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CLOTBUST-ER Trial Investigators

2019-04

CLOTBUST-ER Trial Investigators 2019 , ' Safety and efficacy of sonothrombolysis for acute ischaemic stroke : a multicentre, double-blind, phase 3, randomised controlled trial ' , Lancet Neurology , vol. 18 , no. 4 , pp. 338-347 . [https://doi.org/10.1016/S1474-4422\(19\)30026-2](https://doi.org/10.1016/S1474-4422(19)30026-2)

<http://hdl.handle.net/10138/313690>

[https://doi.org/10.1016/S1474-4422\(19\)30026-2](https://doi.org/10.1016/S1474-4422(19)30026-2)

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Safety and efficacy of sonothrombolysis for acute ischaemic stroke: a multicentre, double-blind, phase 3, randomised controlled trial

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Summary

Background Pulsed-wave ultrasound increases the exposure of an intracranial thrombus to alteplase (recombinant tissue plasminogen activator), potentially facilitating early reperfusion. We aimed to ascertain if a novel operator-independent transcranial ultrasound device delivering low-power high-frequency ultrasound could improve functional outcome in patients treated with alteplase after acute ischaemic stroke.

Methods We did a multicentre, double-blind, phase 3, randomised controlled trial (CLOTBUST-ER) at 76 medical centres in 14 countries. We included patients with acute ischaemic stroke (National Institutes of Health Stroke Scale score ≥ 10) who received intravenous thrombolysis (alteplase bolus) within 3 h of symptom onset in North America and within 4·5 h of symptom onset in all other countries. Participants were randomly allocated (1:1) via an interactive web response system to either active ultrasound (2 MHz pulsed-wave ultrasound for 120 min [sonothrombolysis]; intervention group) or sham ultrasound (control group). Ultrasound was delivered using an operator-independent device, which had to be activated within 30 min of the alteplase bolus. Participants, investigators, and those assessing outcomes were unaware of group assignments. The primary outcome was improvement in the modified Rankin Scale score at 90 days in patients enrolled within 3 h of symptom onset, assessed in the intention-to-treat population as a common odds ratio (cOR) using ordinal logistic regression shift analysis. This trial is registered with ClinicalTrials.gov, number NCT01098981. The trial was stopped early by the funder after the second interim analysis because of futility.

Findings Between August, 2013, and April, 2015, 335 patients were randomly allocated to the intervention group and 341 patients to the control group. Compared with the control group, the adjusted cOR for an improvement in modified Rankin Scale score at 90 days in the intervention group was 1·05 (95% CI 0·77–1·45; $p=0\cdot74$). 51 (16%) of 317 patients in the intervention group and 44 (13%) of 329 patients in the control group died (unadjusted OR 1·24, 95% CI 0·80–1·92; $p=0\cdot37$) and 83 (26%) and 79 (24%), respectively, had serious adverse events (1·12, 0·79–1·60; $p=0\cdot53$).

Interpretation Sonothrombolysis delivered by an operator-independent device to patients treated with alteplase after acute ischaemic stroke was feasible and most likely safe, but no clinical benefit was seen at 90 days. Sonothrombolysis could be further investigated either in randomised trials undertaken in stroke centres that are dependent on patient transfer for endovascular reperfusion therapies or in countries where these treatments cannot yet be offered as the standard of care.

Funding Cerevast Therapeutics.

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Introduction

Intravenous alteplase (recombinant tissue plasminogen activator) is the only approved medical reperfusion treatment for acute ischaemic stroke^{1,2} and should be initiated as early as possible for maximum benefit.³ Yet, half of patients remain disabled or die despite medical treatment because of the initial severity of ischaemic insult and inadequate response to intravenous thrombolysis.^{4,5} Therefore, amplification of alteplase effectiveness in thrombus dissolution remains an important goal for future development of more effective medical stroke treatments, even in the era of mechanical thrombectomy,

because endovascular reperfusion therapies are not readily available in most stroke centres around the world.⁶

Findings of a phase 2 randomised controlled trial⁷ of 2 MHz diagnostic ultrasound equipment (transcranial Doppler), and meta-analyses^{8,9} of similar studies, showed that ultrasound aimed at the residual flow and thrombus interface can at least double the chance of early recanalisation. Sonothrombolysis was also associated with a higher likelihood of favourable functional outcome in the subgroup of patients with pretreatment scores of 10 points or more on the National Institutes of Health Stroke Scale (NIHSS).¹⁰ However, a major obstacle for emergency

Lancet Neurol 2019; 18: 338–47

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Research in context

Evidence before this study

We searched MEDLINE and Scopus up to Nov 17, 2018, without language or any other restrictions, for randomised controlled trials of ultrasonography to enhance the thrombolytic activity of alteplase (recombinant tissue plasminogen activator), referred to as sonothrombolysis, using the keywords “sonothrombolysis”, “ultrasound-enhanced thrombolysis”, “ischemic stroke”, and “clinical trial”. Our search identified six small-scale (phase 2) randomised clinical trials comparing sonothrombolysis with intravenous alteplase or standard-of-care treatment. However, our search found that no large-scale (phase 3) randomised controlled trials using an operator-independent transcranial ultrasound device delivering sonothrombolysis had been undertaken.

