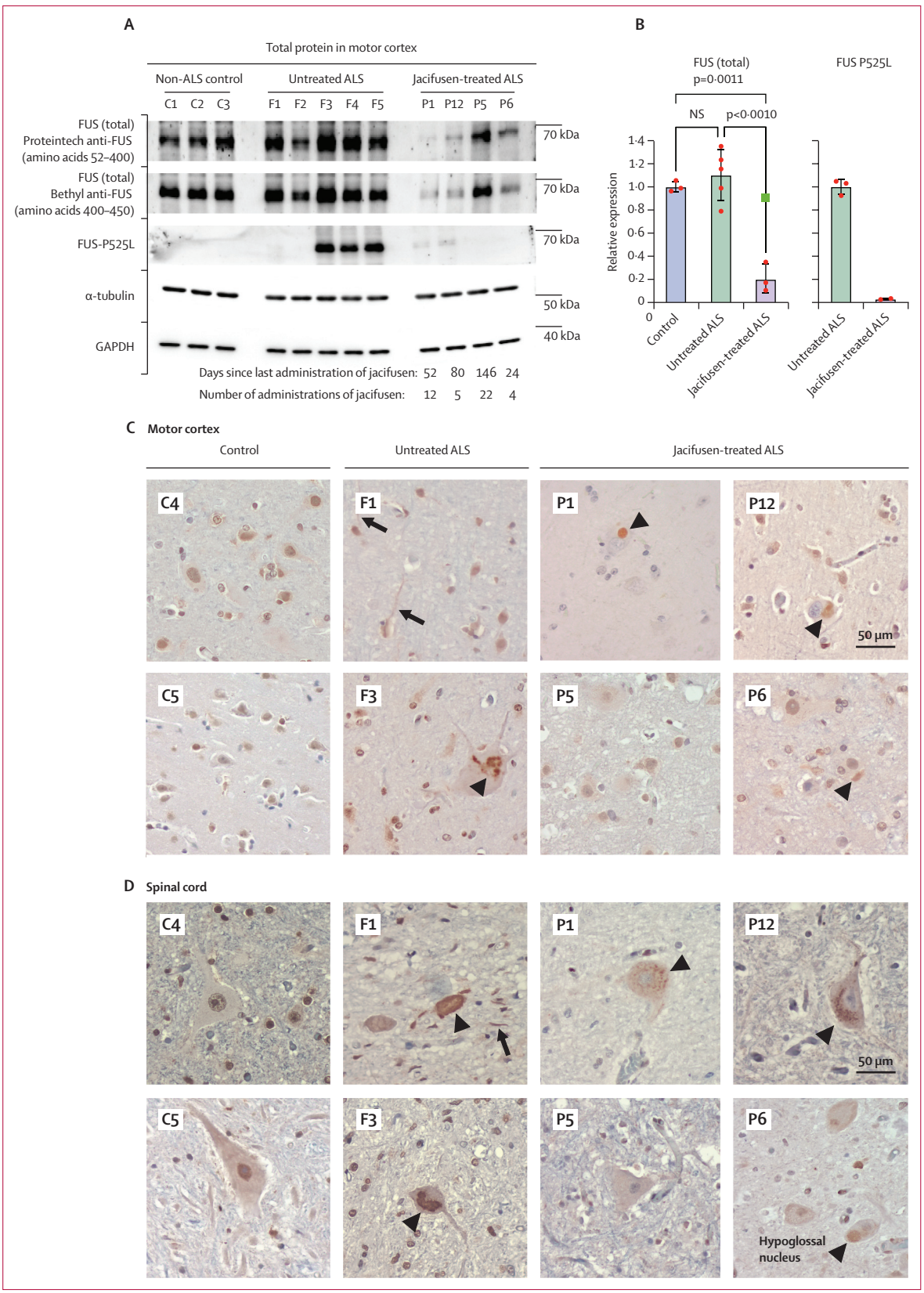
Participant 12, a female participant, had a de-novo pPro525Leu variant and limb-onset ALS. She had a history of hypotonia and developmental delay and was diagnosed with failure to thrive at the age of 1 year. Her first symptoms occurred when she was aged 25 years, and she was diagnosed with ALS 6 months later. Between symptom onset and treatment initiation her ALSFRS-R score declined by approximately 2·2 points per month, from approximately 48 to 28. Her treatment began approximately 9 months after symptom onset, with 120 mg jacifusen administered as three monthly doses followed by two bimonthly doses. Her ALSFRS-R score decreased by a mean of 1·2 points per month, from 28 at treatment initiation to 23 at dose 4 (figure 1A). NfL concentration in CSF decreased from 30711 pg/mL at treatment initiation to 5277 pg/mL at dose 5 (82·8% reduction; figure 1A). This participant died during treatment as a result of respiratory failure secondary to ALS progression after the data cutoff, 9 months after treatment initiation and 18 months after symptom onset.

