Safety, tolerability, and efficacy of fasudil in amyotrophic lateral sclerosis (ROCK-ALS): a phase 2, randomised, double-blind, placebo-controlled trial

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Summary

Background Fasudil is a small molecule inhibitor of Rho-associated kinase (ROCK) and is approved for the treatment of subarachnoid haemorrhage. In preclinical studies, fasudil has been shown to attenuate neurodegeneration, modulate neuroinflammation, and foster axonal regeneration. We aimed to investigate the safety, tolerability, and efficacy of fasudil in patients with amyotrophic lateral sclerosis.

Methods ROCK-ALS was a phase 2, randomised, double-blind, placebo-controlled trial conducted at 19 amyotrophic lateral sclerosis centres in Germany, France, and Switzerland. Individuals (aged 18–80 years) with at least probable amyotrophic lateral sclerosis (as per the revised El Escorial criteria), a disease duration of 6–24 months, and a slow vital capacity greater than 65% of predicted normal were eligible for inclusion. Patients were randomly assigned (1:1:1) to receive 30 mg (15 mg twice daily) or 60 mg (30 mg twice daily) fasudil or matched placebo intravenously for 20 days over a 4-week period. Follow-up assessments were performed at 45, 90, and 180 days after treatment initiation. The co-primary endpoints were safety until day 180 (defined as the proportion without drug-related serious adverse events) and tolerability during the treatment period (defined as the proportion who did not discontinue treatment due to suspected drug-related adverse events). The primary analyses were carried out in the intention-to-treat population, which included all participants who entered the treatment phase. This trial is registered at ClinicalTrials.gov (NCT03792490) and Eudra-CT (2017-003676-31) and is now completed.

Findings Between Feb 20, 2019, and April 20, 2022, 120 participants were enrolled and randomised; two individuals assigned fasudil 30 mg withdrew consent before the baseline visit. Thus, the intention-to-treat population comprised 35 in the fasudil 30 mg group, 39 in the fasudil 60 mg group, and 44 in the placebo group. The estimated proportion without a drug-related serious adverse event was 1·00 (95% CI 0·91 to 1·00) with placebo, 1·00 (0·89 to 1·00) with fasudil 30 mg, and 1·00 (0·90 to 1·00) with fasudil 60 mg; the difference in proportions was 0·00 (95% CI –0·11 to 0·10; p>0·99) for fasudil 30 mg versus placebo and 0·00 (–0·10 to 0·10; p>0·99) for fasudil 60 mg versus placebo. Treatment tolerability (the estimated proportion who did not discontinue) was 0·93 (95% CI 0·81 to 0·99) with placebo, 1·00 (0·90 to 1·00) with fasudil 30 mg, and 0·90 (0·76 to 0·97) with fasudil 60 mg; the difference in proportions was 0·07 (95% CI –0·05 to 0·20; p=0·25) for fasudil 30 mg versus placebo, and –0·03 (–0·18 to 0·10; p=0·70) for fasudil 60 mg versus placebo. Eight deaths occurred: two in the placebo group, four in the fasudil 30 mg group, and two in the fasudil 60 mg group. The most common serious adverse events were respiratory failure (seven events), gastrostomy (five events), pneumonia (four events), and dysphagia (four events). No serious adverse events or deaths were attributed to study treatment. Adverse events, which were mainly related to disease progression, occurred in 139 participants in the placebo group, 108 in the fasudil 30 mg group, and 105 in the fasudil 60 mg group.

Interpretation Fasudil was well tolerated and safe in people with amyotrophic lateral sclerosis. The effect of fasudil on efficacy outcomes should be explored in larger clinical trials with a longer treatment duration, oral administration, and potentially higher dose of the trial drug.

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Introduction

Amyotrophic lateral sclerosis (ALS) is a devastating neurodegenerative disorder that is characterised by motor neuron demise and progressive muscle weakness, usually resulting in death from respiratory failure within 3–4 years after symptom onset.[1] Riluzole is approved

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Research in context

Evidence before this study

We searched PubMed from database inception to May 13, 2024, with the search string "(Fasudil OR ROCK OR "ROCK inhibitor") AND (ALS OR "motor neuron disease")", with no language restrictions. Evidence from cell culture and animal models of neurodegeneration suggests that Rho-associated kinase (ROCK) inhibition improves neuronal survival and axonal regeneration and modulates microglial function. In mouse models of amyotrophic lateral sclerosis, ROCK inhibition improved function and survival. Case reports of compassionate use of the licensed ROCK inhibitor fasudil in individuals with amyotrophic lateral sclerosis suggest that it is well tolerated in this setting. We did not identify any randomised controlled trials assessing the safety, tolerability, or efficacy of fasudil or other drugs with this mechanism of action in individuals with motor neuron diseases.

Added value of this study

This study is, to our knowledge, the first randomised, placebo-controlled trial designed to assess the safety, tolerability, and efficacy of the ROCK inhibitor fasudil for the treatment of amyotrophic lateral sclerosis. Fasudil was investigated at two doses (30 mg per day and 60 mg per day) for a 20-day treatment period, and follow-up was for up to 180 days. Although the study was not powered for secondary outcomes, results from the motor unit number index (MUNIX) assessment suggest that this readout could be a useful outcome measure for subsequent studies assessing the efficacy of fasudil in amyotrophic lateral sclerosis.

Implications of all the available evidence

Fasudil was safe and tolerable in individuals with amyotrophic lateral sclerosis, and results from the MUNIX assessment advocate for further evaluation of this drug in clinical trials with longer treatment durations. These findings also suggest that the neurophysiological MUNIX assessment can be used in multicentre trials and might be more sensitive to change than scale-based measures, such as the well established Revised Amyotrophic Lateral Sclerosis Functional Rating Scale.

globally for the treatment of amyotrophic lateral sclerosis and, in some countries, edaravone is also approved (as well as tofersen for people with *SOD1* mutations).[2] However, for individuals with sporadic disease, these drugs are insufficient at affecting the disease course, calling for the identification of more potent therapies.[2]

The molecular mechanisms contributing to development and progression of amyotrophic lateral sclerosis are only partly understood. Among them are protein aggregation, deregulated RNA metabolism and splicing, excitotoxicity, as well as aberrant immune responses.[2] Although the degeneration of upper and lower motor neurons is a cardinal feature in amyotrophic lateral sclerosis, other cell types—particularly astroglia, microglia, and endothelial cells—have been implicated in disease pathogenesis.[3]

Rho-associated kinase (ROCK) is a serine–threonine kinase and ubiquitously expressed. Its isoform ROCK2 is predominant in the CNS. In-vitro and animal models of neurodegenerative disorders have shown that pharmacological inhibition of ROCK increases neuronal survival, attenuates axonal degeneration, increases regeneration, and mitigates microglial activation.[4] Moreover, increased concentrations of ROCK are found in tissues of individuals with amyotrophic lateral sclerosis.[5] The ROCK inhibitor fasudil has shown beneficial effects in the *SOD1***G93A* mouse model of amyotrophic lateral sclerosis, including prolonged survival in two independent studies.[6,7] Fasudil is licensed in Japan for the treatment of vasospasm after subarachnoid haemorrhage, and it has a beneficial safety profile based on long-standing treatment experience in its licensed indication. Furthermore, it has been tested in several clinical trials, mainly in cardiovascular disease, with a favourable safety profile.[8–10] Known adverse events include allergic skin reactions, a slight drop in systolic blood pressure, and reversible renal impairment without major safety concerns. Case reports of individuals with amyotrophic lateral sclerosis who were treated with fasudil under compassionate-use circumstances also did not reveal any safety concerns.[11] Therefore, we aimed to investigate the safety, tolerability, and efficacy of fasudil compared with placebo in a phase 2 trial in individuals with amyotrophic lateral sclerosis.[12]

Methods

Study design and participants

ROCK-ALS was a phase 2, randomised, double-blind, placebo-controlled trial of fasudil in two different doses as an add-on therapy to riluzole in individuals with amyotrophic lateral sclerosis, conducted at 19 trial centres in Germany, France, and Switzerland. The study protocol is available online[12] and was approved by the respective regulatory authorities and ethics committees in each country, with lead approval in Germany obtained from the ethics committee of the University of Göttingen (approval number 31/5/18). The trial is registered at ClinicalTrials.gov (NCT03792490) and Eudra-CT (2017-003676-31) and is now completed.