Added value of the study

Exposure of patients with acute ischaemic stroke to low-power high-frequency ultrasound using an operator-independent device, after receiving standard-of-care treatment with alteplase, was found to be feasible and most likely safe, but no clinical benefit was seen after 90 days.

Implications of all available evidence

Sonothrombolysis with low-power high-frequency ultrasound seems to be safe but offers no clinical benefit in patients with acute ischaemic stroke. The potential efficacy of sonothrombolysis could be further investigated either in stroke centres that are dependent on patient transfer for endovascular reperfusion therapies or in countries where these treatments cannot yet be offered as standard of care.

doctors, neurologists, and health professionals, which restricts use of diagnostic ultrasound equipment in acute ischaemic stroke, is operator dependency.¹¹ In previous work, we developed a novel hands-free therapeutic device with operator-independent targeting of the intracranial vessels, which has been shown to be safe in early-phase clinical trials.^{12,13} On the basis of this previous work, we undertook the Combined Lysis of Thrombus using Ultrasound and Systemic Tissue Plasminogen Activator for Emergent Revascularization (CLOTBUST-ER) trial—a phase 3 randomised controlled trial of sonothrombolysis in patients with acute ischaemic stroke. Our objective was to establish the safety and therapeutic efficacy of our operator-independent device in combination with intravenous alteplase to improve functional outcome, compared with intravenous alteplase alone, in patients with acute ischaemic stroke presenting within 3 h of symptom onset.

Methods

Study design

We did a multicentre, double-blind, sham-controlled, phase 3, randomised trial (CLOTBUST-ER) at 76 medical centres in 14 countries. 40 centres were based in Europe, 31 in North America, three in Australia, and two in Asia. A list of centres that enrolled patients in CLOTBUST-ER is available in the appendix. Details of methods used have been published elsewhere.¹⁴ The study was undertaken and reported with fidelity to the study protocol.¹⁴ The trial was approved by the institutional review board at every site or national ethics committee, as required.

Participants

We enrolled patients aged 18–80 years with acute ischaemic stroke who had baseline NIHSS scores of 10 points or more and who received intravenous alteplase within a 3 h treatment window in North America or within a 4·5 h treatment window in other participating countries,

as per national approval labels.¹⁴ The NIHSS cutoff was selected on the basis of a sensitivity analysis of phase 2 trial data,^{10,14} which indicated that the beneficial effect of sonothrombolysis was amplified in subgroups of patients with acute ischaemic stroke with NIHSS scores of 10 points or more. All patients were independently functioning in the community immediately before their stroke, with a premorbid modified Rankin Scale score of 0–1. Patients were included irrespective of the anticipated stroke localisation (anterior or posterior circulation). Written informed consent was obtained from the patient or a legal representative before enrolment. A detailed list of inclusion and exclusion criteria (including planned endovascular reperfusion procedures) has been published¹⁴ and is available in the appendix.

Randomisation and masking

We randomly allocated patients in a 1:1 ratio using web-based central randomisation¹⁴ and random permuted blocks stratified by site (random block size of two, four, or six) to either active ultrasound (intervention group) or sham ultrasound (control group). Every patient was assigned a unique site-specific identification number after providing informed consent. Patients were randomised either before or after administration of the alteplase bolus, with the hands-free therapeutic device to be activated within 30 min of the alteplase bolus. The device was programmed based on a randomisation code that maintained masking of treating doctors, patients, and the funder to active versus sham assignments. The interactive web response system for randomisation was provided by ITClinical (Lisbon, Portugal). The system was audited and met all required Good Clinical Practice compliance requirements. Masking was achieved through an algorithm that determined whether “A” setting delivered active insonation and “B” delivered sham (control) insonation, or the reverse. The interactive web response system was programmed to mask the A or B assignments;

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Figure 1: Trial profile

*Treatment was never started, or patient received wrong treatment (crossover). †Recorded insonation time was <105 min (instead of the projected 120 min).

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See Online for appendix

therefore, no user could see which assignment patients were given. The success of masking procedures was not assessed.

Procedures

All eligible patients received standard-of-care treatment, which included full-dose intravenous alteplase (0.9 mg/kg; 90 mg maximum; 10% bolus followed by 90% intravenous infusion over 60 min). The headframe (for delivery of the ultrasound assignment) had to be placed on all patients before or shortly after the alteplase bolus, so as not to delay administration, and had to be activated within 30 min of the alteplase bolus to achieve maximum overlap between exposure to the device and alteplase infusion. All patients—regardless of device activation time—were required to wear the headframe for a total of 120 min. Devices were equipped with a timer showing completion of 120 min exposure and a pause button in case the patient had to have a repeat CT scan done as part of standard-of-care treatment. Interruption time to deliver standard-of-care treatment requiring temporary device removal could not exceed 15 min. A training video was created before study initiation. All site investigators watched the training video and practised assembly and placement of devices under direct supervision of trained clinical monitors in each country. All sites were trained before site initiation. Moreover, all new investigators were required to undergo similar training during the trial. Finally, all global and local investigator meetings had training sessions for new and existing sites.