We used western blot analysis to compare the expression of FUS protein in post-mortem frozen motor cortex tissue from participants 1, 5, 6, and 12; five untreated patients with *FUS*-ALS (pArg521Cys variant, n=2; pPro525Leu variant, n=3), including the identical twin of participant 1; and three age-matched and sex-matched non-neurological controls (figure 2A, B). The results indicated a significant reduction in FUS protein in treated participants versus untreated patients with *FUS*-ALS ($p<0·0010$) or non-neurological controls ($p=0·0011$). In participants 1, 6, and 12, whose deaths occurred less than two drug half-lives after their most recent administration of jacifusen (≤3·7 months), FUS protein expression was 66–90% lower than in untreated patients with *FUS*-ALS. Notably, however, for participant 5, who died 4·8 months after receiving her final administration of jacifusen, the amount of total FUS protein detected by western blotting was similar to that detected in untreated controls (figure 2A, B). Consistent with this finding, antisense oligonucleotide concentration in the motor cortex was lower in participant 5 than in participants 1, 6, and 12 (appendix p 37). As there was no evidence of antisense oligonucleotide activity in participant 5, she was excluded from statistical comparisons of FUS protein. In treated participants 1 and 12, who each carried the pPro525Leu (P525L) variant, FUS-P525L mutant protein levels were 3–4% of those in the three untreated controls with *FUS*-ALS carrying the same variant.

To assess whether the amount of FUS protein in jacifusen-treated participants was associated with the abundance of FUS-related pathological features,[22] we did immunohistochemical staining of total FUS protein in post-mortem tissue samples of motor cortex and spinal cord. Less staining of FUS protein was observed in participants 1, 6, and 12 than in untreated patients with *FUS*-ALS (figure 2C, appendix pp 25–27). Jacifusen antisense oligonucleotide was detected in all tissues (appendix p 29); quantitation in the motor cortex is reported in the appendix (p 37). For participant 1 (12 administrations over 9 months, 1·7 months since last administration), nuclear FUS staining was absent, and rare, dense FUS aggregates were seen in remaining motor neurons in the spinal cord and in layer V neurons of the motor cortex (figure 2C). For participant 6 (four administrations over 2·8 months, 0·8 months since last administration), examination of the brainstem and motor cortex showed somewhat patchy nuclear FUS staining with occasional inclusions in the hypoglossal nucleus motor neurons. For participant 12 (five administrations over 6·3 months, 2·6 months since last administration), patchy loss of nuclear FUS staining was seen, with aggregates throughout the spinal cord motor neurons and layer V neurons of the motor cortex. For participant 5 (22 administrations over 31·3 months, 4·8 months since last administration), nuclear FUS positivity was observed, with no apparent pathological staining. Semiquantitative evaluation of these findings is summarised in table 2.

Discussion

Results from this expanded access programme show the safety of repeated intrathecal administration of jacifusen in 12 participants with *FUS*-ALS. Adverse events were tolerable, with many associated with lumbar puncture



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and assessed as either unrelated or unlikely to be related to the drug. Mild elevation of cell count and protein concentration in CSF were observed in some participants; these transient effects were not associated with treatment duration or any clinical correlate. No evidence of meningitis, myelitis, radiculitis, or papilloedema was observed. Jacifusen treatment was also associated with a