Adults (aged 18–80 years) with clinical probable, laboratory-supported probable, or definite amyotrophic lateral sclerosis (sporadic or familial), according to the revised El Escorial criteria, and a disease duration of more than 6 months and less than 24 months, were eligible for inclusion. Co-medication with riluzole 50 mg twice daily

orally was compulsory. The predicted slow vital capacity (SVC) had to be greater than 65% of normal. Exclusion criteria comprised tracheostomy or assisted ventilation during the preceding 3 months; gastrostomy; known arterial hypotension (<90/60 mm Hg); and a participant or family history of intracranial bleeding, intracerebral aneurysms, or Moyamoya disease (appendix pp 8–9). All participants gave written informed consent before enrolment.

Randomisation and masking

Participants were randomly assigned in a 1:1:1 allocation ratio to receive intravenous fasudil 15 mg, fasudil 30 mg, or matching placebo twice daily. Stratification occurred by geographical region and type of onset (bulbar *vs* spinal). The randomisation list was generated electronically by a member of the statistics core, who was not involved in the design or analysis of this trial, and the list was kept at the central pharmacy at the University of Leipzig Medical Centre (Leipzig, Germany). Participants, medical personnel involved in the intervention or in assessing outcomes, as well as those analysing the data were masked to group assignment.

Procedures

Fasudil hydrochloride hydrate (15 mg/mL ampoules) was imported (Eril; Asahi Kasei Pharma, Tokyo, Japan). The investigational medicinal product contained either 30 mg fasudil (2×1 mL fasudil), 15 mg fasudil (1 mL fasudil and 1 mL sodium chloride [NaCl] 0·9%), or placebo (2×1 mL NaCl 0·9%), all of which were diluted in 100 mL NaCl 0·9% prior to intravenous administration. The high fasudil dose (60 mg, which corresponds to 2×30 mg for 20 days) represents the highest cumulative licensed dose. The investigational medicinal product was infused twice daily over 45 min with a CE-certified infusion pump at an interval of 6–8 h between administrations. Blood pressure and heart rate were measured before infusion and at 0, 10, 20, 30, 45, and 60 min after starting infusion. A total of 24 study visits (visits 0–23) were planned. Screening was done and written informed consent obtained on visit 0, up to 42 days before the baseline visit (visit 1). From visit 1 to 20, patients received trial medication on 20 consecutive working days, excluding weekends and holidays. Three follow-up visits took place at 45 days (visit 21), 90 days (visit 22), and 180 days (visit 23) after baseline. Safety and tolerability were assessed at each visit. Efficacy readouts were acquired at baseline (visit 1, day 0), day 26 (visit 20), day 90 (visit 22), and day 180 (visit 23) after start of treatment. On the last day of treatment (visit 20), a lumbar puncture was performed, if no contraindications applied, to obtain CSF for pharmacokinetic analyses. For pharmacokinetic analyses, blood plasma was also obtained. Simultaneous quantification of fasudil, hydroxyfasudil, and riluzole was performed on these plasma and CSF samples by liquid chromatography with tandem mass spectrometry.[13] Whole genome sequencing and *C9orf72* expansion PCR was performed to detect disease-causing mutations in amyotrophic lateral sclerosis.

Outcomes

The co-primary outcomes were safety from baseline to day 180 and tolerability of intravenous fasudil during the treatment period. The treatment was considered safe for an individual patient if no drug-related serious adverse events were recorded until 180 days after initiation of the 20-day treatment period. The treatment was considered tolerable if participants did not discontinue treatment due to suspected drug-related adverse events.

Secondary efficacy outcomes were survival time (time until death, tracheostomy, or permanent assisted ventilation) and changes from baseline to day 26, day 90, and day 180 in the revised Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R), Amyotrophic Lateral Sclerosis Assessment Questionnaire (ALSAQ-5), Edinburgh Cognitive and Behavioural ALS Screen (ECAS), Motor Unit Number Index (MUNIX), and predicted slow vital capacity (SVC). Because MUNIX has potentially greater sensitivity to functional decline compared with ALSFRS-R,[14] we analysed MUNIX across five muscles on each side, summarised as the MUNIX megascore 10. Separately trained and certified raters measured the biceps brachii, abductor pollicis brevis, abductor digiti minimi, tibialis anterior, and extensor digitorum brevis muscles.[14–16] The MUNIX measurements were calculated as a ratio to baseline (day 1) to ensure comparability across muscles. The lower limit of detection (LLOD) was considered as a fifth of the published 5% quantiles.[17] Values below the LLOD were set a priori to 50% of the LLOD. We also prespecified a MUNIX megascore 8 analysis, excluding the biceps brachii muscle on both sides, because of its known high variability. A further secondary outcome was safety from baseline to the end of treatment.

Exploratory outcomes included changes from baseline to day 26 and day 180 in serum neurofilament light chain (NfL), serum glial fibrillary acidic protein (GFAP; measured by Simoa immunoassay on a HD-X [Quanterix, Lexington, MA, USA]), serum creatine kinase, urinary neurotrophin receptor p75 extracellular domain ($p75^{ECD}$; determined by ELISA), and bodyweight.

Statistical analysis

Sample size calculations were done with nQuery 4.0. We estimated that a sample size of 102 patients (ie, 34 patients per treatment group) would yield sufficiently narrow 95% CIs for the difference in proportions between the placebo group and the treatment groups for both primary endpoints, the proportion of patients without significant drug intolerance, and the proportion of patients without drug-related serious adverse events. Under the assumption of no difference between each of the two treatment groups and the placebo group, the half-width of the