In accordance with parameters mandated by the US Food and Drug Administration (FDA) for currently approved and marketed transcranial Doppler diagnostic ultrasound devices,¹⁵ the intervention group received 2 MHz pulsed-wave transcranial ultrasound for 120 min (total average power 32 mW; maximum spatial peak temporal average intensity 207 mW/cm²; pulse repetition

frequency 8.3 kHz; pulse duration 5 µs). The control group received sham (inactive) ultrasound for 120 min. Details of the operator-independent device, vessel targeting without imaging or Doppler echolocation, safety testing, and phase 2 functional outcomes data have been published elsewhere.^{12,13}

Investigators obtained NIHSS scores before treatment, 2 h and 24 h after treatment, on either day 7 or at discharge from an acute facility to home (if the patient was discharged before day 7), and at day 90. Modified Rankin Scale scores were recorded on either day 7 or at discharge and at day 90. Substantial neurological worsening—defined as a total increase in NIHSS score by 4 points or more from the best score at any time during the first 24 h after alteplase bolus—required a non-contrast CT to rule out symptomatic intracranial haemorrhage. Routine post-stroke imaging was not mandatory but was done at all participating centres as part of standard-of-care treatment for acute ischaemic stroke.

Outcomes

The primary outcome was improvement (defined as a 1-point decrease across all scale scores [shift analysis]) in modified Rankin Scale score¹⁶ at 90 days (range 80–100) after randomisation, for all participants enrolled within 3 h of onset of stroke symptoms (according to FDA regulatory requirements), as assessed by cumulative ordinal logistic regression analysis in the intention-to-treat population (which included all enrolled patients). This analysis was repeated as a secondary outcome for all patients who were enrolled within 4.5 h, which was a criterion for enrolment worldwide (global outcome).¹⁴ Other secondary efficacy endpoints included patients with dichotomous modified Rankin Scale scores of 0–1 and 0–2 at 90 days, dramatic clinical recovery at 120 min, clinical recovery at 24 h, neurological improvement at 24 h, neurological deterioration at 24 h, duration of hospital stay until discharge, independent functional outcome at 90 days, NIHSS score at day 7, NIHSS score at day 90, and modified Rankin Scale score at 7 days or discharge.¹⁴ Secondary efficacy outcomes were assessed both in participants enrolled within 3 h of onset of stroke symptoms (referred to as US outcomes) and in those enrolled within 4.5 h (global outcomes). Dramatic clinical recovery assessed at 120 min (range 105–135) after headframe activation was defined as either a reduction in NIHSS score of 10 points or more compared with pretreatment or a total NIHSS score of 3 points or less. Clinical recovery assessed at 24 h (range 22–26) after headframe activation was defined as either a reduction in NIHSS score of 10 points or more compared with pretreatment or a total NIHSS score of 3 points or less. Neurological improvement assessed at 24 h (range 22–26) after headframe activation was defined as a reduction in NIHSS score of 5 points or more compared with pretreatment. Neurological deterioration assessed at 24 h (range 22–26 h) after headframe activation was defined as an increase in NIHSS score of

4 points or more compared with pretreatment.¹⁴ Independent functional outcome assessed at 90 days (range 80–100) was defined as either a modified Rankin Scale score of 0–1 for patients with a pretreatment NIHSS score of 10–14 and a modified Rankin Scale score of 0–2 for patients with a pretreatment NIHSS score greater than 14.

Safety outcomes were assessed in the safety population, which included all patients who received any part of the treatment and provided at least one assessment of safety. Safety outcomes included symptomatic intracranial haemorrhage within 24 h of alteplase bolus and an overall analysis of adverse events, as described elsewhere.¹⁴ Symptomatic intracranial haemorrhage was defined per study protocol as neurological deterioration (a reduction in NIHSS score of 4 points or more compared with best previous examination) within 24 h after alteplase bolus, with documented parenchymal haemorrhage type 2 or remote parenchymal haemorrhage type 2. Digital images of all intracranial bleeds within 24 h that were associated with neurological deterioration were sent to a central imaging core laboratory (Foothills Medical Centre, Calgary, AB, Canada) for independent adjudication. We also assessed patients with symptomatic intracranial haemorrhage within 36 h,¹⁷ applying the definition and adjudication process used for the 24 h period to all neurological deteriorations reported within 36 h of the alteplase bolus.¹ Three patients with symptomatic intracranial haemorrhage were diagnosed by local investigators, without central adjudication, because of early trial termination by the funder. We included these cases in the final group with symptomatic intracranial haemorrhage, assuming the worst-case scenario. Intracranial haemorrhage that was not associated with neurological deterioration of 4 points or more on the NIHSS scale were subsequently classified as asymptomatic intracranial haemorrhage.

