Safety and efficacy of $GABA_A$ α5 antagonist S44819 in patients with ischaemic stroke: a multicentre, double-blind, randomised, placebo-controlled trial

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Lancet Neurol 2020; 19: 226–33

See **Comment** page 197

*Listed in the appendix

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See **Online** for appendix

Summary

Background S44819, a selective $GABA_A$ α5 receptor antagonist, reduces tonic post-ischaemic inhibition of the peri-infarct cortex. S44819 improved stroke recovery in rodents and increased cortical excitability in a transcranial magnetic stimulation study in healthy volunteers. The Randomized Efficacy and Safety Trial of Oral $GABA_A$ α5 antagonist S44819 after Recent ischemic Event (RESTORE BRAIN) aimed to evaluate the safety and efficacy of S44819 for enhancing clinical recovery of patients with ischaemic stroke.

Methods RESTORE BRAIN was an international, randomised, double-blind, parallel-group, placebo-controlled, multicentre phase 2 trial that evaluated the safety and efficacy of oral S44189 in patients with recent ischaemic stroke. The study was done in specialised stroke units in 92 actively recruiting centres in 14 countries: ten were European countries (Belgium, Czech Republic, France, Germany, Hungary, Italy, Netherlands, Poland, Spain, and the UK) and four were non-European countries (Australia, Brazil, Canada, and South Korea). Patients aged 18–85 years with acute ischaemic stroke involving cerebral cortex (National Institute of Health Stroke Scale [NIHSS] score 7–20) without previous disability were eligible for inclusion. Participants were randomly assigned to receive 150 mg S44819 twice a day, 300 mg S44819 twice a day, or placebo twice a day by a balanced, non-adaptive randomisation method with a 1:1:1 ratio. Treatment randomisation and allocation were centralised via the interactive web response system using computer-generated random sequences with a block size of 3. Blinding of treatment was achieved by identical appearance and taste of all sachets. Patients, investigators and individuals involved in the analysis of the trial were masked to group assignment. The primary endpoint was the modified Rankin Scale (mRS) score 90 days from onset of treatment, evaluated by shift analysis (predefined main analysis) or by dichotomised analyses using 0–1 versus 2–6 and 0–2 versus 3–6 cutoffs (predefined secondary analysis). Secondary endpoints were the effects of S44819 on the NIHSS and Montreal Cognitive Assessment (MoCA) scores, time needed to complete parts A and B of the Trail Making Test, and the Barthel index. Efficacy analyses were done on all patients who received at least one dose of treatment and had at least one mRS score taken after day 5 (specifically, on or after day 30). Safety was compared across treatment groups for all patients who received at least one dose of treatment. The study was registered at ClinicalTrials.gov, NCT02877615.

Findings Between Dec 19, 2016, and Nov 16, 2018, 585 patients were enrolled in the study. Of these, 197 (34%) were randomly assigned to receive 150 mg S44819 twice a day, 195 (33%) to receive 300 mg S44819 twice a day, and 193 (33%) to receive placebo twice a day. 189 (96%) of 197 patients in the 150 mg S44819 group, 188 (96%) of 195 patients in the 300 mg S44819 group, and 191 (99%) patients in the placebo group received at least one dose of treatment and had at least one mRS score taken after day 5, and were included in efficacy analyses. 195 (99%) of 197 patients in the 150 mg S44819 group, 194 (99%) of 195 patients in the 300 mg S44819 group, and 193 (100%) patients in the placebo group received at least one dose of treatment, and were included in safety analyses. The primary endpoint of mRS at day 90 did not differ between each of the two S44819 groups and the placebo group (OR 0·91 [95% CI 0·64–1·31]; p=0·80 for 150 mg S44819 compared with placebo and OR 1·17 [95% CI 0·81–1·67]; p=0·80 for 300 mg S44819 compared with placebo). Likewise, dichotomised mRS scores at day 90 (mRS 0–2 *vs* 3–6 or mRS 0–1 *vs* 2–6) did not differ between groups. Secondary endpoints did not reveal any significant group differences. The median NIHSS score at day 90 did not differ between groups (4 [IQR 2–8] in 150 mg S44819 group, 4 [2–7] in 300 mg S44819 group, and 4 [2–6] in placebo group), nor did the number of patients at day 90 with an NIHSS score of up to 5 (95 [61%] of 156 in 150 mg S44819 group, 106 [66%] of 161 in 300 mg S44819 group, and 104 [66%] of 157 in placebo group) versus more than 5 (61 [39%] in 150 mg S44819 group, 55 [34%] in 300 mg S44819 group, and 53 [34%] in placebo group). Likewise, the median MoCA score (22·0 [IQR 17·0–26·0] in 150 mg S44819 group, 23·0 [19·0–26·5] in 300 mg S44819 group, and 22·0 [17·0–26·0] in placebo group), time needed to complete parts A (50 s [IQR 42–68] in 150 mg S44819 group, 49 s [36–63] in 300 mg S44819 group, and 50 s [38–68] in placebo group) and B (107 s [81–144] in 150 mg S44819 group, 121 s [76–159] in 300 mg S44819 group, and 130 s [86–175] in placebo group) of the Trail Making Test, and the Barthel index (90 [IQR 60–100] in 150 mg S44819 group, 90 [70–100] in 300 mg S44819 group, and 90 [70–100] in placebo group) were similar in all groups. Number and type of adverse events were similar between the three groups. There were no drug-related adverse events and no drug-related deaths.

