Non-immunogenic recombinant staphylokinase versus alteplase for patients with acute ischaemic stroke 4·5 h after symptom onset in Russia (FRIDA): a randomised, open label, multicentre, parallel-group, non-inferiority trial

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Summary

Background Non-immunogenic staphylokinase is modified recombinant staphylokinase with low immunogenicity, high thrombolytic activity, and selectivity to fibrin. We aimed to assess the safety and efficacy of a single intravenous bolus of non-immunogenic staphylokinase compared with alteplase in patients with acute ischaemic stroke within 4·5 h after symptom onset.

Methods We did a randomised, open-label, multicentre, parallel-group, non-inferiority trial in 18 clinical sites in Russia. We included patients aged 18 years and older with a diagnosis of acute ischaemic stroke (up to 25 points on the National Institutes of Health Stroke Scale). The study drug had to be administered within 4·5 h after the onset of symptoms. Patients were randomly assigned to receive either non-immunogenic staphylokinase (10 mg) or alteplase (0·9 mg/kg, maximum 90 mg), both administered intravenously. The randomisation sequence was created by an independent biostatistician using computer-generated random numbers. 84 blocks (block size of four) of opaque sealed envelopes were numbered sequentially from 1 to 336 and were opened in numerical order. Patients were unaware of their assigned treatment and were assessed by the study investigators who were also unaware of the treatment assignment on all trial days. Emergency department staff, who administered the assigned drug and opened the envelopes, were not masked to treatment. The primary efficacy endpoint was a favourable outcome, defined as a modified Rankin scale (mRS) score of 0–1 on day 90. The margin of non-inferiority was established as 16% for the difference in mRS score of 0–1 on day 90. Non-inferiority was tested using Welch's t-test for the primary outcome only. Endpoints were analysed in the per-protocol population, which comprised all randomly assigned patients who completed treatment without any protocol violations; this population was identical to the intention-to-treat population. This trial is completed and registered at ClinicalTrials.gov, NCT03151993.

Findings Of 385 patients recruited from March 18, 2017, to March 23, 2019, 336 (87%) were included in the trial. 168 (50%) patients were randomly assigned to receive non-immunogenic staphylokinase and 168 (50%) to receive alteplase. The median duration of follow-up was 89 days (IQR 89–89). 84 (50%) of 168 patients in the non-immunogenic staphylokinase group had a favourable outcome at day 90 compared with 68 (40%) of 168 patients in the alteplase group (odds ratio [OR] 1·47, 95% CI 0·93 to 2·32; p=0·10). The difference in the rate of favourable outcome at day 90 was 9·5% (95% CI –1·7 to 20·7) and the lower limit did not cross the margin of non-inferiority ($p_{non\text{-}inferiority}$ <0·0001). Symptomatic intracranial haemorrhage occurred in five (3%) patients in the non-immunogenic staphylokinase group and in 13 (8%) patients in the alteplase group (p=0·087). On day 90, 17 (10%) patients in the non-immunogenic staphylokinase group and 24 (14%) patients in the alteplase group had died (p=0·32). 22 (13%) patients in the non-immunogenic staphylokinase group had serious adverse events, compared with 37 (22%) patients in the alteplase group (p=0·044).

Interpretation Non-immunogenic staphylokinase was non-inferior to alteplase for patients with acute ischaemic stroke. Mortality, symptomatic intracranial haemorrhage, and serious adverse events did not differ significantly between groups. Future studies are needed to continue to assess the safety and efficacy of non-immunogenic staphylokinase in patients with acute ischaemic stroke within the 4·5 h time window, and to assess the drug in patients with acute ischaemic stroke outside this time window with reperfusion CT or magnetic resonance angiography followed by thrombectomy if necessary.

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*Study group members listed at the end of the Article

For the Russian translation of the Summary see **Online** for appendix 1

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Research in context

Evidence before this study

We searched PubMed for research articles published in any language from Jan 10, 1995, up to April 19, 2021, using the terms "staphylokinase", "ischemic stroke", and "clinical trial or study". We excluded articles on patients with acute myocardial infarction, cardiac failure, and pulmonary embolism to identify publications reporting the use of staphylokinase for treatment of patients with acute ischaemic stroke. Our search found no articles.

Added value of this study

To the best of our knowledge, the results of FRIDA are the first report of the use of non-immunogenic staphylokinase in patients with acute ischaemic stroke, with results suggesting that this drug is non-inferior to alteplase for the treatment of patients with acute ischaemic stroke within 4·5 h of symptom onset. Mortality, symptomatic intracranial haemorrhage, and serious adverse events did not differ between treatment groups. Non-immunogenic staphylokinase is easy to administer with a rapid single bolus without weighing the patient, simplifying clinical use, and it is available at a low cost in Russia.