All prespecified adverse events,¹⁴ coded and tabulated by MedDRA System Organ Class, were reported by clinical investigators at participating centres who were masked to treatment assignments. Adverse events were reviewed and adjudicated by an independent panel within the Data Safety Monitoring Board, who also were unaware of treatment assignments. If a discrepancy arose between the adjudication panel and the clinical investigator, the adjudication panel's determination was final. Brain herniation, cerebral oedema, and midline shift were not prespecified adverse events of our study and, therefore, were not centrally adjudicated. Information for these adverse events was gathered based on onsite clinical and radiology reports. We used no standardised definition for these adverse events. All adverse events were presented in descending frequency. Adverse events were also tabulated by severity and relation to the investigational device.¹⁴ Death from any cause within 90 days of treatment and deaths due to adverse events were also summarised by treatment group.

	Intervention group (n=335)	Control group (n=341)
Age (years)	70 (60–76)	70 (60–75)
Sex		
Male	187 (56%)	206 (60%)
Female	148 (44%)	135 (40%)
Ethnic origin		
White	261 (78%)	270 (79%)
Black or African-American	18 (5%)	17 (5%)
Hispanic-Latino	37 (11%)	33 (10%)
Asian	12 (4%)	13 (4%)
South Asian or Indian	0	1 (<1%)
Filipino	0	1 (<1%)
American Indian or Alaskan Native	0	2 (1%)
Unknown	7 (2%)	4 (1%)
NIHSS score	15 (11–18)	14 (11–18)
Hypertension	196 (59%)	213 (62%)
Diabetes mellitus	75 (22%)	80 (23%)
Atrial fibrillation	62 (19%)	54 (16%)
Prestroke modified Rankin Scale score 0–1	334 (100%)	339 (100%)
Systolic blood pressure before alteplase bolus (mm Hg)*	150·3 (20·2)	150·3 (20·4)
Diastolic blood pressure before alteplase bolus (mm Hg)†	81·7 (13·2)	81·8 (13·2)
Serum glucose before alteplase bolus (mg/dL)	139·6 (53·0)	137·5 (53·4)
Time from symptom onset to alteplase bolus (min)	117·0 (95·0–156·0)	126·0 (96·0–165·0)
Alteplase bolus within 3 h of symptom onset	279 (83%)	285 (84%)
Time from symptom onset to headframe activation (min)	136·0 (117·0–175·0)	148·0 (115·0–185·5)
Time from alteplase bolus to headframe activation (min)	20·0 (13·0–27·0)	20·0 (13·0–25·0)

Data are median (IQR), mean (SD), or n (%). NIHSS=National Institutes of Health Stroke Scale. *Data missing for nine patients in the intervention group and for 13 patients in the control group. †Data missing for eight patients in the intervention group and for 13 patients in the control group.

Table 1: Baseline characteristics

Statistical analysis

Our prespecified statistical analysis plan, power estimations, and planned interim analyses have been published elsewhere,¹⁴ and are available in the appendix. Interim analyses assessing the primary outcome were scheduled after approximately a third and two-thirds of modified Rankin Scale scores at 90 days were available. O'Brien-Fleming boundaries for the group sequential design, with 90% power and testing at approximately a third and two-thirds of patients, implied critical values of $p=0·0003525$ at the first interim analysis, $p=0·0120085$ at the second interim analysis, and $p=0·0462386$ at the final analysis. Moreover, a conditional power futility analysis was scheduled at each interim analysis point by the Data