Interpretation There was no evidence that S44819 improved clinical outcome in patients after ischaemic stroke, and thus S44819 cannot be recommended for stroke therapy. The concept of tonic inhibition after stroke should be re-evaluated in humans.

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Introduction

Despite considerable progress in reperfusion therapies (that is, intravenous thrombolysis or endovascular treatment),[1] ischaemic stroke remains the leading cause of disability and second cause of death worldwide.[2] The majority of patients with ischaemic stroke exhibit neurological deficits in the long run. Hence, there is a major need for treatments that promote clinical recovery once a stroke has occurred. Pharmacological therapies that aim to promote neuronal survival in the acute stroke phase have not been successful in clinical trials.[3] As a consequence, there has been a shift of focus from the acute to the post-acute stroke phase, where translational neurologists are making major efforts to promote brain remodelling and plasticity. Experimental studies have shown that the remodelling and plasticity of brain tissue in the vicinity of and at a distance from the stroke lesion can successfully be stimulated by therapeutic tools when combined with rehabilitation strategies.[4–6]

The brain region adjacent to the stroke lesion, the so-called peri-infarct tissue, is particularly crucial for stroke recovery. In this region, remodelling of short and long-distance neuronal projections takes place that enables the remapping of lost sensorimotor functions.[6,7] In rodent models of stroke, neuronal inhibition in the peri-infarct tissue is tonically increased by extrasynaptic $GABA_A$ receptors.[8] Interestingly, the reversal of tonic inhibition (by administration of a negative allosteric modulator of the $GABA_A$ α5 receptor, or by genetic knockout of the α5 subunit of the $GABA_A$ receptor) in these models induced an early and sustained improvement of motor recovery.[8,9] Thus, the α5 subunit of the $GABA_A$ receptor could be a key target via which clinical recovery might be induced in patients who have had a stroke.

S44819 is a selective antagonist of the $GABA_A$ α5 receptor that is chemically and pharmacologically distinct from classical, diazepam-like benzodiazepine compounds.[10–12] S44819 binds to the GABA binding site of the $GABA_A$ α5 receptor without affinity to the benzodiazepine site.[13] In rodent models of stroke, the delivery of S44819 in the post-acute stroke phase (starting 72 h after stroke over 28 days) enhanced motor-coordination recovery, increased spatial

Research in context

Evidence before this study

We searched PubMed for articles published in any language between Jan 1, 1980, and Nov 14, 2019, using the keywords "$GABA_A$", "tonic inhibition", and "ischemic stroke". The search yielded four studies, three of which reported original rodent data and one of which reviewed rodent data. According to the three original rodent studies, neuronal excitability is tonically reduced after ischaemic stroke in the peri-infarct tissue by extrasynaptic $GABA_A$ receptors, and the reversal of tonic inhibition by administration of a negative allosteric modulator or antagonist of the $GABA_A$ α5 receptor or by genetic $GABA_A$ receptor α5 subunit knockout induced early and sustained stroke recovery. Hence, the α5 subunit of the $GABA_A$ receptor was suggested to be a key target via which clinical recovery in patients with stroke might be induced. An additional PubMed search using the keyword "S44819" yielded six studies, four of which reported original rodent data, and one presented studies in an ischaemic stroke model. There was one human phase 1 crossover study and one review. According to the four rodent studies, S44819 is a selective $GABA_A$ α5 receptor antagonist that enhanced motor-coordination recovery, increased spatial memory, reduced very delayed neuronal injury, increased perilesional neuroplasticity, and reduced brain atrophy in ischaemic stroke when administered in the post-acute stroke phase starting after 3 days. In the human phase 1 study, which evaluated effects of transcranial magnetic stimulation in healthy human volunteers, S44819 increased cortical excitability, indicating that S44819 accumulates in the brain parenchyma at concentrations high enough to induce physiological drug responses.

Added value of this study

To our knowledge, this is the first phase 2 trial to evaluate the safety and efficacy of S44819 for enhancing clinical recovery in patients with ischaemic stroke. In this international, randomised, double-blind, parallel-group, placebo-controlled, multicentre phase 2 trial we found that the $GABA_A$ receptor α5 subunit antagonist S44819 did not promote clinical recovery over 90 days. Thus, the previously reported efficacy of S44819 in rodents could not be replicated in human patients.

Implications of all the available evidence

The results of an ongoing study in human patients which examines another $GABA_A$ receptor α5 modulator in patients with cognitive deficits in schizophrenia (NCT02953639) are currently awaited. In case of repeated negative findings, the role of the $GABA_A$ receptor α5 subunit in the human brain should be carefully re-evaluated. The lack of side-effects of S44819 encourages further clinical research on $GABA_A$ α5 subunit antagonists.

memory, reduced very delayed neuronal injury, increased perilesional neuroplasticity and reduced brain atrophy.[14,15] In a phase 1 crossover, transcranial magnetic stimulation (TMS) study done in healthy volunteers, 100 mg of oral S44819 increased cortical excitability.[12] These data suggest that S44819 accumulates in the brain parenchyma at concentrations capable of inducing physiological drug responses. Following these observations, and considering that S44819 did not reveal any safety issues in unpublished phase 1 dose-escalation studies, the Randomised Efficacy and Safety Trial of Oral $GABA_A$ α5 antagonist S44819 after Recent ischemic Event (RESTORE BRAIN) aimed to evaluate the safety and efficacy of S44819 for enhancing clinical recovery in patients with ischaemic stroke.