Implications of all the available evidence

Non-immunogenic staphylokinase is an effective and safe thrombolytic agent for the treatment of patients with acute ischaemic stroke within 4·5 h of symptom onset. Future observational studies of non-immunogenic staphylokinase are needed for continued assessment of the safety and efficacy of this drug within the 4·5 h time window. Future studies should also be done with reperfusion CT or magnetic resonance angiography to assess patients later than 4·5 h after symptom onset, with addition of thrombectomy if necessary.

Introduction

Stroke is a leading cause of death and disability worldwide.[1] More than 70% of stroke events are acute ischaemic strokes and require intravenous thrombolytic therapy, but only a small proportion of patients with ischaemic stroke are eligible for such treatment because of the time window after symptom onset.[2] Alteplase, a recombinant tissue-type plasminogen activator, is the only drug approved globally for the treatment of patients with acute ischaemic stroke.[3] However, alteplase has little fibrinolytic efficacy, leading to arterial recanalisation in less than 50% of patients in whom the drug is properly administered. Therefore, the development of an alternative, more effective, and safer thrombolytic therapy is necessary.[4,5]

Staphylokinase was first isolated by Lack in 1948,[6] and methods of purification were improved in subsequent years. The unique fibrin selectivity of staphylokinase made this thrombolytic agent a candidate for first-line treatment of patients with ST-segment elevation myocardial infarction (STEMI) and acute ischaemic stroke in the 1990s.[7] In pilot trials of staphylokinase in patients with STEMI, most individuals had higher reperfusion in the infarct-related coronary artery than in the alteplase group, but they developed neutralising antistaphylokinase IgGs after the infusion, which persisted for several months.[8] Therefore, the high immunogenicity of staphylokinase prevented widespread use. Amino acid substitutions—including Lys74Ala, Glu75Ala, and Arg77Ala—resulted in a more than 200-times reduction in titres of neutralising antistaphylokinase IgGs in patients with STEMI.[9,10]

Non-immunogenic staphylokinase (SuperGene, Moscow, Russia) is a thrombolytic agent with reduced production of antibodies, low immunogenicity, and high biological activity. It was registered in Russia in 2012 as a thrombolytic drug for the treatment of patients with STEMI.[11] In a multicentre, randomised clinical trial in patients with STEMI (FRIDOM 1),[12] non-immunogenic staphylokinase was administered as a single intravenous bolus of 15 mg in all patients, regardless of bodyweight, and showed similar high reperfusion patency and fewer minor bleeding events compared with tenecteplase, as well as absence of neutralising IgGs. The reported low incidence of intracranial haemorrhage and bleeding events and high efficacy of this drug in more than 4000 patients with STEMI suggested that it could be investigated in patients with acute ischaemic stroke.[13] Therefore, we aimed to compare non-immunogenic staphylokinase with alteplase in patients with acute ischaemic stroke.

Methods

Study design and participants

We did a randomised, open-label, multicentre, parallel-group, non-inferiority trial (FRIDA) at 18 clinical sites in Russia. The protocol for the study was initially approved by the Russian Ministry of Health (no 498) on July 15, 2016 (protocol version 1.0), and a subsequent amendment to expand inclusion criteria was approved on June 5, 2018 (protocol version 2.0). Protocol version 2.0 was updated in accordance with the Guidelines for the Early Management of Patients With Acute Ischemic Stroke published by the American Heart Association and American Stroke Association in 2018.[2] The trial protocol and amendments were accepted and approved as a phase 3 trial by the Russian Ministry of Health, the ethics committee of the Russian Ministry of Health, and local ethics committees of the clinical centres.

We initially included patients aged 18–80 years (protocol version 1.0) then additionally considered for inclusion those older than 80 years (ie, including all patients aged

≥18 years; protocol version 2.0). A further inclusion criterion was a diagnosis of acute ischaemic stroke, defined in protocol version 1.0 as 5–25 points on the National Institutes of Health Stroke Scale (NIHSS)[14] and in protocol version 2.0 as up to 25 points on the NIHSS (ie, including patients with a mild neurological deficit [NIHSS score <4]).[15] Non-contrast CT of the brain was required before randomisation to exclude patients with intracranial haemorrhage or severe stroke. Administration of the assigned study drug had to take place within 4·5 h after symptom onset. The major inclusion and exclusion criteria are provided in the appendix 2 (p 2).

All participants or their legal representatives provided written informed consent before enrolment. The treating clinician could proxy consent for patients who were unable to because of the features of their acute ischaemic stroke. The study was done in accordance with all applicable local regulations, Good Clinical Practice guidelines (as outlined at the International Conference on Harmonization), and the Declaration of Helsinki.