	Intervention group	Control group	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Primary outcome*						
Modified Rankin Scale score at 90 days in patients enrolled within 3 h of symptom onset	3.0 (1.0–4.0)	3.0 (1.0–4.0)	1.03 (0.76–1.40)†	0.84	1.05 (0.77–1.45)†	0.74
Secondary outcomes in patients enrolled within 4–5 h of symptom onset (global outcomes)*						
Modified Rankin Scale score at 90 days	3.0 (1.0–4.0)	3.0 (1.0–4.0)	1.00 (0.76–1.32)†	0.99	1.06 (0.80–1.42)†	0.67
Modified Rankin Scale score at 7 days or discharge	3.0 (2.0–4.0)	4.0 (1.0–5.0)	0.99 (0.75–1.31)†	0.97	1.10 (0.82–1.47)†	0.51
Modified Rankin Scale score of 0–1 at 90 days	96/307 (31%)	98/306 (32%)	0.97 (0.69–1.36)†	0.86	1.05 (0.73–1.52)†	0.79
Modified Rankin Scale score of 0–2 at 90 days	149/307 (49%)	142/306 (46%)	1.09 (0.79–1.50)†	0.63	1.25 (0.87–1.79)†	0.22
Independent functional outcome at 90 days	113/307 (37%)	114/306 (37%)	0.98 (0.71–1.36)	0.93	1.07 (0.75–1.51)	0.72
Dramatic clinical recovery at 120 min	60/323 (19%)	65/330 (20%)	0.93 (0.63–1.37)	0.77	0.95 (0.63–1.43)	0.80
Clinical recovery at 24 h	100/313 (32%)	116/322 (36%)	0.83 (0.60–1.16)	0.31	0.88 (0.63–1.24)	0.46
Neurological improvement at 24 h	176/313 (56%)	180/322 (56%)	1.01 (0.74–1.39)	0.94	1.08 (0.78–1.49)	0.66
Neurological deterioration at 24 h	29/313 (9%)	19/322 (6%)	1.63 (0.89–2.97)	0.13	1.47 (0.80–2.75)	0.21
NIHSS at 7 days	5 (1–12)	6 (1–12)	..	0.82	..	..
NIHSS at 90 days	2 (1–6)	2 (1–5)	..	0.68	..	..
Duration of hospital stay until discharge (days)	7 (5–12)	7 (4–11)	..	0.48	..	..
Secondary outcomes in patients enrolled within 3 h of symptom onset (US outcomes)‡						
Modified Rankin Scale score at 7 days or discharge	3.0 (2.0–4.0)	4.0 (1.0–5.0)	1.03 (0.76–1.40)†	0.83	1.09 (0.80–1.50)†	0.58
Modified Rankin Scale score of 0–1 at 90 days	82/255 (32%)	78/254 (31%)	1.07 (0.73–1.55)†	0.77	1.16 (0.77–1.75)†	0.48
Modified Rankin Scale score of 0–2 at 90 days	127/255 (50%)	118/254 (47%)	1.14 (0.81–1.62)†	0.48	1.27 (0.85–1.89)†	0.24
Independent functional outcome at 90 days	96/255 (38%)	93/254 (37%)	1.04 (0.73–1.50)	0.85	1.11 (0.76–1.63)	0.58
Dramatic clinical recovery at 120 min	58/269 (22%)	60/279 (22%)	0.99 (0.66–1.49)	>0.99	0.99 (0.65–1.52)	0.97
Clinical recovery at 24 h	83/261 (32%)	102/271 (38%)	0.77 (0.54–1.10)	0.17	0.79 (0.54–1.15)	0.22
Neurological improvement at 24 h	148/261 (57%)	154/271 (57%)	0.99 (0.71–1.40)	>0.99	1.04 (0.73–1.49)	0.83
Neurological deterioration at 24 h	23/261 (9%)	17/271 (6%)	1.44 (0.75–2.77)	0.32	1.37 (0.70–2.71)	0.36
NIHSS at 7 days	5 (1–12)	6 (1–12)	..	0.80	..	..
NIHSS at 90 days	2 (0–6)	2 (0–5)	..	0.84	..	..
Duration of hospital stay until discharge (days)	7 (5–12)	7 (4–11)	..	0.60	..	..
Data are median (IQR) or n/N (%). NIHSS=National Institutes of Health Stroke Scale. OR=odds ratio. *Analyses were done in the intention-to-treat population, comprising 335 patients allocated to the intervention group and 341 allocated to the control group. †Data for modified Rankin Scale score outcomes are common odds ratios. ‡279 patients from the intervention group and 285 from the control group were enrolled within 3 h of symptom onset.						
Table 2: Primary and secondary efficacy outcomes						

Safety Monitoring Board, at which time the study would stop should the conditional power fall below 15%.

Analyses reported here were undertaken in the intention-to-treat population using a program written in Matlab version R2018b, and one master datafile was generated. All further statistical analyses were done in R version 3.4, running under an R Studio environment, and primary outcomes were cross-checked in the Matlab environment. All planned statistical analyses were done before unblinding of data.

The primary outcome was specified as the proportional odds logistic regression (polr command in R) over

the 90-day modified Rankin Scale score distribution (scores range from 0 to 6), after combining data for grades 5 and 6.¹⁴ By doing univariate logistic regressions for each of the six groupings (ie, 0, 1, 2, 3, 4, and 5–6), we noted that the odds ratios (ORs) cluster around one, with negligible differences attributed to random variation, giving credit to the hypothesis of proportional odds across the groupings of the modified Rankin Scale score. Further, we did two imputation analyses on the primary endpoint. Missing modified Rankin Scale scores were estimated using multiple imputation methodology¹⁸ in the first analysis, based on the strongest predictors of

90-day modified Rankin Scale score, as prespecified in our statistical analysis protocol (ie, baseline NIHSS score, NIHSS score at 24 h, or modified Rankin Scale score at day 7 or discharge, along with assignment to treatment or control). In the second analysis, missing modified Rankin Scale scores were imputed to the worst case (eg, modified Rankin Scale score of 6). For the primary outcome of interest, we analysed alterations in the distribution of patients over the entire range of the six groupings of modified Rankin Scale scores (shift analysis—ie, analysis over ranks) and reported the corresponding common odds ratios (cORs) to express the change in odds for each unit (1-point) decrease in modified Rankin Scale score between the two groups.