Methods

Study design

RESTORE BRAIN was an international, randomised, double-blind, parallel-group, placebo-controlled, multicentre phase 2 trial that evaluated the safety and efficacy of oral S44189 in patients with recent ischaemic stroke. The study was done in specialised stroke units in 92 actively recruiting centres in 14 countries: ten were European countries (Belgium, Czech Republic, France, Germany, Hungary, Italy, Netherlands, Poland, Spain, and the UK) and four were non-European countries (Australia, Brazil, Canada, and South Korea). The trial was approved by the ethics committees of the study centres. An independent data and safety monitoring board regularly reviewed the safety data of the trial.

Participants

Patients were eligible for the RESTORE BRAIN study if they were 18–85 years of age and had an ischaemic stroke at least 48 h (2 days) but less than 144 h (6 days) before selection. Additional eligibility criteria were a National Institutes of Health Stroke Scale (NIHSS) score of 7–20, no previous disability or clinically significant pre-stroke cognitive impairment, and an acute ischaemic cortical or combined cortical-subcortical lesion confirmed by CT or MRI. Patients had to be clinically stable according to the investigator's judgment, and able to undertake rehabilitation if required after discharge from the neurological department. Intravenous thrombolysis and thrombectomy were permitted.

Exclusion criteria included absence of informed consent, acute haemorrhagic stroke or symptomatic haemorrhagic transformation, carotid endarterectomy or endovascular therapy of brain or neck vessels required during the study, any disease or condition that would place the patient at undue risk or likely to interfere with the study evaluation, known severe renal or hepatic impairment, brain MRI showing severe microangiopathy (grade 3 on Fazekas scale), brain CT showing confluent ischaemic white matter lesions, corrected QT interval by Fredericia above 480 ms in at least two out of three electrocardiograph (ECG) recordings, and pharmacological treatments interacting with $GABA_A$ receptors (eg, benzodiazepines) that could not be stopped for inclusion.

The study protocol was amended on May 24, 2017 (5 months after initiation of patient enrolment) following observations that patient recruitment was behind schedules at that time. In this amendment, inclusion criteria for patient age were widened from 18–80 years originally to 18–85 years, the maximum selection time was increased from 96 h (4 days) originally to 144 h (6 days), and brain CT was allowed for stroke diagnosis besides brain MRI. The brain CT was allowed because some study centres had been unable to provide brain MRIs as needed.

All patients (in some countries, their authorised caregivers) gave written informed consent for study participation. A detailed medical history was taken of all patients, which included anamnestic information on previous stroke, arterial hypertension, type 2 diabetes, hyperlipidemia, smoking, atrial fibrillation, and chronic kidney disease and coronary heart disease or previous myocardial infarcts, as well as information on the intake of platelet inhibitors, antihypertensive drugs, lipid-modifying drugs, and anticoagulants.

Randomisation and masking

Participants were randomly assigned to receive 150 mg S44819 twice a day, 300 mg S44819 twice a day, or placebo twice a day by a balanced, non-adaptive randomisation method with a 1:1:1 ratio. Treatment randomisation and allocation were centralised via the interactive web response system using computer-generated random sequences with a block size of 3. Blinding of treatment was achieved by identical appearance and taste of all sachets. Patients, investigators, and individuals involved in the analysis of the trial were masked to group assignment. Access to the randomisation code was strictly limited to spatially separate, non-trial team functions, including clinical trial supply unit staff who were responsible for packaging and labelling, and a dedicated contract research organisation responsible for the interactive response system. Investigators did not have access to the randomisation code. In emergency situations (eg, if knowledge of the treatment of a patient was required to provide appropriate medical treatment, or to assure the safety of trial participants) a code break was available to the investigators via an interactive voice system that was part of the internet-based interactive web response system. The success of masking was assessed by a dedicated contract research organisation.

Procedures

The trial consisted of three study periods: a selection period without study drug intake that lasted 2–6 days; a double-blind treatment period of 90 days from onset of treatment at day 0 (3–8 days following stroke), during which patients were allocated in a balanced ratio to three groups (150 mg S44819 twice a day, 300 mg S44819 twice a