Randomisation and masking

Participants were randomly assigned (1:1) to receive either non-immunogenic staphylokinase or alteplase by block randomisation (block sizes of four), stratified by age and NIHSS score. The independent biostatistician created the randomisation sequence, using computer-generated random numbers. 84 blocks of opaque sealed envelopes were numbered sequentially from 1 to 336. To ensure proper randomisation and to minimise the predictability of the contents in the envelope, the block size was hidden from researchers and the envelopes were opened in numerical order by the emergency department staff. This was an open-label study: the emergency department staff were aware of the assigned study drug. Patients were unaware of their assigned treatment and were assessed by the study investigators who were also unaware of treatment assignments at all trial visits. On day 90, for endpoint assessments, the modified Rankin scale[16] (mRS) score, NIHSS score, and Barthel index[17] score were recorded on a separate case-report form so that investigators could not identify the name of the study drug. The CT done to assess symptomatic intracranial haemorrhage was done independently at each centre and the name of the drug was hidden from treatment allocation.

Procedures

Non-immunogenic staphylokinase (10 mg reconstituted in 10 mL of NaCl solution [0·9%]) was administered as a single intravenous bolus for 10 s, regardless of bodyweight. Alteplase (Boehringer Ingelheim, Ingelheim, Germany; 0·9 mg/kg; maximum dose 90 mg) was administered as an intravenous bolus of 10% of the total dose for 1–2 min and the remaining dose by continuous intravenous infusion within 60 min. Patients were assessed by study investigators at the time of enrolment and 1–24 h, 2 days, 7 days, 14 days, and 90 days after drug administration. Additionally, patients' vital signs (blood pressure, heart rate, degree of oxygenation, pulse, and respiratory rate) were monitored during the first 24 h and on the days of the examinations. The initial assessments included a physical examination; scores on NIHSS, mRS, and Barthel index; and CT or MRI. NIHSS and mRS were scored at baseline and on days 1, 2, 7, 14, and 90 after drug administration. The Barthel index was scored on days 7, 14, and 90 after drug administration. At baseline and on days 1 and 7 after drug administration, a CT or MRI was done and the Alberta Stroke Program Early CT score[18] was assessed. The Trial of Org 10172 in Acute Stroke Treatment[19] (TOAST) classification was assessed the day after drug administration.

See Online for appendix 2

Outcomes

The primary efficacy endpoint was a favourable outcome, defined by an mRS score of 0–1 on day 90 after drug administration. The secondary efficacy endpoint was defined as simultaneous outcomes of mRS score 0–1, NIHSS score 0–1, and Barthel index score of 95 or more on day 90. Additional secondary endpoints were NIHSS score after 24 h and on day 90. An mRS score of 0–2 on day 90 was included as a post-hoc analysis of outcome. Safety endpoints were mortality on day 90, intracranial haemorrhage, symptomatic intracranial haemorrhage (European Cooperative Acute Stroke Study III [ECASS III] definition[24]), and other serious adverse events. A list of serious adverse events and their relationship to the study drug was sent to the Russian Ministry of Health for verification and approval. The ECASS III definition of symptomatic intracranial

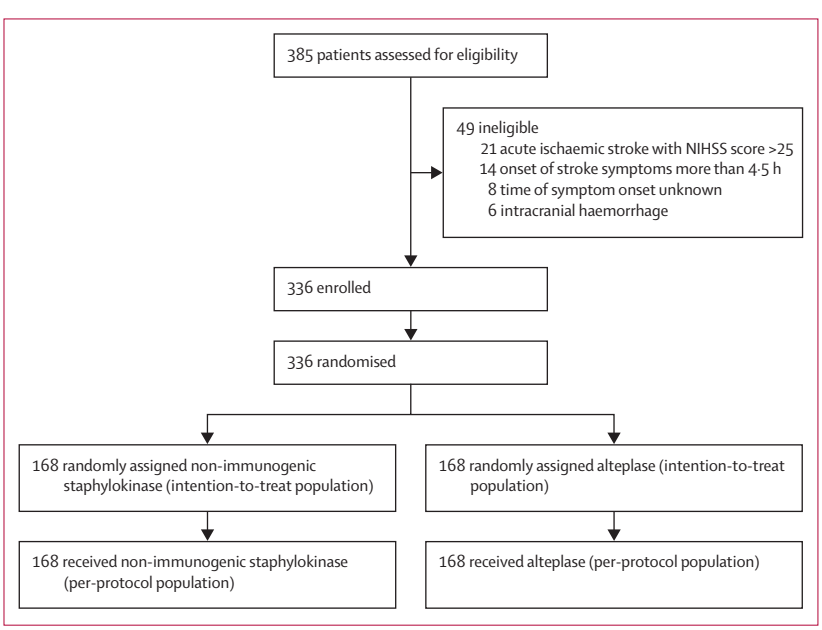

Figure 1: **Trial profile**
NIHSS=National Institutes of Health Stroke Scale.