Unadjusted and adjusted analyses are reported separately. Both unadjusted and adjusted statistical analyses for secondary endpoints were prespecified. The unadjusted approach was the primary analytical approach whereas the adjusted approach served as a secondary analysis. Prespecified secondary efficacy outcomes were tested in unadjusted analyses with Fisher's two-sided test of proportions, and CIs were provided according to the methodology of Bland and Altman.¹⁹ Prespecified safety outcomes were also tested using Fisher's two-sided test of proportions. Adjustment was done in terms of baseline NIHSS score, age, baseline serum glucose, and time to alteplase bolus. These factors were chosen post hoc by the steering committee before unmasking of data. Adjustment for these factors was applied uniformly for all safety and efficacy outcomes. In all analyses, no allowance for multiplicity was made. To allow for the interim analyses, a spend adjustment was not done while calculating p values in all analyses. Also, point estimates were crude and not bias-adjusted for the interim analyses.

This trial is registered with ClinicalTrials.gov, number NCT01098981.

Role of the funding source

The funder contributed to study design, data monitoring, and database maintenance. The funder had no role in data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study. The steering committee of CLOTBUST-ER, which included representatives of the funder, had final responsibility for the decision to submit for publication.

Results

Between August, 2013, and April, 2015, 676 participants underwent randomisation, of whom 335 were allocated to the intervention group (active ultrasound) and 341 to the control group (sham ultrasound); these patients comprised the intention-to-treat population (figure 1). Patients assigned to the intervention and control groups did not differ in any baseline characteristics (table 1). The median elapsed time from alteplase bolus to headframe activation was similar in the intervention group (20 min [IQR 13–27]) and control group (20 min [13–25]).

Figure 2: Modified Rankin Scale scores at 90 days in patients treated with intravenous thrombolysis within 3 h (intention-to-treat population)

Scores on the modified Rankin Scale range from 0 to 6, with 0 indicating no symptoms, 1 no clinically significant disability, 2 slight disability (patient is able to look after own affairs without assistance but is unable to carry out all previous activities), 3 moderate disability (patient requires some help but is able to walk unassisted), 4 moderately severe disability (patient is unable to attend to bodily needs without assistance and unable to walk unassisted), 5 severe disability (patient requires constant nursing care and attention), and 6 death.

CLOTBUST-ER was stopped early because of futility, according to prespecified stopping rules, by the Data Safety and Monitoring Board after the per-protocol defined second interim analysis, at which time two-thirds of 90-day modified Rankin Scale scores were available. The results of the first and second interim analyses of the primary outcome are available in the appendix. Patients who were enrolled in the study at the time of the futility determination were followed up until 90 days after the alteplase bolus by site investigators, despite discontinuation of the study by the funder. We present here the results for the total sample of patients randomised in CLOTBUST-ER.

Among participants randomised within 4.5 h of symptom onset, 28 patients in the intervention group and 35 patients in the control group had missing data for 90-day modified Rankin Scale scores. Patients with missing follow-up data were censored from analyses of the primary endpoint and secondary endpoints that were assessed at 90 days after symptom onset. Patients in the intervention and control groups enrolled within 3 h of symptom onset did not differ in terms of modified Rankin Scale scores at 90 days (adjusted cOR 1.05, 95% CI 0.77–1.45; $p=0.74$; table 2; figure 2). Moreover, modified Rankin Scale scores at 90 days in patients enrolled within 4.5 h (secondary global outcome) did not differ between the intervention and control groups (adjusted cOR 1.06, 95% CI 0.80–1.42; $p=0.67$; table 2). We also detected no difference between groups in modified Rankin Scale scores at 90 days in patients enrolled within 3 h of symptom onset after adjusting for per-protocol defined covariates (site, baseline NIHSS, premorbid modified Rankin Scale score, and age) in the statistical analysis plan (adjusted cOR 0.93, 95% CI 0.69–1.24; $p=0.61$; data not shown). Furthermore, no difference was noted in the