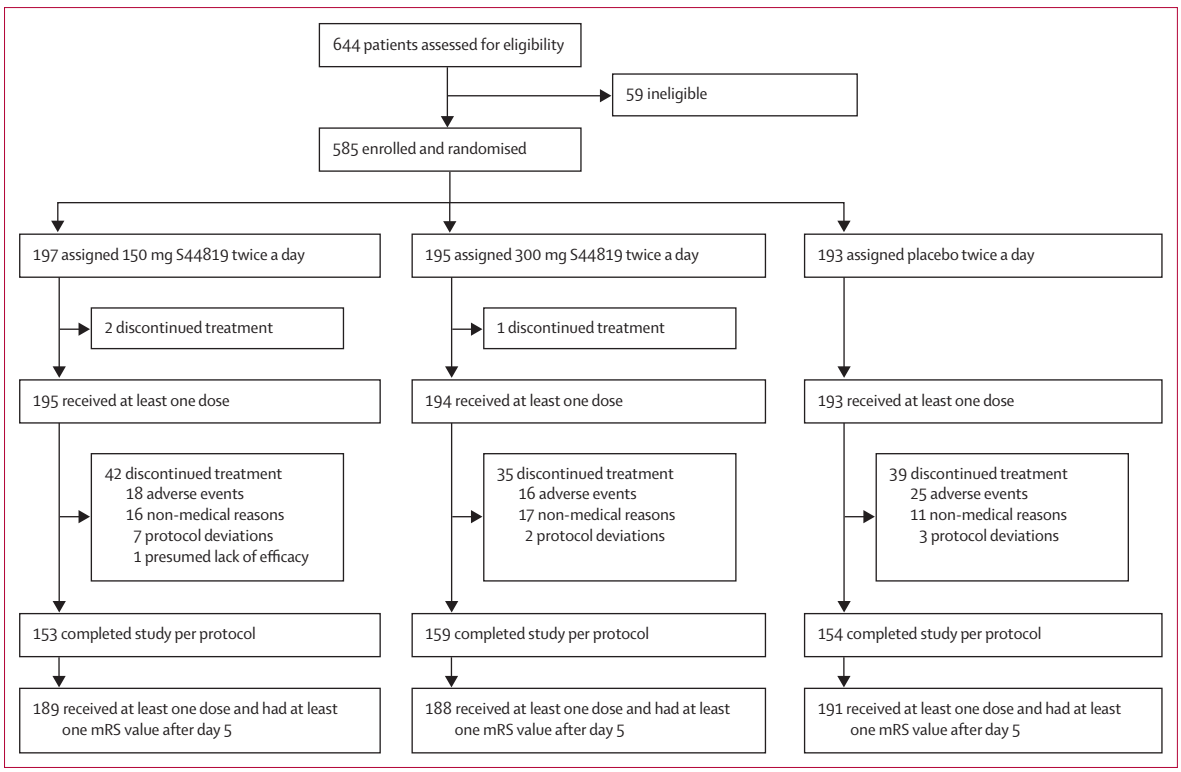

Figure 1: **Trial profile**
Patients who received at least one dose of treatment and had at least one modified Rankin Scale score taken after day 5 from onset of treatment were included in the full analysis set. Patients who received at least one dose of treatment were included in the safety set.

day, or placebo twice a day); and a follow-up period of 15 days without any study drug intake. Patients had to stay in the recruiting neurological department for at least 48 h after their first study drug intake and could then be discharged either to a rehabilitation department or home with outpatient rehabilitation if necessary. Physical and other rehabilitation therapies were provided throughout the study, on the basis of treatment decisions by physicians in charge.

Patients received the assigned study treatment twice daily for 90 days in sachets. Twice daily doses of 150 mg and 300 mg S44819 were chosen mainly on the basis of results of the TMS study[12] and additional phase 1 safety and tolerability studies. The TMS study is a more relevant translational approach than rodent studies. It has shown that S44819—at least at the dose of 100 mg—reaches the human cortex and increases corticospinal excitability by specifically reducing $GABA_A$ receptor-mediated inhibition.[12] In RESTORE BRAIN, doses were elevated (to 150 mg or 300 mg twice a day) to further augment treatment responses.

Following the baseline visit at day 0, participants had safety and efficacy (ie, modified Rankin Scale [mRS] and NIHSS) assessments at visits scheduled at days 5, 30, 60, and 90. The Barthel index was also obtained at days 30, 60, and 90. Cognitive evaluation was performed using the Montreal Cognitive Assessment (MoCA) and parts A and B of the Trail Making Test at days 30 and 90. All measures at baseline and at follow-ups were taken by study investigators and their teams who were qualified in stroke diagnosis and treatment.

Outcomes

The primary endpoint of this study was the mRS score at day 90, evaluated by shift analysis (predefined main analysis) or by dichotomised analyses using 0–1 versus 2–6 and 0–2 versus 3–6 cutoffs (predefined secondary analysis). Secondary endpoints were the effects of S44819 on the NIHSS and MoCA scores, time needed to complete parts A and B of the Trail Making Test, and the Barthel index. The safety of S44819 was evaluated by the number and type of adverse events (specified according to the European Medicines Agency guideline ICH topic E2A and directive 2001/20/EC of the European Parliament and Council with respect to their seriousness, severity, and causality). Besides clinical patient observation, S44819 safety was assessed by paraclinical tests which included supine systolic and diastolic blood pressure, body weight, 12-lead ECG recordings, blood laboratory tests (haemoglobin, platelets, leucocytes, creatinine, aspartate transaminase, alanine aminotransferase, and bilirubin), and a urinary pregnancy test if needed. Suicidal ideation and behaviour was assessed using the Columbia Suicide Severity Rating Scale.