	Non-immunogenic staphylokinase (n=168)	Alteplase (n=168)
Sex		
Male	106 (63%)	112 (67%)
Female	62 (37%)	56 (33%)
Age, years	64·4 (9·6)	64·6 (10·6)
Bodyweight, kg	80 (74–90)	80 (75–90)
Body-mass index, kg/m²	27·1 (27·7–30·65)	27·5 (25·1–30·95)
Stroke risk factors		
Hypertension	159 (95%)	159 (95%)
Diabetes*	16 (10%)	21 (13%)
Hyperlipidaemia	33 (20%)	40 (24%)
Current smoker	44 (26%)	43 (26%)
Cardiovascular history		
Previous stroke	22 (13%)	23 (14%)
Previous transient ischaemic attack	2 (1%)	4 (2%)
Stroke in relatives	8 (5%)	7 (4%)
Previous myocardial infarction	63 (38%)	54 (32%)
Atrial fibrillation	65 (39%)	52 (31%)
Mitral prolapse	8 (5%)	0
TOAST classification		
Atherothrombotic	44 (26%)	48 (29%)
Cardioembolic	60 (36%)	54 (32%)
Lacunar	6 (4%)	11 (7%)
Undetermined	58 (34%)	55 (32%)
Other	0	0
Stroke localisation		
Right middle cerebral artery	74 (44%)	63 (38%)
Left middle cerebral artery	80 (48%)	88 (52%)
Basilar artery	14 (8%)	17 (10%)
Stroke characteristics		
Baseline NIHSS score	11 (8–14)	11 (8–16)
Baseline mRS score	4 (4–5)	4 (4–5)
ASPECT score†	10 (10–10; n=154)	10 (9–10; n=151)
Onset to treatment time, h		
0–4·5	2·9 (0·8)	2·9 (0·7)
<3·0	2·3 (0·5)	2·3 (0·4)
3·0–4·5	3·5 (0·4)	3·5 (0·4)
Clinical characteristics		
Baseline systolic blood pressure, mm Hg	156 (18)	157 (19)
Baseline diastolic blood pressure, mm Hg	89 (11)	90 (10)
Baseline heart rate, beats per min	79 (13)	80 (14)
Baseline blood glucose, mmol/L	6·2 (5·4–7·8)	6·2 (5·4–7·5)

Data are n (%), mean (SD), or median (IQR). NIHSS=National Institutes of Health Stroke Scale. TOAST=Trial of ORG 10172 in Acute Stroke Treatment. mRS=modified Rankin scale. ASPECT=Alberta Stroke Program Early CT score. *Of any type. †Included only patients with hemispheric acute ischaemic stroke.

Table 1: **Baseline demographic data, clinical characteristics, and comorbidities**

haemorrhage was any haemorrhage with neurological deterioration (as indicated by an NIHSS score that was higher by 4 points or more than the value at baseline or the lowest value in the first 7 days) or any haemorrhage leading to death. Additionally, haemorrhage must have been identified as the predominant cause of the neurological deterioration, assessed independently after thrombolysis.

Statistical analysis

The sample size calculation was based on findings of the Safe Implementation of Thrombolysis in Stroke-MOnitoring STudy (SITS-MOST),[21] a study done to confirm the safety and efficacy of alteplase after its European approval, and three randomised trials in patients who received alteplase or placebo after onset of stroke and had an mRS score of 0–1 on day 90.[22–24] In SITS-MOST,[21] 3553 (54·8%) of 6483 patients with acute ischaemic stroke who received alteplase had an mRS score of 0–1 at day 90, whereas 405 (37·9%) of 1069 patients in the placebo group in randomised controlled trials had an mRS score of 0–1. Because the difference between the alteplase and placebo group was 16·9% in SITS-MOST, the margin of non-inferiority for our trial was defined as 16%. We calculated that 168 patients would be required for each treatment group (including a 10% potential dropout rate) to achieve 80% statistical power with a two-sided p value of less than 0·05. The non-inferiority hypothesis would be declared if the lower limit of the 95% CI for an mRS score of 0–1 on day 90 did not cross the margin of non-inferiority of 16%. The non-inferiority hypothesis was tested using Welch's t-test for the primary outcome only. Endpoints were analysed in the per-protocol population, which comprised all randomly assigned patients who completed treatment without any protocol violations; this population was identical to the intention-to-treat population.