	Intervention group (n=317)	Control group (n=329)	Unadjusted OR (95% CI)	p value	Adjusted OR (95% CI)	p value
Death	51 (16%)	44 (13%)	1.24 (0.80–1.92)	0.37	1.19 (0.74–1.92)	0.48
Death due to serious adverse event	34 (11%)	34 (10%)	1.04 (0.63–1.72)	0.90	1.00 (0.58–1.73)	>0.99
Serious adverse events	83 (26%)	79 (24%)	1.12 (0.79–1.60)	0.53	1.08 (0.74–1.57)	0.69
Symptomatic intracranial haemorrhage at 24 h	8 (3%)	6 (2%)	1.39 (0.48–5.06)	0.60	1.43 (0.49–4.44)	0.51
Symptomatic intracranial haemorrhage at 36 h	9 (3%)	7 (2%)	1.34 (0.49–3.65)	0.62	1.39 (0.51–3.95)	0.52
Asymptomatic intracranial haemorrhage at 24 h	34 (11%)	20 (6%)	1.86 (1.04–3.30)	0.046	1.78 (0.98–3.31)	0.061
Cerebral oedema	17 (5%)	8 (2%)	2.27 (0.97–5.35)	0.066	2.15 (0.93–5.40)	0.08
Brain herniation	11 (3%)	5 (2%)	2.33 (0.80–6.78)	0.13	2.09 (0.73–6.87)	0.19
Midline shift	9 (3%)	9 (3%)	1.04 (0.41–2.65)	>0.99	0.98 (0.35–2.72)	0.97
Study discontinuation due to adverse events	21 (7%)	22 (7%)	0.99 (0.53–1.84)	>0.99	1.01 (0.53–1.96)	0.96
Headache	57 (18%)	50 (15%)	1.22 (0.81–1.85)	0.40	1.30 (0.85–2.00)	0.23
Pyrexia	30 (9%)	37 (11%)	0.82 (0.50–1.37)	0.52	0.81 (0.48–1.36)	0.43
Nausea	33 (10%)	27 (8%)	1.30 (0.76–2.22)	0.35	1.32 (0.77–2.29)	0.31
Pneumonia or aspiration pneumonia	34 (11%)	27 (8%)	1.34 (0.79–2.28)	0.28	1.33 (0.76–2.36)	0.32
Constipation	24 (8%)	33 (10%)	0.73 (0.42–1.27)	0.33	0.69 (0.39–1.20)	0.19
Atrial fibrillation as adverse event	28 (9%)	14 (4%)	2.18 (1.12–4.22)	0.025	2.25 (1.17–4.52)	0.018
Atrial fibrillation as adverse event after exclusion of patients with atrial fibrillation at baseline	23/312 (7%)	13/328 (4%)	1.93 (0.96–3.88)	0.085	1.91 (0.96–3.97)	0.072

OR=odds ratio.

Table 3: Safety outcomes and serious adverse events within 90 days after randomisation in the safety population

adjusted analyses of modified Rankin Scale score at 90 days in patients enrolled within 3 h of symptom onset using either multiple imputation methodology (unadjusted cOR 0.98, 95% CI 0.73–1.31; $p=0.87$; adjusted cOR 0.99, 95% CI 0.74–1.34; $p=0.97$; appendix) or imputation to the worst case (unadjusted cOR 1.08, 95% CI 0.80–1.45; $p=0.60$; adjusted cOR 1.14, 95% CI 0.84–1.54; $p=0.39$; appendix). The intervention and control groups did not differ with respect to any secondary outcomes (table 2).

The safety population comprised 317 patients in the intervention group and 329 in the control group. 51 (16%) patients assigned to the intervention group and 44 (13%) assigned to the control group died (unadjusted OR 1.24, 95% CI 0.80–1.92; $p=0.37$) and 83 (26%) and 79 (24%) patients, respectively, had serious adverse events (unadjusted OR 1.12, 95% CI 0.79–1.60; $p=0.53$; table 3). The occurrence of symptomatic intracranial haemorrhage

did not differ between the intervention and control groups at 24 h (eight [3%] of 317 patients *vs* six [2%] of 329 patients; unadjusted OR 1.39, 95% CI 0.48–5.06; $p=0.60$) or at 36 h (nine [3%] of 317 patients *vs* seven [2%] of 329 patients; 1.34, 0.49–3.65; $p=0.62$). The only safety outcome that differed between treatment groups was asymptomatic intracranial haemorrhage at 24 h, which was more prevalent in the intervention group than in the control group (34 [11%] of 317 patients *vs* 20 [6%] of 329 patients; unadjusted OR 1.86, 95% CI 1.04–3.30; $p=0.046$), but after adjustment for baseline NIHSS score, age, baseline serum glucose, and time to alteplase bolus, this difference became non-significant. The only adverse event that differed between intervention and control groups was atrial fibrillation (28 [9%] of 317 patients *vs* 14 [4%] of 329 patients; unadjusted OR 2.18, 95% CI 1.12–4.22; $p=0.025$). However, after excluding patients with atrial fibrillation at baseline assessment, this difference became non-significant (23 [7%] of 312 patients *vs* 13 [4%] of 328 patients; unadjusted OR 1.93, 95% CI 0.96–3.88; $p=0.085$). Two (1%) of 317 patients in the intervention group had partial seizures compared with no patients in the control group (OR 5.22, 95% CI 0.25–109.20; $p=0.49$).