	S44819 150 mg (n=197)	S44819 300 mg (n=195)	Placebo (n=193)
Age (years)			
<65	75 (38%)	57 (29%)	79 (41%)
≥65	122 (62%)	138 (71%)	114 (59%)
Median	67 (59–76)	71 (62–77)	69 (59–76)
Sex			
Female	91 (46%)	93 (48%)	80 (41%)
Male	106 (54%)	102 (52%)	113 (59%)
Ethnicity			
Caucasian	176/195 (90%)	173/190 (91%)	170/187 (91%)
African	1/195 (1%)	3/190 (2%)	4/187 (2%)
Asian	11/195 (6%)	13/190 (7%)	11/187 (6%)
Other	7/195 (4%)	1/190 (1%)	2/187 (1%)
Delay of treatment from stroke onset (days)	5 (4–6)	5 (4–6)	5 (4–6)
Acute therapy			
Only intravenous thrombolysis	45 (23%)	48 (25%)	41 (21%)
Only thrombectomy	35 (18%)	32 (16%)	26 (14%)
Intravenous thrombolysis and thrombectomy	34 (17%)	30 (15%)	42 (22%)
None	83 (42%)	85 (44%)	84 (44%)
Previous ischaemic stroke			
No	175 (89%)	175 (90%)	164 (85%)
Yes	22 (11%)	20 (10%)	29 (15%)
Arterial hypertension	150 (76%)	153 (79%)	145 (75%)
Type 2 diabetes	55 (28%)	46 (24%)	57 (30%)
Hyperlipidaemia	21 (11%)	23 (12%)	22 (11%)
Atrial fibrillation	44 (22%)	46 (24%)	47 (24%)
Smoking			
Current smoker	49 (25%)	49 (26%)	58 (30%)
Former smoker	50 (26%)	39 (20%)	55 (29%)
Never	97 (50%)	104 (54%)	79 (41%)
Chronic kidney disease	8 (4%)	3 (2%)	8 (4%)
Coronary heart disease or previous myocardial infarction	29 (15%)	22 (11%)	34 (17%)
Platelet inhibitors	47 (24%)	47 (24%)	66 (34%)
Antihypertensive drugs	12 (6%)	10 (5%)	16 (8%)
Lipid-lowering drugs	49 (25%)	36 (19%)	47 (24%)
Anticoagulants	26 (13%)	30 (15%)	25 (13%)

Data are n (%) or median (IQR).

Table 1: **Baseline demographic and clinical characteristics of the randomly assigned population**

Statistical analysis

Estimations using Whitehead's formula for ordered categorical data with Bonferroni correction for multiplicity, which assumed a dropout rate of 5% and a power of 85% with a two-sided type 1 error of 5%, revealed that a sample size of 192 patients per group was needed to detect a difference of 15% in the cumulative proportion of patients with a mRS score of 0–2 at day 90 between at least one treatment group and the placebo group (eg, 45% in placebo group vs 60% in one of the S44819 groups). All efficacy analyses were done in the full analysis set, defined as all patients who received at least one dose of treatment and had at least one mRS score taken after day 5 (specifically, on or after day 30).

The primary endpoint was analysed using ordinal logistic regression adjusted for country and previous revascularisation therapy (that is, thrombolysis or endovascular therapy or both versus none). The ordinal logistic regression based on cumulative logits provides a treatment effect in the form of a common estimate of the odds ratio (OR) for improvement above considered cut points. The OR was the adjusted ordinal cumulative logistic OR that estimates a common effect size measure along the first five cut points on the mRS ordinal scale. The treatment effect was measured by estimating the OR and its 95% CIs by means of the model coefficient derived from the ordinal logistic regression model. The choice of the shift analysis is based on the assumption that the mRS is a true interval scale when categories 5 and 6 are combined, meaning that any one step increment in the scale has the same value across the scale. The shift analysis is now an accepted methodology in the stroke community and by regulatory agencies. To check the proportionality assumption, the cumulative logits were plotted for each value of the mRS score. Indeed the assumptions for an ordinal logistic model implied that the curves on the various cumulative logits were parallel. This assumption was assessed visually for a given predictor by plotting it against the empirical logits. Missing data at the day 90 time-point were imputed using a last observation carried forward approach. Multiplicity was handled using the step-down Holm procedure.

Secondary endpoints were analysed by non-parametric Mann-Whitney U tests. Where applicable (that is, for the NIHSS score), statistical estimates using the Hodges-Lehmann approach were computed. Missing data at the day 90 time-point were again imputed using the last observation carried forward approach. In post-hoc analyses, we evaluated the number and percentage of patients with an NIHSS score of up to 5 versus more than 5 in the three treatment groups. Further post-hoc analyses were done on the mRS score at day 90 in patients categorised by revascularisation therapy (yes *vs* no), median NIHSS score at day 0 (≤14 *vs* >14 points), or median delay of treatment onset (≤4 *vs* >4 days). All safety analyses were done in the safety set, defined as all patients who received at least one dose of treatment. p values below 0·05 were considered significant. This trial was supervised by a data monitoring committee. Statistical analyses were done using SAS (version 9.2). The study was registered at ClinicalTrials.gov, NCT02877615.