We judged outcomes statistically significant if the p value was less than 0·05. Continuous variables are presented as mean (SD) if Pearson's non-parametric skewness coefficient was less than 0·2.[20] Skewed parameters are presented as median (IQR). Categorical variables are presented as n (%). We used the Mann-Whitney *U* test to compare continuous variables and the two-sided Fisher's exact test to compare categorical variables. Odds ratios (ORs) and Hodges-Lehman estimation of location shift are presented with 95% CIs. All statistical analyses were done using R (version 3.5.1).

This trial is registered at ClinicalTrials.gov, NCT03151993.

Role of the funding source

The funder of the study participated in study design and writing of the report. The funder had no role in data collection, data analysis, or data interpretation.

Results

Between March 18, 2017, and March 23, 2019, 385 patients from 18 clinical sites in Russia were screened for the study. 49 patients did not meet inclusion criteria and were judged ineligible (figure 1). Thus, 336 individuals

	Non-immunogenic staphylokinase	Alteplase	Odds ratio (95% CI)	p value
Efficacy outcomes				
mRS score 0–1 on day 90	84/168 (50%)	68/168 (41%)	1·47 (0·93 to 2·32)	0·10
mRS score 0–1, NIHSS score 0–1, and Barthel index score ≥95 on day 90	59/168 (35%)	52/168 (31%)	1·21 (0·75 to 1·95)	0·49
NIHSS score after 24 h	6 (3–11)	6 (3–12)	0 (–1·0 to 1·0)	0·68
NIHSS score on day 90	2 (1–5)	2 (1–5)	0 (–1·0 to 0·0)	0·95
mRS score 0–2 on day 90	115/168 (68%)	105/168 (63%)	1·30 (0·81 to 2·10)	0·30
Safety outcomes				
Mortality on day 90	17/168 (10%)	24/168 (14%)	0·68 (0·33–1·37)	0·32
Intracranial haemorrhage	31/168 (19%)	28/168 (17%)	1·13 (0·62–2·07)	0·77
Symptomatic intracranial haemorrhage	5/168 (3%)	13/168 (8%)	0·37 (0·10–1·13)	0·087

Data are n/N (%) or median (IQR), unless otherwise specified. mRS=modified Rankin scale. NIHSS=National Institutes of Health Stroke Scale.

Table 2: **Efficacy and safety outcomes**

were randomly allocated either non-immunogenic staphylokinase (168 [50%]) or alteplase (168 [50%]). All patients were followed up at each timepoint and were included in the final analysis.

Baseline demographic data, clinical characteristics, and comorbidities were similar between treatment groups (table 1). 218 (65%) patients were male and the mean age of participants was 64·5 years (SD 10·1). The non-immunogenic staphylokinase group had a higher rate of mitral prolapse than did the alteplase group. Both groups had moderate stroke severity and had similar mean onset-to-treatment times.

The last follow-up visit was completed on June 20, 2019. Median duration of follow-up was 89 days (IQR 89–89). In the non-immunogenic staphylokinase group, 84 (50%) patients had an mRS score of 0–1 compared with 68 (41%) patients in the alteplase group (OR 1·47, 95% CI 0·93 to 2·32; p=0·10; table 2). The distribution of mRS scores on day 90 by treatment group is shown in figure 2. The difference in the rate of favourable outcomes was 9·5% (–1·7 to 20·7), and the lower limit of the 95% CI did not cross the margin of non-inferiority ($p_{non\text{-}inferiority}$<0·0001; appendix 2 p 1). The association between drug use and the reduction of mRS score was investigated using an ordinal logistic regression model, adjusted for sex, age, and treatment time. Non-immunogenic staphylokinase was not associated with an increased reduction in mRS score, compared with that of alteplase (OR 1·27, 95% CI 0·85 to 1·92; p=0·24).

The secondary efficacy endpoint of mRS score 0–1, NIHSS score 0–1, and Barthel index score of 95 or more at day 90 was met in 59 (35%) patients in the non-immunogenic staphylokinase group and in 52 (31%) patients in the alteplase group (OR 1·21, 95% CI 0·75–1·95; p=0·49; table 2). The additional secondary endpoint of the NIHSS score after 24 h was median 6 (IQR 3–11) in the non-immunogenic staphylokinase group and 6 (3–12) in the alteplase group (p=0·68) and on day 90 was 2 (1–5) in both groups (p=0·95; table 2).