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See **Online** for appendix

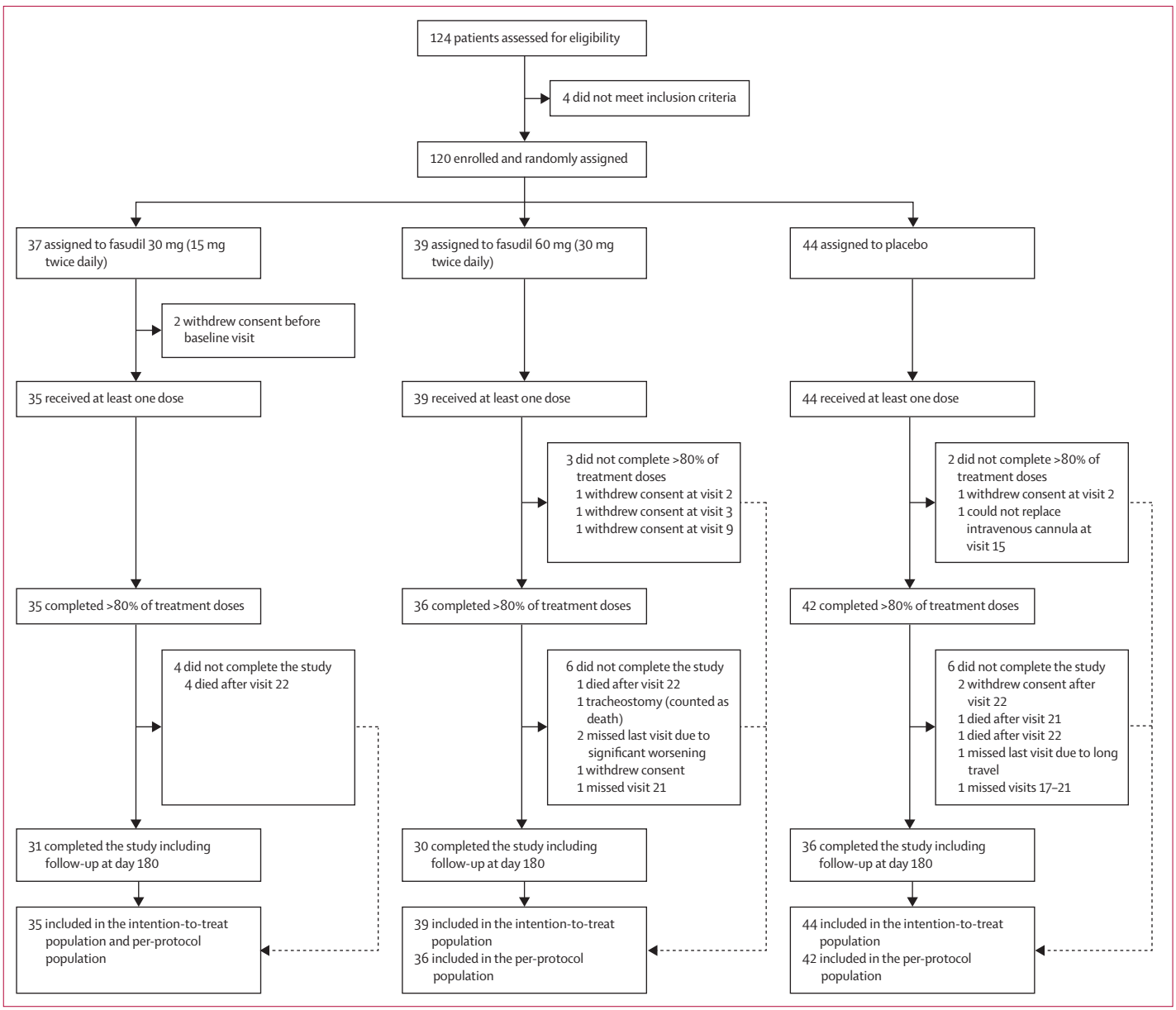

Figure 1: **Trial profile**
118 patients who received at least one dose of the study drug were included in the intention-to-treat population. Five patients discontinued treatment due to the given reasons. The per-protocol population comprised 113 patients who received at least 80% of the scheduled treatment doses. 97 patients completed the study (all trial visits).

95% CI for the difference in proportions is, at most, 0·24. We expected high proportions of tolerability and safety, in which case the 95% CI becomes narrower. Adjusting for a dropout rate of 15%, we aimed to recruit 120 patients (ie, 40 patients per treatment group).

The primary analyses of safety and tolerability were carried out in the intention-to-treat population, which included all patients who entered the treatment phase. An analysis of the per-protocol population, which excluded patients who received less than 80% of the investigational medicinal product, was performed as a sensitivity analysis on all endpoints. Safety results were reviewed by an independent data safety and monitoring board every 3 months during the trial with recommendations to the sponsor on whether to continue, modify, or terminate the trial. For the tolerability analyses, in a first analysis, participants who discontinued during the treatment period were considered as worst case (ie, no drug tolerability). Exact two-sided 95% CIs for the difference between treatment groups in proportions of tolerability were calculated as the intersection of the two one-sided 97·5% CIs derived with the inductive method.[18] In an additional analysis of tolerability, patients who dropped out during the treatment period

with an explicitly non-drug-related reason were considered as competing events. Proportions at day 26 were estimated with 95% CIs with the Aalen-Johansen estimator. Differences between treatment groups were estimated with 95% CIs with the method from Scosyrev.[19] Using the time to first drug-related serious adverse event, the proportions free of any drug-related serious adverse event at day 180 were assessed with Kaplan-Meier estimates, and 95% CIs for the differences between treatment groups were calculated with the beta product confidence procedure for right censored data.[20]

Secondary efficacy outcomes (ALSFRS-R, ALSAQ-5, ECAS, SVC, and MUNIX until day 180) and exploratory outcomes were also analysed in the intention-to-treat population by means of the Gaussian linear model for repeated measures (so-called mixed models for repeated measures), with treatment group, time (days 26, 90, and 180), treatment-by-time interaction, region, stratum of onset, and sex as factors, and with baseline measurements of the outcome as well as pre-trial estimated ALSFRS-R progression (change per month) as covariates. Least squares (expected marginal) mean changes from baseline are reported for the treatment groups with 95% CIs as well as the difference between the least squares treatment group means with 95% CIs and p value testing the null hypothesis of no treatment effect. For the prespecified secondary ALSFRS-R and SVC outcomes, and the exploratory NfL and bodyweight outcomes, subgroup analyses were conducted by sex (male and female), by stratum of onset (spinal *vs* bulbar), and by pre-study disease progression speed (faster *vs* slower, split at the median). The Kaplan-Meier method was applied to estimate the survival probabilities in each group, and 95% CIs for the differences between treatment groups were calculated with the beta product confidence procedure for right censored data.[20]

p values smaller than or equal to 0·05 were considered statistically significant. In this phase 2 trial, the statistical analysis plan did not prespecify hierarchical testing, nor did it correct for multiple testing. p values are therefore descriptive only for secondary and exploratory analyses and should be interpreted cautiously. Statistical analyses were performed in R (version 4.2.3). More details on the methods are provided in the appendix (pp 21–25).

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, writing of the manuscript, or the decision to submit the manuscript for publication.

Results

Between Feb 20, 2019, and April 20, 2022, 120 people were enrolled into the ROCK-ALS trial and randomly allocated to either fasudil (30 mg or 60 mg) or placebo

	Total (n=118)	Placebo (n=44)	Fasudil 30 mg (n=35)	Fasudil 60 mg (n=39)
Age, years	60 (56–67)	61 (56–67)	63 (58–68)	59 (53–66)
18–64	77 (65%)	30 (68%)	20 (57%)	27 (69%)
64–83	41 (35%)	14 (32%)	15 (43%)	12 (31%)
Sex				
Female	54 (46%)	19 (43%)	19 (54%)	16 (41%)
Male	64 (54%)	25 (57%)	16 (46%)	23 (59%)
Ethnicity				
White	114 (96%)	43 (98%)	35 (100%)	36 (92%)
Black	1 (1%)	0 (0%)	0 (0%)	1 (3%)
Hispanic	1 (1%)	1 (2%)	0 (0%)	0 (0%)
Other	2 (2%)	0 (0%)	0 (0%)	2 (5%)
Height, cm	170 (164–177)	171 (163–176)	170 (164–175)	172 (164–182)
Bodyweight, kg	72 (64–84)	75 (64–84)	67 (61–83)	69 (65–82)
BMI, kg/m²	24 (23–27)	25 (23–28)	24 (22–27)	24 (23–27)
Site of disease onset				
Bulbar	39 (33%)	15 (34%)	13 (37%)	11 (28%)
Spinal	79 (67%)	29 (66%)	22 (63%)	28 (72%)
Age at onset of muscle weakness, years	60 (55–66)	60 (56–67)	62 (57–66)	58 (52–66)
Time to diagnosis from onset of weakness, months	9·0 (5·9–12)	9·0 (6·0–12)	8·0 (5·5–13)	9·0 (6·2–11)
Time since initial diagnosis until visit 0, months	3·9 (2·1–6·9)	3·9 (1·7–6·7)	3·8 (1·3–6·7)	4·1 (2·6–7·3)
Time from onset of weakness to visit 0, months	14 (12–17)	14 (12–17)	14 (9·3–15)	15 (12–17)
Sporadic disease	110 (93%)	39 (89%)	35 (100%)	36 (92%)
C9orf72 expansion	11 (10%)	3 (8%)	4 (11%)	4 (11%)
Other class 4–5 mutations	3 (3%)	1 (3%)	0 (0%)	2 (6%)
No genetic testing performed	4 (3%)	0	1 (3%)	3 (8%)
Familial disease	8 (7%)	5 (11%)	0 (0%)	3 (8%)
C9orf72 expansion	3 (50%)	2 (67%)	0 (0%)	1 (33%)
Other class 4–5 mutations	1 (17%)	0 (0%)	0 (0%)	1 (33%)
No genetic testing performed	2 (2%)	2 (5%)	0	0
Revised El Escorial category				
Laboratory-supported probable	3 (3%)	1 (2%)	0 (0%)	2 (5%)
Clinical probable	32 (27%)	15 (34%)	9 (26%)	8 (21%)
Definite	83 (70%)	28 (64%)	26 (74%)	29 (74%)
Number of regions involved				
1	0	0	0	0
2	20 (17%)	10 (23%)	5 (14%)	5 (13%)
3	60 (51%)	23 (52%)	20 (57%)	17 (44%)
4	38 (32%)	11 (25%)	10 (29%)	17 (44%)
ALSFRS-R total score	39 (36–42)	39 (36–42)	39 (36–43)	39 (36–42)
Change in ALSFRS-R (loss in points per month)	0·58 (0·39–0·90)	0·60 (0·41–0·89)	0·56 (0·35–0·92)	0·54 (0·43–0·88)
Slower progressor (<0·58 points per month)	59 (50%)	19 (43%)	18 (51%)	22 (56%)
Faster progressor (>0·58 points per month)	59 (50%)	25 (57%)	17 (49%)	17 (44%)
Slow vital capacity, % predicted	89 (78–101)	89 (76–103)	88 (77–98)	91 (82–101)
MUNIX, megascore-10	68 (39–91)	76 (42–93)	63 (39–85)	66 (35–102)
ALSAQ-5, points	28 (15–40)	30 (20–40)	30 (15–42)	25 (15–35)