Role of the funding source

The funder of the study (represented by UM, M-LA-I, AS, EL, and MW) was responsible for the protocol design, data collection, data analysis, patient recruitment, and data monitoring in close discussion with the advisory board (HC, CLB, and DMH). All members of the advisory board had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between Dec 19, 2016, and Nov 16, 2018, a total of 644 patients were assessed for eligibility, of which 585 patients were enrolled (figure 1). 197 (34%) were randomly assigned to receive 150 mg S44819 twice a day, 195 (33%) to receive 300 mg S44819 twice a day, and 193 (33%) to receive placebo twice a day. 189 (96%) of 197 patients in the 150 mg S44819 group, 188 (96%) of 195 patients in the 300 mg S44819 group, and 191 (99%) of 193 patients in the placebo group were included in efficacy analyses. 496 (87%) of 568 patients had an mRS score available at day 90. This means that 72 (13%) patients of the full analysis set were imputed for the primary analysis. 195 (99%) of 197 patients in the 150 mg S44819 group, 194 (99%) of 195 patients in the 300 mg S44819 group, and 193 (100%) patients in the placebo group were included in safety analyses.

Baseline characteristics were similar between treatment groups (table 1). The median age was 67 years. 519 (91%) of 572 patients were of Caucasian origin. Patients received treatment a median of 5 days from stroke onset. 333 (57%) of 585 patients received acute therapy (thrombolysis, thrombectomy, or both). 71 (12%) had a previous history of stroke, 448 (77%) had arterial hypertension, 158 (27%) had type 2 diabetes, 66 (11%) had hyperlipidaemia, and 137 (23%) had atrial fibrillation.

The primary endpoint of mRS at day 90 (evaluated as shift analysis using ordinal logistic regression) did not differ between each of the two S44819 groups and the placebo group (OR 0·91 [95% CI 0·64–1·31]; p=0·80 for 150 mg S44819 compared with placebo and OR 1·17 [95% CI 0·81–1·67]; p=0·80 for 300 mg S44819 compared with placebo; figure 2). Likewise, dichotomised mRS scores at day 90 (mRS 0–2 *vs* 3–6 or mRS 0–1 *vs* 2–6) did not differ between groups (table 2).

Secondary endpoints did not reveal any significant group differences. The median NIHSS score at day 90 did not differ between groups (4 [IQR 2–8] in 150 mg S44819 group, 4 [2–7] in 300 mg S44819 group, and 4 [2–6] in placebo group; appendix p 1), nor did the number of patients at day 90 with an NIHSS score of up to 5 (95 [61%] of 156 in 150 mg S44819 group, 106 [66%] of 161 in 300 mg S44819 group, and 104 [66%] of 157 in placebo group) versus more than 5 (61 [39%] in 150 mg S44819 group, 55 [34%] in 300 mg S44819 group, and 53 [34%] in placebo group; appendix p 2). Likewise, the median MoCA score (22·0 [IQR 17·0–26·0] in 150 mg S44819 group, 23·0 [19·0–26·5] in 300 mg S44819 group, and 22·0 [17·0–26·0] in placebo group), time needed to complete parts A (50 s [IQR 42–68] in 150 mg S44819 group, 49 s [36–63] in 300 mg S44819 group, and 50 s[38–68] in placebo group) and B (107 s [81–144] in 150 mg S44819 group, 121 s [76–159] in 300 mg S44819 group, and 130 s [86–175] in placebo group) of the Trail Making Test, and the Barthel index (90 [IQR 60–100] in 150 mg S44819 group, 90 [70–100] in 300 mg S44819 group, and 90 [70–100] in placebo group) were similar in all groups (appendix p 3).

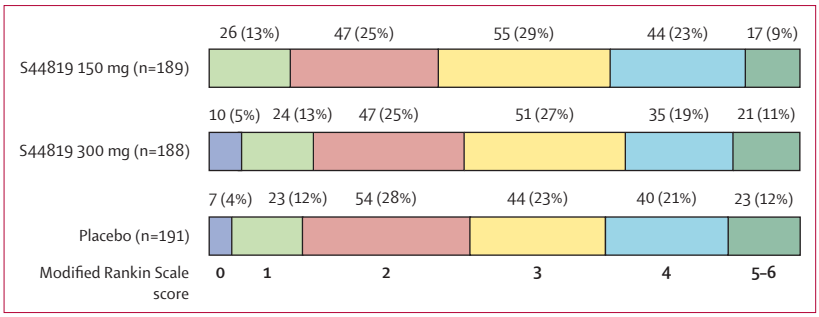

Figure 2: **Modified Rankin Scale score at day 90 in the full analysis set**

	S44819 150 mg (n=189)	S44819 300 mg (n=188)	Placebo (n=191)
0–2; 3–6	73 (39%); 116 (61%)	81 (43%); 107 (57%)	84 (44%); 107 (56%)
Comparison with placebo	0·78 (0·51–1·20)	0·98 (0·64–1·51)	1 (ref)
0–1; 2–6	26 (14%); 163 (86%)	34 (18%); 154 (82%)	30 (16%); 161 (84%)
Comparison with placebo	0·81 (0·44–1·49)	1·22 (0·68–2·19)	1 (ref)

Data are n (%) or odds ratio (95% CI).