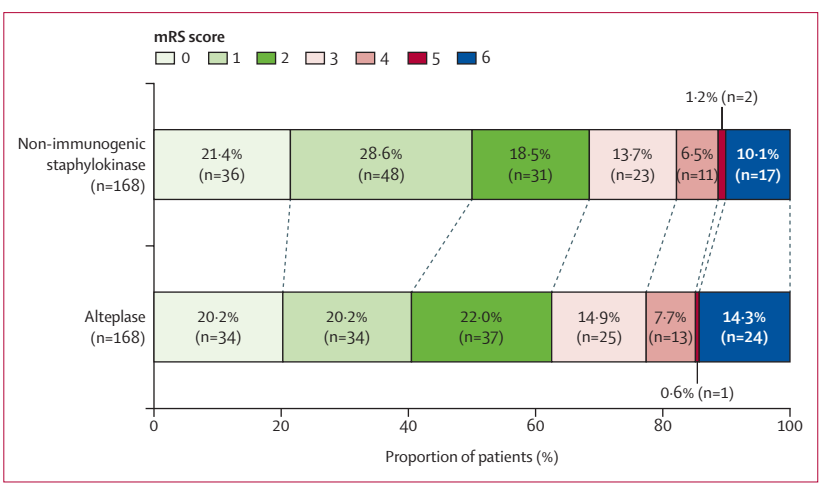

Figure 2: **Distribution of mRS scores on day 90**
mRS=modified Rankin scale.

The post-hoc outcome of an mRS score of 0–2 on day 90 was met in 115 (68%) patients in the non-immunogenic staphylokinase group and in 105 (63%) patients in the alteplase group in (OR 1·30, 95% CI 0·81–2·10; p=0·30; table 2).

17 (10%) patients in the non-immunogenic staphylokinase group and 24 (14%) patients in the alteplase group had died by day 90 (p=0·32; table 2). 31 (19%) patients in the non-immunogenic staphylokinase group and 28 (17%) patients in the alteplase group had intracranial haemorrhage (p=0·77). Symptomatic intracranial haemorrhage occurred in five (3%) patients in the non-immunogenic staphylokinase group and in 13 (8%) patients in alteplase group (p=0·087; table 2). Serious adverse events are shown in table 3. 22 (13%) patients in the non-immunogenic staphylokinase group had one or more serious adverse events, compared with 37 (22%) patients in the alteplase group (p=0·044; table 3). Efficacy and safety outcomes did not differ between treatment groups when considering patient's bodyweight (appendix 2 p 5).

	Non-immunogenic staphylokinase (n=168)	Alteplase (n=168)	p value
All serious adverse events on day 90	39 (23%)	62 (37%)	..
Patients with one or more serious adverse event	22 (13%)	37 (22%)	0·044
All-cause mortality	17 (10%)	24 (14%)	0·32
Death from acute ischaemic stroke	14 (8%)	19 (11%)	0·46
Death from cardiovascular disease	2 (1%)	5 (3%)	0·45
Death from other causes	1 (1%)	0	>0·99
Cerebral oedema	7 (4%)	14 (8%)	0·18
Symptomatic intracranial haemorrhage	5 (3%)	13 (8%)	0·087
Surgery	5 (3%)	3 (2%)	0·72
Neurosurgery	2 (1%)	1 (1%)	>0·99
Acute myocardial infarction	1 (1%)	1 (1%)	>0·99
Pulmonary thromboembolism	1 (1%)	3 (2%)	0·62
New acute ischaemic stroke	1 (1%)	0 (0)	>0·99
Gastric ulcer	0	2 (1%)	0·49
Takotsubo cardiomyopathy	0	1 (1%)	>0·99
Serious adverse event probably related to study drug	7/39 (18%)	8/62 (13%)	0·57
Serious adverse event possibly related to study drug	1/39 (3%)	12/62 (19%)	0·015

Data are n (%) or n/N (%), unless otherwise specified.

Table 3: **Serious adverse events**

Discussion

Administration of non-immunogenic staphylokinase within 4·5 h after onset of symptoms was found to be non-inferior to alteplase in patients with acute ischaemic stroke. To the best of our knowledge, the findings of the FRIDA study are the first to report use of non-immunogenic staphylokinase in patients with acute ischaemic stroke.