	Nuclear FUS staining			Dense neuronal cytoplasmic inclusions			Granular neuronal cytoplasmic inclusions			Skein-like neuronal cytoplasmic inclusions			Glial cytoplasmic inclusions			Neuropil threads		
	Motor cortex	Cervical spinal cord	Lumbar spinal cord	Motor cortex	Cervical spinal cord	Lumbar spinal cord	Motor cortex	Cervical spinal cord	Lumbar spinal cord	Motor cortex	Cervical spinal cord	Lumbar spinal cord	Motor cortex	Cervical spinal cord	Lumbar spinal cord	Motor cortex	Cervical spinal cord	Lumbar spinal cord
Total FUS immunohistochemistry																		
C4	+++	+++	+++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	-
C5	+++	+++	+++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	-
C6	+++	+++	+++	–	–	–	–	–	–	–	–	–	–	–	–	–	–	-
F1 pArg521Cys	+++	+++	+++	–	+/–	–	+	+++	+++	+/–	+++	++	+/–	+	++	+	+++	++
F2 pArg521Cys	+++	+++	+++	–	+	+	+	++	++	–	+++	+++	+/–	++	++	++	+++	+++
F3 pPro525Leu	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	–	–	–	–	–	–	–	-
F4* pPro525Leu	+++	+++	+++	+	++	NA	++	+++	NA	–	+	NA	–	–	–	–	–	-
P1 pPro525Leu	+	+	–	+	–	–	+	++	++	–	–	–	–	–	–	–	–	-
P5 pArg521Leu	+++	+++	++	–	–	–	–	+/–	–	–	–	–	–	–	–	–	–	-
P6† pGln519IlefsTer9	++	++	++	+	+	+	++	++	++	–	–	–	–	+/–	+/–	–	+/–	
P12 pPro525Leu	++	++	+	++	++	+	+++	+++	++	–	–	–	–	–	–	–	–	-
FUS P525L-specific immunohistochemistry‡																		
F3 pPro525Leu	+++	+++	+++	+++	+++	+++	+++	+++	+++	–	–	–	–	–	–	–	–	-
F4 pPro525Leu	+++	+++	+++	+	++	NA	+++	+++	NA	–	+	NA	–	–	–	–	–	-
P1 pPro525Leu	–	–	–	+	–	–	+	+	–	–	–	–	–	–	–	–	–	-
P12 pPro525Leu	+	+	+	++	++	+	+++	+++	++	–	–	–	–	–	–	–	–	-

Semiquantitative grading of pathological features with respect to two distinct pathological patterns described in *FUS*-ALS: absent (–), extremely rare (+/–), rare (+), occasional (++), or common (+++). C4–C6 are healthy controls. F1–F4 are untreated patients with *FUS*-ALS. P1, P5, P6, and P12 are study participants. NA indicates that tissue was not available for assessment. ALS=amyotrophic lateral sclerosis. *FUS*-ALS=*FUS*-associated amyotrophic lateral sclerosis. NA=not applicable. *No lumbar spinal cord was available for examination. †For P6, medulla at the hypoglossal level was examined in the absence of spinal cord tissue. ‡Non-neurological control tissue was used as a negative control for FUS pPro525Leu-specific staining.

Table 2: **Semiquantitative assessment of FUS-related pathological features in post-mortem tissue**