(Table 1 continues on next page)

(figure 1). Two individuals allocated to fasudil 30 mg withdrew their consent before treatment initiation. Therefore, 118 participants received the investigational medicinal product and were included in the intention-to-treat population for assessment of the primary and secondary endpoints (35 people in the 30 mg fasudil group, 39 in the 60 mg fasudil group, and 44 in the placebo group). 39 (33%) had bulbar onset disease and 79 (67%) had spinal onset. The baseline characteristics of participants were similar across the three groups (table 1). 113 of 118 patients received at least 80% of the investigational medicinal product and comprised the per-protocol population (figure 1).

Because no treatment-related serious adverse events were recorded in any of the treatment groups up to day 180 (table 2), the treatment was considered safe for all participants. The estimated proportion of patients without an event was 1·00 (95% CI 0·91 to 1·00) with placebo, 1·00 (0·89 to 1·00) with fasudil 30 mg, and 1·00 (0·90 to 1·00) with fasudil 60 mg; the difference in proportions was 0·00 (95% CI –0·11 to 0·10, p>0·99) for fasudil 30 mg versus placebo, and 0·00 (–0·10 to 0·10, p>0·99) for fasudil 60 mg versus placebo (table 2, appendix pp 2–3). The treatment was tolerable for 41 of 44 participants in the placebo group (0·93; 95% CI 0·81 to 0·99), 35 of 35 in the fasudil 30 mg group (1·00; 0·90 to 1·00), and 35 of 39 in the fasudil 60 mg group (0·90; 0·76 to 0·97). The difference in proportions was 0·07 (–0·05 to 0·20, p=0·25) for fasudil 30 mg versus placebo and –0·03 (–0·18 to 0·10], p=0·70) for fasudil 60 mg versus placebo. When dropouts unrelated to treatment were not considered worst case (ie, intolerability events), but were modelled as competing events instead, treatment intolerability occurred in 0·05 (95% CI 0·00 to 0·12) of patients in the placebo group, in 0·00 (0·00 to 0·05) in the fasudil 30 mg group, and in 0·08 (0·00 to 0·17) in the fasudil

	Total (n=118)	Placebo (n=44)	Fasudil 30 mg (n=35)	Fasudil 60 mg (n=39)
(Continued from previous page)				
ECAS (total score), points	111 (102–120)	114 (106–119)	110 (99–116)	107 (102–124)
Serum NfL, pg/mL	110 (71–156)	105 (73–154)	99 (57–180)	124 (76–152)
Riluzole use	118 (100%)	44 (100%)	35 (100%)	39 (100%)
Edaravone use	10 (8%)	4 (9%)	3 (9%)	3 (8%)

Data are median (IQR) or n (%). Other class 4–5 mutations were detected in the following genes: *TARDBP* (881G>T [Gly294Val], class 5, in one male patient with sporadic ALS in the fasudil 60 mg group), *SOD1* (341T>C [Ile114Thr], class 5, in one female patient with familial ALS in the fasudil 60 mg group) and *NEK1* (379C>T [Arg127Ter], class 5, in one male patient with sporadic ALS in the placebo group and 1791del [Phe597LeufsTer32], class 4, in one female patient with sporadic ALS in the fasudil 60 mg group). ALSFRS-R=Amyotrophic Lateral Sclerosis Functional Rating Scale Revised. MUNIX=Motor Unit Number Index. ALSAQ-5=Amyotrophic Lateral Sclerosis Assessment Questionnaire-5. ECAS=Edinburgh Cognitive and Behavioural ALS Screen. NfL=neurofilament light chain.

Table 1: **Baseline demographic and clinical characteristics of participants in intention-to-treat population**

	Placebo (n=44)	Fasudil 30 mg (n=35)	Fasudil 60 mg (n=39)	Difference between fasudil 30 mg and placebo	p value for fasudil 30 mg *vs* placebo	Difference between fasudil 60 mg and placebo	p value for fasudil 60 mg *vs* placebo
Primary endpoints							
Safety until day 180, number of events	0	0	0	..	..	..	..
Safety until day 180, proportion without event	1·00 (0·91 to 1·00)	1·00 (0·89 to 1·00)	1·00 (0·90 to 1·00)	0·00 (–0·11 to 0·10)	>0·99	0·00 (–0·10 to 0·10)	>0·99
Tolerability, number of events	3	0	4	..	..	..	..
Tolerability, proportion without event	0·93 (0·81 to 0·99)	1·00 (0·90 to 1·00)	0·90 (0·76 to 0·97)	0·07 (–0·05 to 0·20)	0·25	–0·03 (–0·18 to 0·10)	0·70
Secondary endpoints							
Safety until day 26, number of events	0	0	0	..	..	..	..
Safety until day 26, proportion without event	1 (0·92 to 1·00)	1·00 (0·90 to 1·00)	1·00 (0·91 to 1·00)	0·00 (–0·10 to 0·08)	>0·99	0·00 (–0·10 to 0·08)	>0·99
Survival until day 180, number of events	2	4	2	..	..	..	..
Survival until day 180, % alive	97·6% (78·3 to 99·9)	85·9% (60·5 to 96·3)	94·4% (74·0 to 99·3)	–11·7% (–40·3 to 13·3)	0·52	–3·2% (–26·1 to 19·4)	>0·99
ALSFRS-R, points difference from day 1							
Day 26	–0·72 (–1·55 to 0·11)	–0·86 (–1·73 to 0·00)	–0·38 (–1·30 to 0·55)	0·14 (–0·89 to 1·17)	0·79	–0·35 (–1·36 to 0·67)	0·50
Day 90	–2·53 (–3·74 to –1·33)	–3·64 (–4·92 to –2·35)	–3·11 (–4·42 to –1·80)	1·10 (–0·55 to 2·76)	0·19	0·58 (–1·06 to 2·21)	0·49
Day 180	–6·50 (–8·16 to –4·84)	–7·94 (–9·72 to –6·17)	–6·85 (–8·63 to –5·07)	1·44 (–0·91 to 3·80)	0·23	0·35 (–1·98 to 2·67)	0·77

(Table 2 continues on next page)