Mortality at day 90 in the non-immunogenic staphylokinase group was similar to mortality in the alteplase group in our study and in previous randomised controlled trials.[21–24] The incidence of symptomatic intracranial haemorrhage is difficult to compare with other studies because it has been defined differently between trials. Nevertheless, the incidence of symptomatic intracranial haemorrhage in the non-immunogenic staphylokinase group (ECASS III definition) was similar to that in the alteplase group in ECASS III.[24]

Some important differences exist between the two study interventions. Non-immunogenic staphylokinase was administered as one 10 mg dose for all patients, regardless of bodyweight, whereas alteplase was administered as weight-dependent doses (0·9 mg/kg), even in patients weighing more than 100 kg, who received the maximum dose of alteplase of 90 mg. In the ECASS trial,[25] for example, patients weighing more than 100 kg were not included. A single dose of 10 mg of non-immunogenic staphylokinase simplifies the treatment of patients with acute ischaemic stroke without weighing the patient and reduces the risk of errors when calculating the dose of alteplase. The rapid single bolus administration of non-immunogenic staphylokinase can be used in mobile stroke units (specifically designed ambulances equipped with CT scanners) and can help simplify thrombolytic therapy for patients with acute ischaemic stroke, as has been done for the treatment of patients with STEMI. Further, non-immunogenic staphylokinase is cheaper than alteplase for the treatment of patients with acute ischaemic stroke in Russia (approximately US$350 *vs* $625), which will possibly increase the availability and prevalence of thrombolysis therapy.

A strength of FRIDA is that we included patients with all subtypes of acute ischaemic stroke not only those with hemispheric acute ischaemic stroke. We also included analyses by time to symptom onset (0–4·5 h as well as <3·0 h and 3·0–4·5 h; appendix 2 pp 3–4). Our findings with non-immunogenic staphylokinase might be generalisable to countries other than Russia, considering the non-inferiority result for efficacy, no differences in safety between treatment groups, the convenience of administration, a reduction in treatment delays, and advantages in cost, compared with alteplase.

FRIDA was not without limitations. The main limitation is the 16% non-inferiority margin, especially when compared with the non-inferiority margin in trials of thrombectomy in the treatment of patients with acute ischaemic stroke.[26,27] Although absolute minimal clinically important differences in stroke trials are viewed by experts to be 3–5%,[28] non-inferiority trials are often designed around much wider margins that invoke criticisms for allowing a greatly inferior or even ineffective treatment or technology to claim non-inferiority against established treatment.[29] The 16% margin of non-inferiority in FRIDA was liberal, stemming from the interval of 15–20% used for previous non-inferiority trial designs,[30,31] thus ensuring we only needed a small sample size for the FRIDA trial.

The FRIDA trial was designed to compare non-immunogenic staphylokinase with alteplase, not with placebo. Alteplase was used as an active control drug. This approach is allowed for patients with acute ischaemic stroke by European and US authorities if previous results of active control drug versus placebo exist and a historical evidence of sensitivity to drug effects is presented. New trials must be similar to the historical one.[32,33] FRIDA was indentical in trial design to the historical trials of alteplase versus placebo in patients with acute ischaemic stroke, which met the so-called constancy assumption concerning patient's characteristics, inclusion criteria, study end-points, dose of previously used alteplase, and analytical approaches. Another reason for using alteplase rather than placebo in FRIDA was an ethical one. Alteplase is the only registered thrombolytic drug for the treatment of patients with acute ischaemic stroke in Russia and, according to basic rules and limitations of the ethics committee of the Russian Ministry of Health, a study with placebo in such a case would be considered not ethical and would be unlikely to affect the reliability of the results. The same principles were observed in studies of tenecteplase, in which the drug was compared with

alteplase and not with placebo in patients with acute ischaemic stroke.[34,35]

In FRIDA, we included only a few patients older than 80 years and individuals with a mild neurological deficit, which was because of a protocol amendment that happened after 2 years of the trial.

Non-immunogenic staphylokinase was registered in Russia in 2020, as a thrombolytic agent for patients with acute ischaemic stroke to be given within 4·5 h after symptoms onset. In the future, observational studies of non-immunogenic staphylokinase must be done to continue to assess the safety and efficacy of the drug within this time window. In future studies of non-immunogenic staphylokinase, the superiority of non-immunogenic staphylokinase compared with alteplase should also be investigated. Future studies should also use reperfusion CT or magnetic resonance angiography to assess patients beyond 4·5 h after symptom onset, with addition of thrombectomy if necessary.