Figure 2: **Pathological findings**

(A) Western blot comparing FUS protein in post-mortem motor cortex tissue samples from three individuals without ALS as controls (C1–3), five untreated patients with *FUS*-ALS (two [F1 and F2] with the variant pArg521Cys and three [F3–5] with pPro525Leu), and four jacifusen-treated participants with *FUS*-ALS (two with pPro525Leu [P1 and P12], one with pArg521Leu [P5], and one with pGln519IlefsTer9 [P6]). F5 is the identical twin of participant P1. Total FUS protein was detected using two antibodies with non-overlapping epitopes (from Proteintech [amino acids 52–400] and Bethyl [amino acids 400–450]). FUS-P525L protein (only detectable in individuals with a pPro525Leu variant—ie, F3–5, P1, and P12) was measured using a FUS-P525L-specific antibody (appendix pp 36–37); α-tubulin and GAPDH were used as loading controls. Days since last administration and number of administrations are listed for treated participants. (B) Quantification of total FUS protein (detected with the Bethyl anti-FUS antibody; left); for participant P5 (indicated by the green square in the third bar of the graph), who died 146 days (more than two jacifusen half-lives) after her last dose of jacifusen and had the lowest concentration of antisense oligonucleotide in the motor cortex, the amount of total FUS protein was similar to that in untreated patients with *FUS*-ALS and healthy controls without ALS, probably indicating restoration of FUS protein production due to an extended period without the antisense oligonucleotide; thus, P5 was not included in the statistical comparison of treated participants. Quantification of FUS-P525L protein for individuals with the pPro525Leu variant (F3–F5, P1, and P12) is shown in the graph on the right. (C) Immunohistochemical staining of total FUS protein in post-mortem motor cortex, layer V (FUS protein is brown, nuclei are blue). Sections from controls without ALS (C4 and C5) have ubiquitous nuclear FUS staining. Sections from untreated patients with *FUS*-ALS caused by the pPro525Leu variant (F3; F4 not shown) have numerous dense neuronal cytoplasmic aggregates (arrowhead). Tissue from untreated patients with *FUS*-ALS caused by the pArg521Cys variant (F1; F2 not shown) has very rare, dense aggregates of FUS, scattered skein-like inclusions, glial cytoplasmic inclusions, and widespread neuropil threads and dystrophic neurites (arrows). Tissue sections from P1 have virtually absent nuclear FUS staining, with very sparse dense aggregates (arrowhead). Tissue sections from P12 have patchy nuclear FUS staining and multiple FUS aggregates (arrowhead). Tissue sections from P5 have widespread nuclear FUS positivity with negligible pathological staining. P6 has patchy nuclear FUS positivity with rare aggregates (arrowhead). Images are ×40 magnification; scale bar indicates 50 μm. (D) Immunohistochemical staining of total FUS protein in motor neurons from post-mortem spinal cord (and brainstem for P6); FUS protein is brown, nuclei are blue. Controls (C4 and C5) have ubiquitous nuclear FUS staining in the spinal cord. Tissue sections from untreated patients with *FUS*-ALS caused by the pPro525Leu variant (F3; F4 not shown) have numerous dense aggregates in residual motor neurons (arrowhead). Tissue sections from untreated patients with the pArg521Cys variant (F1 and F2 [not shown]) have rare, dense aggregates of FUS, scattered skein-like inclusions, glial cytoplasmic inclusions, and widespread neuropil threads (arrows). Nuclear FUS staining is virtually absent in tissue sections from P1, with very sparse, dense aggregates of FUS; some residual motor neurons show fine granular cytoplasmic FUS staining (arrowhead). Of note, Schwann cells of the peripheral nerve roots retain FUS nuclear positivity (not shown). Tissue sections from P12 have patchy nuclear FUS staining and aggregates in few residual motor neurons (arrowhead). Tissue sections from P5 have patchy nuclear FUS positivity with negligible pathological staining. Tissue sections from P6 have patchy nuclear FUS positivity with rare aggregates in motor neurons in the hypoglossal nuclei (arrowhead). All images are ×40 magnification; scale bar indicates 50 μm. Neuropathological findings are summarised semiquantitatively in table 2. ALS=amyotrophic lateral sclerosis. ALSFRS-R=Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised. *FUS*-ALS=*FUS*-associated amyotrophic lateral sclerosis. NS=non-significant.

substantial decrease in the concentration of NfL—a marker of axonal injury and neurodegeneration—in CSF. Here, in seven symptomatic individuals with known pathogenic *FUS* variants treated with jacifusen for longer than 3 months, we observed a 46–83% reduction in NfL concentration overall; in four participants carrying the pPro525Leu variant—which causes an aggressive, early-onset form of *FUS*-ALS—there was a 62–83% reduction in NfL in CSF, similar to the reduction observed in patients with *SOD1*-ALS treated with tofersen (52–86%).[23] This finding is in contrast to natural history data, which show an initial rise during onset followed by stabilisation of NfL concentration in CSF, plasma, and serum during the untreated ALS disease course.[24–28] However, in participants with a *FUS* variant of uncertain significance, for which there are few data regarding pathogenicity,[29] we found that concentrations of NfL in CSF were not consistently reduced during treatment.