	Placebo (n=44)	Fasudil 30 mg (n=35)	Fasudil 60 mg (n=39)	Difference between fasudil 30 mg and placebo	p value for fasudil 30 mg *vs* placebo	Difference between fasudil 60 mg and placebo	p value for fasudil 60 mg *vs* placebo
(Continued from previous page)							
ALSAQ-5 total score, points difference from day 1							
Day 26	−1·79 (−5·95 to 2·37)	−1·40 (−5·81 to 3·02)	0·69 (−3·90 to 5·28)	−0·39 (−5·70 to 4·91)	0·88	−2·48 (−7·71 to 2·74)	0·35
Day 90	3·52 (−1·16 to 8·20)	5·17 (0·27 to 10·08)	7·04 (2·01 to 12·08)	−1·65 (−7·74 to 4·43)	0·59	−3·52 (−9·50 to 2·46)	0·25
Day 180	13·12 (7·91 to 18·33)	14·92 (9·45 to 20·39)	12·65 (7·09 to 18·21)	−1·80 (−8·73 to 5·13)	0·61	0·47 (−6·33 to 7·27)	0·89
ECAS total score, points difference from day 1							
Day 26	3·64 (0·56 to 6·71)	5·27 (1·91 to 8·63)	4·22 (0·84 to 7·60)	−1·63 (−5·64 to 2·37)	0·42	−0·59 (−4·45 to 3·28)	0·76
Day 90	6·58 (3·43 to 9·74)	2·92 (−0·42 to 6·26)	6·07 (2·66 to 9·48)	3·67 (−0·39 to 7·73)	0·08	0·515 (−3·39 to 4·42)	0·79
Day 180	6·18 (2·88 to 9·49)	4·90 (1·25 to 8·55)	7·59 (4·04 to 11·14)	1·28 (−3·13 to 5·70)	0·57	−1·41 (−5·58 to 2·77)	0·50
Slow vital capacity, percentage of normal, difference from day 1							
Day 26	−8·34 (−15·24 to −1·44)	−2·87 (−10·27 to 4·54)	−0·07 (−7·68 to 7·53)	−5·48 (−14·54 to 3·60)	0·23	−8·27 (−17·13 to 0·59)	0·067
Day 90	−9·24 (−15·72 to −2·75)	−5·82 (−12·80 to 1·16)	−4·46 (−11·57 to 2·65)	−3·41 (−11·74 to 4·91)	0·42	−4·78 (−12·84 to 3·29)	0·24
Day 180	−17·24 (−25·12 to −9·36)	−15·76 (−24·50 to −7·01)	−13·27 (−21·95 to −4·60)	−1·48 (−12·29 to 9·32)	0·79	−3·97 (−14·44 to 6·51)	0·45
MUNIX megascore 10, ratio relative to day 1							
Day 26	0·94 (0·89 to 0·99)	0·98 (0·93 to 1·04)	1·01 (0·95 to 1·07)	0·95 (0·90 to 1·02)	0·13	0·93 (0·88 to 0·99)	0·015
Day 90	0·79 (0·73 to 0·86)	0·89 (0·81 to 0·97)	0·89 (0·81 to 0·97)	0·89 (0·80 to 1·00)	0·042	0·89 (0·80 to 0·99)	0·038
Day 180	0·73 (0·64 to 0·85)	0·74 (0·64 to 0·86)	0·73 (0·63 to 0·85)	0·99 (0·81 to 1·21)	0·94	1·00 (0·82 to 1·22)	0·99

Data are proportions and differences in proportions for tolerability and safety endpoints, Kaplan-Meier-based survival estimates, and expected marginal mean estimates of differences from baseline or ratios to baseline, as indicated, expressed with 95% CIs in parentheses. No adjustment for multiplicity was done, so p values for secondary efficacy and safety outcomes should be interpreted cautiously. ALSFRS-R=Amyotrophic Lateral Sclerosis Functional Rating Scale Revised. ALSAQ-5=Amyotrophic Lateral Sclerosis assessment questionnaire-5. ECAS=Edinburgh Cognitive and Behavioural ALS Screen. MUNIX=Motor Unit Number Index, presented here as the MUNIX megascore 10 comprising the following muscles on both sides: abductor pollicis brevis, abductor digiti minimi, biceps brachii, tibialis anterior, and extensor digitorum brevis.

Table 2: **Primary and secondary endpoints in the intention-to-treat population**

60 mg group (appendix p 12). No significant differences were observed between groups.

The frequency of all adverse events and serious adverse events leading to dropout from the study was similar between groups (table 3). 352 adverse events were reported in 92 (78%) of 118 patients. Overall, 89 (25%) adverse events were deemed possibly, probably, or definitely related to the investigational medicinal product. The most common non-serious adverse events were injuries (72 overall [21 in the placebo group, 28 in the 30 mg fasudil group, and 23 in the 60 mg fasudil group], of which there were 36 falls [11 in the placebo group, 16 in the 30 mg fasudil group, and nine in the 60 mg fasudil group]), headache (45 overall [15 in the placebo group, 19 in the 30 mg fasudil group, and 11 in the 60 mg fasudil group]), gastrointestinal disorders (41 overall [22 in the placebo group, eight in the 30 mg fasudil group, 11 in the 60 mg fasudil group], of which there were six dysphagia events [four in the placebo group, one in the 30 mg fasudil group, and one in the 60 mg fasudil group]), respiratory disorders (37 overall [15 in the placebo group, 11 in the 30 mg fasudil group, and 11 in the 60 mg fasudil group], of which there were 11 dyspnoea events [six in the placebo group, none in the 30 mg fasudil group, and five in the 60 mg fasudil group] and ten respiratory failures [three in the placebo group, six in the 30 mg fasudil group, and one in the 60 mg fasudil group]), and infections (34 [15 in the placebo group, 14 in the 30 mg fasudil group, and five in the 60 mg fasudil group]). 40 serious adverse events were recorded in 30 (25%) of 118 patients, but none was related to the investigational medicinal product. The most common serious adverse event was respiratory failure (seven events), followed by gastrostomy (five events), pneumonia (four events), and dysphagia (four events).

	Total (n=118)	Placebo (n=44)	Fasudil 30 mg (n=35)	Fasudil 60 mg (n=39)
All adverse events (number of events)	352	139	108	105
Grade 1 (mild)	205 (58%)	79 (57%)	63 (58%)	63 (60%)
Grade 2 (moderate)	125 (36%)	50 (36%)	36 (33%)	39 (37%)
Grade 3 (severe)	22 (6%)	10 (7%)	9 (8%)	3 (3%)
All adverse events (number of participants relative to intention-to-treat population)	92 (78%)	35 (80%)	29 (83%)	28 (72%)
Adverse events leading to dropout from the study	5	1	0	4
Adverse events (relation to study drug)				
Highly probable or definitely related	3 (1%)	0 (0%)	1 (1%)	2 (2%)
Probably related	17 (5%)	7 (5%)	9 (8%)	1 (1%)
Possibly related	69 (20%)	20 (14%)	17 (16%)	32 (30%)
Unlikely to be related	65 (18%)	36 (26%)	11 (10%)	18 (17%)
Not related	198 (56%)	76 (55%)	70 (65%)	52 (50%)
Most frequent adverse events				
Injuries	72 (20%)	21 (15%)	28 (26%)	23 (22%)
Falls	36 (10%)	11 (8%)	16 (15%)	9 (9%)
Headache	45 (13%)	15 (11%)	19 (18%)	11 (10%)
Gastrointestional disorders	41 (12%)	22 (16%)	8 (7%)	11 (10%)
Dysphagia	6 (2%)	4 (3%)	1 (1%)	1 (1%)
Respiratory disorders	37 (11%)	15 (11%)	11 (10%)	11 (10%)
Dyspnoea	11 (3%)	6 (4%)	0	5 (5%)
Respiratory failure	10 (3%)	3 (2%)	6 (6%)	1 (1%)
Infections	34 (10%)	15 (11%)	14 (13%)	5 (5%)
Serious adverse events (number of events)	40	15	14	11
Grade 1 (mild)	5 (12%)	3 (20%)	0 (0%)	2 (18%)
Grade 2 (moderate)	17 (42%)	3 (20%)	7 (50%)	7 (64%)
Grade 3 (severe)	18 (45%)	9 (60%)	7 (50%)	2 (18%)
Serious adverse events (number of participants relative to intention-to-treat population)	30 (25%)	10 (23%)	12 (34%)	8 (21%)
Serious adverse events related to the study drug	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Serious adverse events (by system organ class)*				
Respiratory, thoracic, and mediastinal disorders	15	6	7	2
Respiratory failure	7	1	5	1
Injuries, poisoning, and procedural complications	9	1	6	2
Post-lumbar puncture syndrome	2	1	1	0
Falls	3	0	2	1
Gastrointestinal disorders	5	4	1	0
Dysphagia	4	3	1	0
Infections and infestations	5	0	2	3
Pneumonia	4	0	2	2
Nervous system disorders	4	1	2	1
Cardiac disorders	3	2	0	1
Ear and labyrinth disorders	1	1	0	0

(Table 3 continues on next page)

Two serious adverse events were related to the lumbar puncture procedure (table 3). No suspected serious adverse reaction was reported.