Contributors

EIG contributed to the conceptualisation; supervision; manuscript writing, review, and editing; and verified the data. MYM and AAN contributed to the investigation, methodology, writing the original draft, and verified the data. NAS contributed to the investigation, methodology, and writing of the original draft. LLK contributed to the formal analysis, investigation, methodology. JYC, VIG, SEC, LVT, VVB, SBA, SAZ, NVZ, and AAN contributed to the formal analysis and investigation. EAP, VNN, IVG, AMA, DVP, SAP, OVA, UAE, and SAF contributed to the data curation, formal analysis, and investigation. ANC, KVC, AIG, ONJ, IVK, YAL, AAV, and DSY contributed to the data curation. EAG contributed to the investigation and data validation. MPS, ASS, and AMS contributed to project administration and resources. AVD, VlAK, AAO did statistical analysis. EBY did statistical analysis and verified the data. VaAK and RIA contributed to manuscript writing and review. AIA contributed to supervision, manuscript writing, review, and editing. SSM contributed to the methodology and project administration. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

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Declaration of interests

EIG, MYM, AAN, and MPS declare a patent issued for a method for treatment of ischemic stroke. NAS participated in clinical trials by Bayer, Sanofi, Ever Neuro Pharm, AstraZeneca, and Pharmasoft; and has received lecture fees from Sanofi, Ever Neuro Pharm, AstraZeneca, Pharmasoft, Nutricia, Boehringer Ingelheim, Generium, Geropharm, and Pfizer. OVA participated in clinical trials by AstraZeneca, Boehringer Ingelheim, Pharmasoft, and Takeda; and has received lecture fees from Boehringer Ingelheim, Bayer, Ever Neuro Pharm, Krka, Ipsen, Takeda, Pharmasoft, and Soteks. VVB participated in clinical trials by AstraZeneca and Quantum Genomics; and has received lecture fees from Bayer, SuperGene, Pfizer, Boehringer Ingelheim, AstraZeneca, and Sanofi. AMA participated in clinical trials by Boehringer Ingelheim, Bayer, and Pharmasoft; and has received lecture fees from Boehringer Ingelheim, Generium, and SuperGene. SBA participated in clinical trials by AstraZeneca, Boehringer Ingelheim, Sanofi, Servier, Pfizer, GlaxoSmithKline, Novartis, Abbott, Generium, SuperGene, Amgen, Vifor, and R-Pharm; and has received lecture fees from AstraZeneca, Boehringer Ingelheim, Bayer, Sanofi, Servier, GlaxoSmithKline, Abbott, SuperGene, Krka, and Alfasigma. SEC participated in clinical trials by Materia Medica, AstraZeneca, Pharmasoft, and Geropharm; and has received lecture fees from Bayer, Dr Reddys, Boehringer Ingelheim, Merz, Ever Neuro Pharm, and Pharmasoft. JYC participated in clinical trials by Basilea. VIG participated in clinical trials by Boehringer Ingelheim; and has received lecture fees from Polisan and Pharmasoft. IVG participated in clinical trials by Materia Medica, Roche, Biocad, and Aspen; and has received lecture fees from Roche, Novartis, R-Pharm, and Generium. ONJ participated in clinical trials of Geropharm, Pharmsyntes, Boehringer Ingelheim, Biocad, and Biogen. VNN participated in clinical trials by Boehringer Ingelheim, Geropharm, Materia Medica, and Bayer; and has received lecture fees from Boehringer Ingelheim, Bayer, Pfizer, and Takeda. EAP participated in clinical trials by Biocad; and has received lecture fees from Bayer, Servier, and Alfasigma. DVP participated in clinical trials by Bayer; and has received lecture fees from AstraZeneca, Bayer, Pfizer, and Portola. YAL participated in clinical trials by Biogen, Biocad, and Geropharm. LVT participated in clinical trials by Boehringer Ingelheim, Geropharm, Pharmsyntes, Biogen, and Biocad. KVC participated in clinical trials by Roche; and has received lecture fees from Bayer and Jannssen. ANC participated in clinical trials of Generium; and has received lecture fees from Boehringer Ingelheim, Takeda, and Polisan. SAZ participated in clinical trials by Janssen, Bayer, and Novo Nordisk; and has received lecture fees from Boehringer Ingelhaim, AstraZeneca, and Aspen. DSY participated in clinical trials by AstraZeneca, Servier, GlaxoSmithKline, Novartis, Generium, and SuperGene; and has received lecture fees from AstraZeneca, Bayer, and SuperGene. SAP participated in clinical trials by Alvogen, Pharmstandart, Pharmadiol, and Aquavia. NVZ participated in clinical trials by Teva, Sanofi, and Geropharm. SSM and AMS are employees of SuperGene and declare a patent issued for a method for the treatment of patients with ischaemic stroke. All other authors declare no competing interests.

Data sharing

The funder of the study is committed to the responsible sharing of data from clinical trials. Data will be provided to any qualified investigator on reasonable request. Deidentified participant data will be available after the publication of the results of the completed study on request to the corresponding author. Proposals will be reviewed and approved by the funder, researchers, local regulatory authorities, and the ethics committee of the Russian Ministry of Health. Once the proposal has been approved, data can be transferred through a secure online platform after the signing of a data access agreement and a confidentiality agreement.

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