In ALS, growing evidence suggests that a reduction in NfL concentration in response to an investigational therapeutic can be used as a potential surrogate endpoint predicting clinical benefit.[25] However, in this programme, despite the substantial reduction in NfL concentrations in CSF in most participants, several individuals continued to decline functionally, raising the question of how well this biomarker predicts clinical benefit and which additional factors determine whether a clinical response occurs after NfL reduction. Although a controlled trial with a larger sample size is necessary to definitively show the predictive value of NfL and efficacy of jacifusen in the context of patients with *FUS*-ALS the clinical and genetic complexity of our cohort offers insight into the relationship between NfL reduction and subsequent molecular, pathological, and functional evidence of recovery. Factors including the pathogenicity of the specific *FUS* variant, age at disease onset, clinical status (eg, ALSFRS-R score) at treatment initiation, and duration of treatment all appear to influence clinical response following NfL reduction. In the case of participant 10, who had objective functional recovery, clinical benefit was delayed relative to NfL lowering. From our post-mortem studies of participants and untreated patients with *FUS*-ALS carrying the pPro525Leu variant, which indicated decreased amounts of FUS protein and reduced abundance of FUS aggregates in the CNS in response to jacifusen, we presume that this delayed clinical response is preceded by and dependent on clearance of FUS pathology, leading to repair of the dysfunctional neurons and circuitry that control motor behaviour.

In participants last treated within two half-lives of jacifusen, western blotting showed a substantial (66–90%) decrease in the total amount of FUS protein in the motor cortex. In carriers of pPro525Leu, FUS-P525L and total FUS protein were reduced by similar amounts. Immunohistochemical analysis showed an apparent reduction in the amount of nuclear FUS protein in treated versus untreated participants and a relative decrease in aggregate burden in participants who had prolonged treatment. Interestingly, for participant 5, who died 146 days after her last dose of jacifusen (~2·6 half-lives), we observed ubiquitous nuclear FUS staining, similar to the non-neurological controls, and no pathological FUS staining.

Possible clinical benefit was observed in some cases. For example, participant 11 was asymptomatic at the initiation of treatment with jacifusen; however, a single-patient investigational new drug application was approved by the US Federal Drug Administration, partly on the basis of electomyographic evidence of a diffuse neurogenic process affecting thoracic and lumbosacral segments. In isolation, such electrophysiological findings are not diagnostic of ALS, but in the context of this participant's family history and presence of the pArg521Leu mutation, they were taken as evidence of transition to a preclinical stage of disease, in line with presymptomatic classification frameworks.[30–34] While continuing to receive jacifusen over the past 3 years, participant 11 has remained asymptomatic, with documented improvement in the distribution of neurogenic changes after 1 year of treatment in a repeated electromyography study by the same examiner and maintenance of normal concentrations of NfL in CSF, as established for his age group in healthy controls, for the duration of this programme. Although we cannot be certain if or how participant 11 would have progressed clinically had he not been treated, this case suggests the possibility that jacifusen could be used in carriers of pathogenic *FUS* variants to delay or prevent disease onset. Furthermore, participant 10 had an unprecedented functional recovery after 10 months of treatment. Her ALSFRS-R score declined during the first 10 months of treatment, even as the concentration of NfL in her CSF decreased substantially. However, she subsequently had a marked functional recovery in fine motor, gross motor, and respiratory domains that was inconsistent with the natural history of pPro525Leu FUS-ALS[35] and not observed in patients receiving other treatments, including tofersen.[23] Indeed, at data cutoff for this report—approximately 4 years after symptom onset and 3 years after treatment initiation—participant 10 remained ambulatory and required only intermittent ventilatory support. Possibly contributing to her exceptional response, participant 10 was treated earlier in her disease course than were other participants with the pPro525Leu variant, such as participant 1 (starting ALSFRS-R scores of 28 *vs* 17) suggesting the importance of earlier intervention. She was also the youngest participant and had a long, consistent treatment course (eg, compared with participant 2, who had a prolonged treatment interruption) supported by tracheotomy and mechanical ventilation after acute respiratory failure due to a concurrent illness. Of the participants carrying the pPro525Leu variant, participant 10 also had the lowest

starting concentration of NfL in CSF, which declined to a concentration close to that observed in controls.