Safety during the treatment period (until day 26) was separately assessed as a secondary outcome, which also did not reveal any safety events related to the investigational medicinal product in any treatment group, and there were no significant differences between treatment groups (table 2). Because fasudil has known vasodilatory effects, the trial protocol required blood pressure monitoring during and after drug infusion. No clinically significant changes in arterial blood pressure or heart rate were observed in either group (appendix pp 4, 10–11).

Survival outcomes until the end of the trial did not significantly differ across the treatment groups (table 2, figure 2A). There were two deaths in the placebo group, four deaths in the fasudil 30 mg group and two deaths in the fasudil 60 mg group (table 3). Most deaths were related to respiratory failure as part of disease progression; none was judged to be related to the study drug or study procedures.

All secondary efficacy analyses were done in the intention-to-treat population. Measures of significance for these analyses should be interpreted cautiously, since adjustment for multiplicity was not done. The expected marginal means for the difference in the ALSFRS-R score from baseline to day 180 did not differ between groups: –6·50 (95% CI –8·16 to –4·84) with placebo, –7·94 (–9·72 to –6·17) with fasudil 30 mg, and –6·85 (–8·63 to –5·07) with fasudil 60 mg. Moreover, there were no differences at days 26 and 90 (table 2, figure 2B). Post-hoc analyses of the bulbar (Q1–3) and respiratory subscores (Q10–12) showed no differences (appendix p 16). The subjective health status of patients was measured by ALSAQ-5 (figure 2E) and cognitive and behavioural status was assessed by ECAS (figure 2F), both of which did not show differences between treatment groups at any timepoint (table 2). Respiratory function, assessed by SVC, declined over time in all groups. The expected marginal means of the difference in calculated SVC at day 26 from baseline did not differ between treatment groups: –8·34% (95% CI –15·24 to –1·44) with placebo, –2·87% (–10·27 to 4·54) with fasudil 30 mg, and –0·07% (–7·68 to 7·53) with fasudil 60 mg (p=0·067 for fasudil 60 mg *vs* placebo). The expected marginal means of the SVC at days 90 and 180 from baseline also did not differ between treatment groups (table 2, figure 2C). In a prespecified subgroup analysis of female and male patients, there was a significantly slower SVC decline in females in the fasudil 60 mg group at all timepoints (appendix p 5).

At day 26, the expected marginal means for the ratio of MUNIX megascore 10 relative to baseline were 0·94 (95% CI 0·89 to 0·99) with placebo, 0·98 (0·93 to 1·04) with fasudil 30 mg, and 1·01 (0·95 to 1·07) with fasudil 60 mg. Pairwise contrast tests showed significant differences between the placebo and fasudil 60 mg groups

(p=0·015). At day 90, the expected marginal means for the ratio relative to baseline were 0·79 (95% CI 0·73 to 0·86) with placebo, 0·89 (0·81 to 0·97) with fasudil 30 mg, and 0·89 (0·81 to 0·97) with fasudil 60 mg. Pairwise contrast tests again showed significant differences between placebo and fasudil 30 mg (p=0·042) as well as placebo and fasudil 60 mg (p=0·038). No significant differences between groups were observed at day 180 (table 2, figure 2D). A prespecified megascore 8 secondary analysis (excluding the biceps brachii due to frequent technical challenges associated with analysing this muscle) largely resembled the megascore 10 results (appendix pp 13–15).

Several exploratory analyses were done to characterise the molecular effects of ROCK inhibition, which should be interpreted with much caution, since no adjustment for multiplicity was done. There were no significant effects of fasudil treatment on serum NfL concentrations in the prespecified analysis (appendix pp 17–18). However, a directional NfL decline was observed in the fasudil 60 mg group, from 126 ng/mL at baseline to 108 ng/mL at day 180, whereas NfL concentrations increased slightly in the other groups. A post-hoc analysis showed a significant effect of fasudil 60 mg at day 180 on the ratio of NfL values relative to baseline in the bulbar subgroup (1·92 [95% CI 1·26 to 2·92]; p=0·003; appendix p 6). We did exploratory analyses of creatine kinase, $p75^{ECD}$, GFAP, and bodyweight. Creatine kinase in serum and $p75^{ECD}$ in urine did not differ significantly between groups. A significant reduction in serum GFAP was observed on day 180 in the fasudil 60 mg group (0·75; 95% CI 0·60 to 0·95) compared with placebo (0·90 [0·75 to 1·08]; p=0·041; appendix pp 17–19). Bodyweight decreased in all groups over the study period, but this decrease was less pronounced in the fasudil-treated groups, with a directional benefit for fasudil 60 mg especially at day 90 (p=0·074 for fasudil 60 mg *vs* placebo; appendix pp 17–19).

To understand whether fasudil acts in a differential manner on patients with fast or slow disease progression, we did prespecified subgroup analyses after dichotomising progression rate into faster (>0·58 points loss in ALSFRS-R per month) and slower (<0·58 points loss in ALSFRS-R per month). None of the results was specific to these subgroups (appendix pp 5–6).

We assessed plasma concentrations of fasudil and its active metabolite hydroxyfasudil at the first and last day of drug treatment (all drug concentrations are reported as exploratory post-hoc analyses, and analyses should be interpreted cautiously). Although fasudil was almost non-detectable due to its fast metabolism, concentrations of hydroxyfasudil were expectedly higher in the fasudil 60 mg group than in the fasudil 30 mg group and were non-detectable in the placebo group. On day 26, lumbar puncture was performed in 67 patients and CSF was analysed for fasudil, hydroxyfasudil, and riluzole. Corresponding to the serum results, fasudil concentrations were nearly undetectable in the CSF. Hydroxyfasudil was detected in all fasudil-treated patients (fasudil 30 mg, mean 5·8 [SD 2·0] µg/L; fasudil 60 mg, 9·4 [4·5] µg/L; p=0·001), but in none of the placebo-treated patients. To rule out an effect of fasudil by interaction with riluzole, we analysed riluzole concentrations before treatment, at day 26, and day 180. No significant changes in riluzole concentrations were observed between groups and concentrations remained stable over time (appendix pp 7, 20). In addition to the intention-to-treat analysis, we also performed a sensitivity analysis on the per-protocol population, comprising all patients who received at least 80% of the investigational medical product. These results largely resembled the intention-to-treat analysis.

	Total (n=118)	Placebo (n=44)	Fasudil 30 mg (n=35)	Fasudil 60 mg (n=39)
(Continued from previous page)				
General disorders and administration site conditions	2	1	0	1
Chest pain	1	0	0	1
Gait disturbance	1	1	0	0
Vascular disorders	1	0	0	1
Thrombosis	1	0	0	1
Surgical and medical procedures	7	2	2	3
Gastrostomy	5	1	2	2
Tracheostomy	1	0	0	1
Mechanical ventilation	1	1	0	0
Investigations	1	1	0	0
Bodyweight decrease	1	1	0	0
Deaths	8 (7%)	2 (5%)	4 (11%)	2 (5%)
Discontinuations during treatment phase	4 (3%)	2 (5%)	0 (0%)	2 (5%)
Discontinuations during follow-up phase	16 (14%)	6 (14%)	4 (11%)	6 (15%)

Data are n or n (%). No p values are given here since statistical testing of safety and tolerability was already performed for the primary endpoints and did not reveal any significant differences. *Serious adverse events by system organ class might contain multiple organ class listings for a single serious adverse event, thus the total number of events listed here might be higher than the actual number of serious adverse events.