Table 2: **Dichotomised modified Rankin Scale scores at day 90**

In additional post-hoc analyses, mRS scores at day 90 did not significantly differ between the two S44819 groups and the placebo group, when patients were categorised by revascularisation therapy (yes *vs* no), median NIHSS score at day 0 (≤14 *vs* >14 points), or median delay of treatment onset (≤4 *vs* >4 days; appendix p 4).

Safety analyses revealed that the number and type of adverse events were similar in the three groups (table 3). 17 (9%) of 195 patients in the 150 mg S44819 group, 12 (6%) of 194 patients in the 300 mg S44819 group, and 25 (13%) of 193 patients in the placebo group had adverse events suspected to be related to the study which resulted in treatment withdrawal. In order of frequency, the most frequent adverse events were arterial hypertension, urinary tract infection, fall, constipation, depression, nausea, diarrhoea, arterial hypotension, vomiting, atrial fibrillation, dehydration, pneumonia, hypokalaemia, headache, depressed mood, insomnia, and deep vein thrombosis. There was no evidence of suicidal ideations or behaviours.

Discussion

This international, randomised, double-blind, parallel-group, placebo-controlled, multicentre phase 2 trial showed that administration of S44819, a $GABA_A$ α5 antagonist, in patients with ischaemic stroke at doses of 150 mg or 300 mg twice daily does not improve clinical recovery over 90 days when initiated 3–8 days after stroke onset. Disability evaluated by the mRS, neurological and cognitive deficits examined by the NIHSS and MoCA scores, time to complete parts A and B of the Trail Making Test, and daily life activities assessed by the

	S44819 150 mg (n=195)		S44819 300 mg (n=194)		Placebo (n=193)	
	Events	Patients (%)	Events	Patients (%)	Events	Patients (%)
Adverse events suspected to be related to the study drug	35	22 (11%)	32	20 (10%)	49	29 (15%)
Serious adverse events	128	71 (36%)	106	56 (29%)	133	65 (34%)
Serious adverse events suspected to be related to the study drug	3	2 (1%)	2	2 (1%)	17	11 (6%)
Severe adverse events	30	21 (11%)	31	19 (10%)	21	14 (7%)
Severe adverse events suspected to be related to the study drug	0	0	1	1 (1%)	4	4 (2%)
Adverse events leading to death	9	7 (4%)	10	9 (5%)	6	4 (2%)
Adverse events leading to death suspected to be related to the study drug	0	0	0	0	1	1 (1%)
All reported adverse events*	524	154 (79%)	460	139 (72%)	562	152 (79%)
Arterial hypertension	16	16 (8%)	17	16 (8%)	14	14 (7%)
Urinary tract infection	16	14 (7%)	18	15 (8%)	17	17 (9%)
Fall	5	5 (3%)	21	14 (7%)	23	17 (9%)
Constipation	24	24 (12%)	13	13 (7%)	18	18 (9%)
Depression	13	13 (7%)	13	13 (7%)	10	10 (5%)
Nausea	7	7 (4%)	11	11 (6%)	6	6 (3%)
Diarrhoea	8	7 (4%)	11	10 (5%)	11	9 (5%)
Arterial hypotension	8	8 (4%)	9	9 (5%)	6	6 (3%)
Vomiting	6	6 (3%)	9	8 (4%)	12	10 (5%)
Atrial fibrillation	13	13 (7%)	7	7 (4%)	7	7 (4%)
Dehydration	7	7 (4%)	8	7 (4%)	1	1 (1%)
Pneumonia	5	5 (3%)	7	7 (4%)	5	5 (3%)
Hypokalaemia	1	1 (1%)	8	7 (4%)	4	4 (2%)
Headache	9	9 (5%)	6	6 (3%)	10	9 (5%)
Depressed mood†	7	7 (4%)	6	6 (3%)	6	6 (3%)
Insomnia	5	5 (3%)	6	6 (3%)	10	10 (5%)
Deep vein thrombosis	1	1 (1%)	6	6 (3%)	4	4 (2%)

Data are n (%). *Subsequently listed adverse events are shown if they were noted in more than 3% of patients in any of the S44819 groups. †Not fulfilling clinical criteria for depression.

Table 3: **Summary of adverse events in the safety population**

Barthel index did not differ between groups. None of the preplanned analyses showed any difference between the treatment groups.

This study was based on the solid pathophysiological concept of peri-infarct tonic inhibition,[8,9] which was targeted by the $GABA_A$ α5 antagonist. In the preparation of RESTORE BRAIN, two rodent studies had been performed (one in mice and one in rats),[14,15] in which S44819 enhanced functional motor-coordination recovery, increased spatial memory, and improved perilesional brain remodelling and neuroplasticity when administered in the post-acute stroke phase starting 3 days post-stroke for 28 days. A pitfall of most pharmacological studies in animal models is the absence of drug biodistribution and pharmacodynamics data in human brain tissue. By showing that S44819 increased cortical excitability in a phase 1 crossover TMS study in healthy volunteers,[12] evidence had been provided that S44819 accumulates in the brain parenchyma after oral delivery at concentrations capable of inducing physiological drug responses.