A limitation of this report is that it is a case series, without a control group. In addition, sample size was small, and there were no strict inclusion or exclusion criteria beyond the participant either having *FUS*-related ALS or carrying a FUS variant and showing signs of preclinical disease.[30] Indeed, many participants initiated treatment at a later stage of disease than would be typical in a controlled trial. However, this design allowed for the inclusion of a wide spectrum of *FUS*-ALS participants, including informative cases that might have been excluded due to their disease stage (ie, participants 1 and 10) or for not meeting diagnostic criteria (ie, participants 4 and 11). Furthermore, although participants were generally treated according to a paradigm of monthly intrathecal injections, there was no uniform treatment protocol, with standards evolving in response to experience gained from the first-in-human use of this therapeutic in participant 1. With human clinical data from this investigator-initiated programme, the treatment protocol refined and informed the protocol used in the ongoing registrational trial.

Overall, data from this investigator-initiated, multicentre case series provide evidence for the safety of jacifusen and insight into early biomarker and clinical responses in a heterogeneous cohort of patients with *FUS*-ALS. These results provided sufficient safety and early efficacy data for jacifusen and the antisense therapeutic approach to *FUS*-ALS to enable the ongoing, global, phase 1–3, double-blind, randomised, placebo-controlled trial of this antisense oligonucleotide (NCT04768972) as the next step in its clinical development.

Contributors

NAS, SA-Z, JS, and JAA designed the programme. NAS, MBH, SA-Z, BNH, JS, AJ, VRM, IL, DW, DN, RMP, MW, and CN oversaw acquisition of expanded access programme data. EAH, VAK, MMA, MS, JKW, SDC, AS, RL, SH, JM, PJ-N, DN, FR, RHB, and SM contributed to data collection. LEB contributed to regulatory toxicology strategy. NAS, VAK, OMR, MMA, JKW, SM, RC, and CFB interpreted the data. VAK, OMR, and MMA were responsible for statistical analysis. All authors contributed to drafting the manuscript, made the decision to submit the manuscript for publication, and vouch for the accuracy of the data and analyses.

Declaration of interests

AS, RL, SH, JM, PJ-N, DN, FR, SM, and CFB are employees of Ionis Pharmaceuticals and own stock and/or stock options in Ionis Pharmaceuticals. DN has received support for attending meetings and travel from Ionis Pharmaceuticals. NAS received research funding to their institution from Ionis Pharmaceuticals in support of this investigator-initiated study of jacifusen; receives research funding to their institution from Cellenkos and Merck, unrelated to this study; has served as a consultant for Merck and Regeneron; has received payment and/or honoraria from Alnylam and support for attending meetings and/or travel from Merck; and has served as a Safety Review Committee member for Regeneron. Unrelated to the current study, MBH receives funding from Ionis Pharmaceuticals and has served as a consultant for Biogen, uniQure, Sarepta, and Amylyx and as an expert panel Chair for ClinGen ALS Gene Curation. JAA receives research funding to their institution from Biogen, Cytokinetics, Amylyx, the ALS Platform Trial (RaPharma, Biohaven, Clene, Prilenia, Denali, and Calico), Corcept, and the National Institute of Neurological Disorders and Stroke (part of the US National Institutes of Health [NIH]). JAA has served as a consultant for Amylyx, Apellis, Biogen, Cytokinetics, and Regeneron; on the data safety monitoring board for AL-S Parma, QurAlis, and Sanofi; and as Co-chair for the Northeast ALS Consortium. RMS's spouse owns stock in Amgen, AbbVie, Merck, and Pfizer. LEB has received support for attending meetings and/or travel from the Northeast ALS Consortium. DW has received funding from the US Federal Drug Administration and Minnesota Office of Higher Education and royalties from Springer Publishing; has served as a consultant for Biogen, Mitsubishi Tanabe Pharma America, and Amylyx; has received payment or honoraria from Mitsubishi Tanabe Pharma America; has served on data safety monitoring or advisory boards for NIH, Mitsubishi Tanabe Pharma America, and Biogen; and has held a leadership or fiduciary role at the ALS Association. CN has received payment or honoraria and support for attending meetings and/or travel from data safety monitoring or advisory boards related to myasthenia gravis and spinal muscular atrophy, and served on data safety monitoring or advisory boards related to myasthenia gravis and spinal muscular atrophy. CN has served a leadership or fiduciary role for the European Academy of Neurology. None of these activities are relevant to this study. All other authors declare no competing interests.

Data sharing

De-identified individual participant data can be requested by contacting the corresponding author and shared under data use agreements and to the extent permissible by informed consent documents within 36 months of publication.

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