Table 3: **Adverse events during the 6-month trial period in intention-to-treat population**

Discussion

ROCK-ALS is the first placebo-controlled, double-blind, randomised clinical trial to test a ROCK inhibitor, fasudil, in patients with amyotrophic lateral sclerosis; therefore, the trial was primarily focused on safety and tolerability. Although the current approval in Japan for fasudil (for treatment of vasospasm) restricts the treatment period to 2 weeks and the maximum cumulative dose (1260 mg), our study was designed to assess immediate and potential long-term effects on safety and efficacy up to 180 days after treatment start. To modify the disease early, we included individuals with a short interval from onset of weakness to baseline (median 14 months), but we did not enrich the population for people who had fast disease

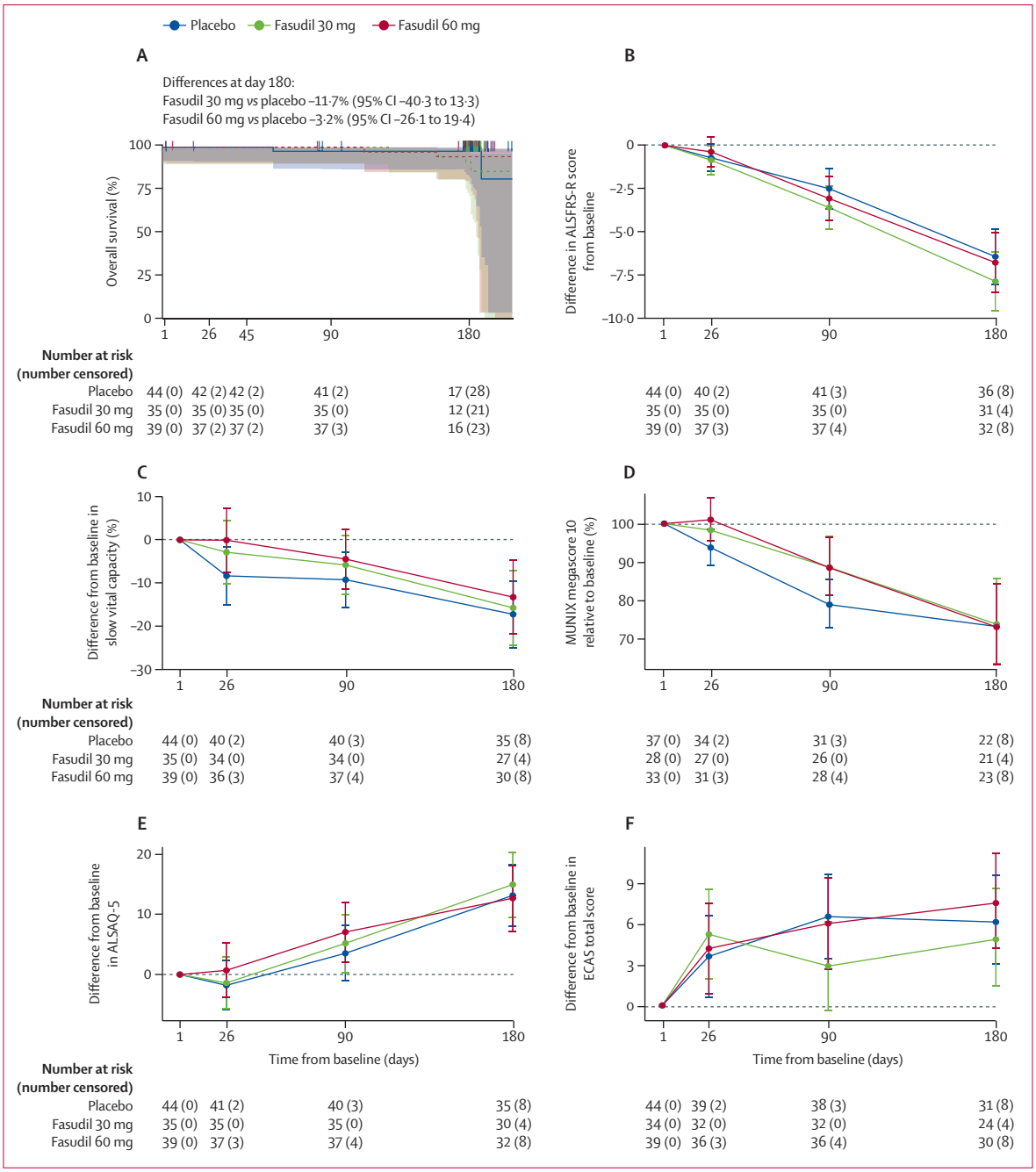

Figure 2: **Efficacy outcomes in the intention-to-treat population**
(A) Kaplan-Meier survival curves for overall time to death, tracheostomy, or permanent assisted ventilation up to day 180. (B–F) Estimated point difference from baseline up to day 180 for ALSFRS-R total score (B), predicted SVC (C), MUNIX megascore 10 (comprising the abductor pollicis brevis, abductor digiti minimi, biceps brachii, tibialis anterior, and extensor digitorum brevis muscles on both sides; (D), ALSAQ-5 (E), and ECAS (F). For overall survival (A), the event and censoring times are reported exact to the day whereas other investigations (B–F) were performed at certain visits with visit windows (±5 days maximum). Therefore, the numbers of patients for survival and the other outcomes vary. Error bars represent 95% CIs. ALSFRS-R=Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised. MUNIX=Motor Unit Number Index. SVC=slow vital capacity. ALSAQ-5=Amyotrophic Lateral Sclerosis Assessment Questionnaire-5. ECAS=Edinburgh Cognitive and Behavioural ALS Screen.

progression. Thus, the median progression rate of 0·58 points lost in ALSFRS-R per month was rather low,[21] which made recognition of small therapeutic effects after the short treatment period more challenging.

Fasudil was well tolerated and safe, with no treatment-related serious adverse events. The adverse events were mainly attributed to disease progression, and they did not differ between treatment groups. This observation aligns

with previous clinical trials of fasudil in individuals with cardiovascular disorders[9,22,23] but the finding is particularly noteworthy because fasudil was given twice daily intravenously over 20 days, representing a burden for participants. Fasudil is a potent vasodilator through reduced expression of angiotensin-converting enzyme[24] and inhibition of calcium channels.[25] In our study, we did not find any acute effects on blood pressure or heart rate during treatment.

During our trial, fasudil treatment over 20 days did not significantly affect the ALSFRS-R, survival, ALSAQ-5, or ECAS, which was not unexpected because of the short treatment duration. However, we observed a numerical difference in SVC favouring fasudil, which was significant in a prespecified analysis for females. However, this finding must be interpreted cautiously, because we did not adjust for multiple testing, and p values should be considered descriptive only. In addition to its established efficacy in pulmonary hypertension, fasudil has previously been shown to exert positive effects on lung vascular remodelling, lung smooth muscle, endothelial cells, and pulmonary inflammation.[26] Given that respiratory failure is by far the most common cause of death in amyotrophic lateral sclerosis, this finding might be of particular interest. Our data suggest that some effects of fasudil could be more sex-specific than others, but this possibility needs further exploration in future trials.[27]

MUNIX has been suggested to be more sensitive than ALSFRS-R for monitoring disease progression in amyotrophic lateral sclerosis,[14] detecting changes already present in presymptomatic muscles[16] and predicting the rate of disease progression earlier than ALSFRS-R.[28] Therefore, MUNIX might be more apt for detection of subtle therapeutic effects, as expected with a short-term treatment with a putatively neuroprotective drug such as fasudil. Here, we found a significant attenuation in MUNIX decline (corresponding to the loss of motor units) at day 90 in the fasudil 30 mg group and at days 26 and 90 in the fasudil 60 mg group. This effect was dose dependent, but no longer sustained at day 180. Given that these p values were not adjusted for multiplicity, these findings should be interpreted with caution; we hypothesise that a longer intervention period is needed to observe more sustainable effects. Moreover, fewer MUNIX measurements were performed at day 180, possibly because of the laborious examination technique, thus making the data at that timepoint less robust. Preclinical studies have shown that fasudil improves motor neuron survival, induces axonal regeneration, and inhibits the pathological activation of microglia.[4] In vitro, the effects on microglia occurred within hours.[7] Because such effects could lead to better preservation of motor neurons, it is plausible that an attenuated decay of motor units is among the earliest signs of a therapeutic effect exerted by fasudil. As the ALSFRS-R reflects a functional composite of different muscle groups, modulation of this scale would require a stronger effect size and might be insensitive to short-term treatment.