In view of these promising pharmacodynamics data, the negative results of this clinical trial could be explained in different ways. The clinical stroke severity of our enrolled patients was similar to those enrolled in the NEST-1 trial.[16] Thus, the negative results were unlikely to be caused by a non-representative patient sample. Instead, other explanations are more likely.

The efficacy of S44819 in patients with acute ischaemic stroke might have been overestimated on the basis of rodent studies. The rodent studies were done in highly standardised stroke models in young, otherwise healthy male animals.[14,15] In human patients, more variable clinical deficits and cortex involvement, advanced age, female sex, associated vascular risk factors and diseases, revascularising therapies, and concomitant medications might have decreased drug responses, and thus reduced the ability to detect clinical recovery effects. Hence, an absolute improvement in clinical outcome equivalent to a 15% shift in the mRS, as expected in the sample size calculation, may have been too optimistic.

The accumulation of S44819 in the brain of patients who had a stroke may have been misjudged from the TMS study in healthy volunteers.[10] Notably, the healthy volunteers were young (mean age 27·5 years [SD 6·0]), whereas the patients enrolled in this study with recent ischaemic stroke were older and had vascular risk factors and comorbidities. Differences in age, risk factors, and comorbidities could have led to alterations in drug biodistribution, and insufficient drug concentrations could have resulted in the loss of plasticity-promoting effects. Pharmacodynamics studies, ahead of future clinical trials, should be considered in older human patients and possibly also in patients who had a stroke.

The choice of primary endpoint might have impeded the detection of a drug effect. Ranging from 0 to 6, the mRS is a comparably rough scale. Besides motor performance, the score is affected by a variety of non-motor factors that include the general health status, language abilities, and psychosocial variables. More targeted scales of upper limb function such as the Fugl-Meyer Assessment scale might be better choices for detecting moderate motor improvements, although this scale is not supported by drug authorities. Thorough discussions about the selection of appropriate endpoints will be needed in the field.

The time window of S44819 delivery in humans compared with rodents must also be discussed. The median delay of treatment onset in our patient sample was 5 days and the median treatment duration was 90 days, compared with 3 and 28 days in rodents. There is no consensus on how the time window of brain remodelling and plasticity in rodents should be translated to human patients with ischaemic stroke, and the biochemical milieu of these patients and its development over time is insufficiently characterised. In rodents, effects of S44819 on motor-coordination recovery were noted within

7–14 days post-stroke, and functional improvements persisted after 28 days of treatment.[14,15] Whether 28 days in rodents adequately reflect 90 days in humans is unknown. The human brain is far larger than the rodent brain. As such, the formation of new neuronal circuits could take longer in humans than in rodents.

A true null effect of $GABA_A$ α5 antagonist on stroke recovery in the human brain should also be considered. Hence, the contribution of extrasynaptic $GABA_A$ α5 receptors to post-stroke tonic inhibition could differ between rodents and humans, and tonic inhibition might have limited effect on stroke recovery in humans. As a consequence, the $GABA_A$ α5 antagonist may have had no effect on clinical stroke outcome.

S44819 could be reliably administered and was well tolerated in patients with ischaemic stroke. The number and type of adverse events did not differ from placebo, and no safety signals were noted in laboratory tests. Therefore, future studies in humans should further explore the concept of peri-infarct tonic inhibition in ischaemic stroke and measure potential benefits of the drug in neuropsychiatric conditions.

In conclusion, 150 mg or 300 mg S44819 twice daily cannot be recommended for promoting clinical recovery in patients with ischaemic stroke. Further studies are needed to delineate whether and how the pharmacological modulation of $GABA_A$ α5 receptors affects cortical excitability in patients with ischaemic stroke. An ongoing clinical study of negative allosteric modulators in cognitive impairment associated with schizophrenia (NCT02953639) might help to define the therapeutic potential of $GABA_A$ α5 antagonists.

Contributors

All authors contributed substantially to the preparation of this manuscript. UM, M-LA-I, AS, EL, and MW were responsible for protocol design, data collection, and data analysis in close discussion with the advisory board (represented by HC, CLB, and DMH). All authors interpreted the data. HC, UM, and DMH prepared the figures and tables and wrote the manuscript. All authors revised and finalised the manuscript.

Declaration of interests

HC, CLB, and DMH were members of the RESTORE BRAIN advisory board and received fees for it. UM, M-LA-I, AS, EL, and MW are Servier employees. HC reports personal fees from Hovid for steering committee activities. DMH reports grants from Servier.

Data sharing

Anonymised patient-level and study-level clinical trial data (including the clinical study report) and the study protocol will be shared in agreement with the Servier Data Sharing Policy. Data access will be granted to researchers upon submission of a research proposal to the data request portal, provided the request is approved by a dedicated committee and the data sharing agreement is signed by the requesting person.

Acknowledgments

We are grateful to Heinrich Mattle (Department of Neurology, University Hospital Berne, Berne, Switzerland), Joachim Röther (Department of Neurology, Asklepios Klinik Altona, Hamburg, Germany) and François Montestruc (eXYSTAT Malakoff, Malakoff, France) for their participation in the data monitoring committee. We would also like to thank all participating investigators and patients for their motivation, cooperation, and confidence.

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For **data sharing** see https://clinicaltrials.servier.com/data-request-portal/