Our pharmacokinetic analyses showed that the active metabolite, hydroxyfasudil, was present in the CSF in a dose-dependent manner. Although the treatment regimens are not directly comparable, the high fasudil dose (60 mg per day) resulted in CSF concentrations (mean 9·4 [SD 4·5] µg/L) that were similar to those achieved in the *SOD1*G93A* mouse model that showed significant effects on survival (mean 8·8 [SD 3·0] µg/L).[7] We cannot rule out, however, that higher doses of fasudil could result in a stronger clinical effect. Since all patients in this trial were treated with riluzole, and concentrations of this drug in serum and CSF were not altered by fasudil, we can largely exclude a riluzole-mediated effect of fasudil.

The molecular biomarkers NfL and $p75^{ECD}$ did not differ between treatment groups, even though the absolute NfL values showed a numerical difference in the fasudil 60 mg group from baseline to day 180. We hypothesise that the magnitude of the effect of fasudil was not high enough because of the short treatment duration. Interestingly, a significant decrease in GFAP was noted at day 180 in the fasudil 60 mg group compared with placebo, potentially implying a decrease in astrogliosis, which was also one of the strongest effects of fasudil in *SOD1*G93A* mice.[7] Since this effect was only observed at one timepoint, it should be interpreted with caution and validated in a larger cohort. Notably, sensitivity analyses in the per-protocol population were largely identical to the results in the intention-to-treat population.

Our study has several limitations. Most importantly, the current label of fasudil restricted the total applicable dose and we were unable to implement a more sustained treatment framework. We are aware that a longer treatment duration might have resulted in more pronounced effects and will also be needed for the treatment of patients with amyotrophic lateral sclerosis. We observed more pronounced effects in the 60 mg fasudil group, suggesting that these effects were drug mediated. However, even if CSF concentrations of hydroxyfasudil were similar to those in the *SOD1*G93A* mouse model, we cannot rule out the possibility that even higher concentrations could elicit more potent results. An extended-release formulation of fasudil used in a trial for pulmonary hypertension resulted in two to three times higher hydroxyfasudil concentrations than the current study and was well tolerated.[23] To comply with the label, fasudil was administered intravenously, which is challenging for both patients and trial staff. The same licensed preparation has been shown to be usable as an oral solution, which would greatly facilitate its administration. To achieve the equivalent drug plasma concentrations, the oral formulation requires an approximately 1·5 times higher dose.[13] Subsequent trials with fasudil, therefore, should explore chronic administration of oral formulations, including higher doses.

The number of patients enrolled in this trial was calculated to assess the primary endpoints of safety and

tolerability. To improve the power for assessing efficacy endpoints, a higher number of patients will be needed, which would also permit generation of more robust data on defined subgroups, such as fast versus slow disease progressors. As ROCK-ALS was not powered for efficacy endpoints, the statistical analysis plan did not prespecify hierarchical testing, nor did it correct for multiplicity. Our trial protocol also did not foresee an open-label extension or any other follow-up after day 180, which would be informative for assessment of long-term efficacy.

The use of MUNIX comes with methodological challenges, such as training requirements and potential inter-rater variation. To address these challenges effectively, we provided comprehensive manuals to ensure standardised procedures. Moreover, we implemented rigorous on-site training sessions followed by controlled test-retest measurements. These measures optimised the reliability and consistency of MUNIX measurements, ultimately enhancing its utility in assessing disease progression and treatment efficacy. Notably, the trial population was mainly of White ethnicity and therefore our results cannot be generalised to a more diverse population.

Overall, our results suggest that fasudil 30 mg and 60 mg delivered intravenously is safe and tolerable. These findings support further investigation of this drug as a potential disease-modifying treatment for patients with amyotrophic lateral sclerosis.

ROCK-ALS Study group

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Contributors

JCK, AL, TF, and PL planned the study. JCK, IC, JD, RG, DZ, NB, MM, PC, EDLC, PW, TM, JG, M-HS, SA, JHW, UW, JK, MD, CN, ACL, JS, WC, and MW were study investigators. HB was the responsible project manager at Münchner Studienzentrum. JS was the head of the clinical trial. YR was in charge of the preparation of the study drug at the central pharmacy. IC, GZ, M-LR, and EF performed biomarker analyses. IC, JW, and MB contributed to genetic analyses. CN and MW conceived and interpreted the MUNIX examinations. AL, TF, JCK, and PL verified, analysed, and interpreted the data based on full access to the raw data. JCK, AL, and PL wrote the manuscript. All authors had full access to all data, critically revised the manuscript, and accept responsibility to submit the manuscript for publication.

Declaration of interests

JCK reports a grant from the Deutsche Gesellschaft für Muskelkranke and consulting fees from AbbVie, Biogen, Ipsen, Roche, and Zambon. RG reports grants from the Deutsche Gesellschaft für Muskelkranke, Initiative SMA, and Bundesministerium für Bildung und Forschung, as well as consulting fees from Biogen, Roche, ITF Pharma, and Zambon. DZ has received consulting fees from Novartis and Angelini Pharma and has served on an advisory board for Biogen. NB has received compensations from Mitsubishi Tanabe Pharma. PC serves on the editorial advisory boards of *ALS* and *The Revue Neurologique*; reports consultancy work or participation on advisory boards for Amylyx, Biogen, Cytokinetics, Ferrer, Mitsubishi Tanabe Pharma, VectorY, and Zambon; serves on the drug safety monitoring board for Quralis, and has received a research grant from Biogen. EDLC has received travel grants from Biogen and EFFIK. PW reports grants from the Boris Canessa Foundation and the Bundesministerium für Bildung and Forschung; and has received consulting fees from ITF Pharma, Zambon, Novartis, Biogen, and Roche. TM reports institutional grants from Cytokinetics, Ferrer, AL-S Pharma, Sanofi, Amylyx, Mitsubishi Tanabe, and Apellis Pharmaceuticals, as well as personal fees from Biogen, Amylyx, and ITF Pharma; and is co-founder and shareholder of the Ambulanzpartner Soziotechnologie APST. JG has received personal fees from UCB, Alexion, Amylyx, Roche, and Zambon; and is a member of advisory boards of the European Network to Cure ALS, Neuroimaging Society in amyotrophic Lateral Sclerosis, EU ALS coalition, and the World Federation of Neurology Motoneuron Disease group. M-HS received compensations for consulting from Amylyx, Zambon, EFFIK-Italfarmaco, and SOS Oxygene. M-LR reports grants from FightMND, MND Research Australia and the US National Institutes of Health. CN has received fees for non-related services for Biogen, Mitsubishi Tanabe, Roche, and Argenx. JW reports grants from the US National Institutes of Health. JS has received payments for participation on advisory boards, talks, travel, and research projects from

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Data sharing

De-identified individual participant data will be available 12 months after publication via a restricted-access, online data repository accessible at https://doi.org/10.25625/HOZIRN. These data will include demographics, vital signs, ALSFRS-R, SVC, ALSAQ-5, MUNIX, ECAS (total scores, each), and others, including a data dictionary. The study protocol is available as an open access publication.[12] Data will be available for researchers or investigators whose use has been reviewed and approved by the ROCK-ALS data access committee to be used in scientific analyses of the individual data or for merging with other data in meta-analyses. Applicants must sign a data access agreement. Requests should be addressed via email to the corresponding author.

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