We did not detect any differences ($p \geq 0.1$ for interaction) in the effect of sonothrombolysis in prespecified subgroup analyses by sex, age, baseline NIHSS score, and time from symptom onset to alteplase bolus (figure 3). Sensitivity analyses did not detect any difference in primary and secondary efficacy outcomes, mortality, or symptomatic intracranial haemorrhage after excluding patients with 90-day modified Rankin Scale scores after completion of the second interim analysis (47 patients in the intervention group and 52 in the control group). Further details on sensitivity analyses are in the appendix. Analyses of efficacy outcomes in the per-protocol and safety populations yielded similar results to analyses in the intention-to-treat population (appendix). Similarly, analyses of safety outcomes yielded almost identical results in the intention-to-treat population, per-protocol population, and safety population (appendix).

Discussion

The CLOTBUST-ER trial was stopped early because of futility, according to prespecified rules. Findings of the trial showed that, compared with standard-of-care treatment alone (alteplase bolus), additional use of low-power high-frequency ultrasound with an operator-independent device provided no benefit for functional outcome. However, the results indicated the potential feasibility and safety of sonothrombolysis for patients with acute ischaemic stroke.

Our findings regarding sonothrombolysis safety corroborate the conclusions of two independent meta-analyses suggesting the potential safety of high-frequency ultrasound coupled with intravenous thrombolysis as an investigational reperfusion treatment for acute ischaemic

stroke.^{8,9} The proportion of patients in the intervention group of CLOTBUST-ER with symptomatic intracranial haemorrhage (3% at 36 h) is less than the pooled proportion reported in previous smaller randomised controlled trials of sonothrombolysis (4%),⁸ but it is similar to data reported in the European Cooperative Acute Stroke Study III (2%)² and the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (2%).¹⁷ Moreover, the proportion with symptomatic intracranial haemorrhage was lower than the proportion reported in an individual patient data meta-analysis of nine intravenous thrombolysis trials (4%),²⁰ despite the fact that pretreatment stroke severity was higher in our trial.

A potential safety concern in our trial was the high proportion of patients with cerebral oedema, brain herniation, and asymptomatic intracranial haemorrhage detected in the intervention group in unadjusted analyses (table 3). These adverse events were recorded based on radiology reports by local investigators, without undergoing central adjudication. In previous randomised controlled trials, no association was detected between ultrasound-enhanced thrombolysis and risk for cerebral oedema.^{7,21,22} Likewise, contrary to symptomatic intracranial haemorrhage, asymptomatic intracranial haemorrhage is not related to clinical outcome in patients treated with intravenous thrombolysis.²³ Moreover, the proportions of patients with midline shift were similar in the intervention and control groups of CLOTBUST-ER, and no difference was noted between treatment groups in neurological deterioration at 24 h. Finally, associations of sonothrombolysis with cerebral oedema, brain herniation, and asymptomatic intracranial haemorrhage were not significant after adjustment for prespecified confounders. Nevertheless, the potential relation between 2 MHz sonothrombolysis and cerebral oedema deserves further investigation in future randomised controlled trials with central adjudication of brain herniation.

Sonothrombolysis did not improve functional outcome in CLOTBUST-ER. This finding could be partly explained by design features and limitations of our trial. First, unlike previous studies of ultrasound-enhanced thrombolysis, in which imaging documentation of proximal intracranial occlusions was required,^{7,21,22} stroke severity was used as the surrogate measure of large-vessel occlusion and vascular imaging was not mandatory. As a result, some of our patients might not have had a proximal occlusion within the target area of our operator-independent device. We postulate that our findings parallel the results of the Interventional Management of Stroke III (IMS III) trial,²⁴ which confirmed the need to select patients with a proximal arterial occlusion using vessel imaging to test acute reperfusion treatments (instead of enrolling patients with severe stroke as a surrogate for an occlusion). Second, compared with use of a handheld device in previous positive studies,^{7,8} it is possible that our operator-independent device provided less direct thrombus exposure to ultrasound because of the multitransducer

Figure 3: Common odds ratio for improvement on the modified Rankin Scale at 90 days in patients treated with intravenous thrombolysis within 4.5 h

Data were analysed according to ordinal logistic regression after collapsing modified Rankin Scale scores 5 and 6 and adjusting for age, NIHSS score at baseline, time from stroke onset to alteplase bolus, and baseline serum glucose, across the different prespecified subgroups. NIHSS scores range from 0 to 42, with higher scores indicating more severe neurological deficits. Thresholds for age and NIHSS score were chosen at the median. The threshold for time from stroke onset to alteplase bolus was prespecified. cOR=common odds ratio. NIHSS=National Institutes of Health Stroke Scale.

headframe design.^{25,26} Third, data for functional outcome at 90 days were unavailable in 63 patients (9% of the study population), because of early discontinuation of CLOTBUST-ER after the second interim analysis. After study termination by the funder, 90-day follow-up assessments were completed for most patients because of the efforts of onsite investigators, who were asked to complete the trial in their own time. Nevertheless, it should be noted that our sensitivity analysis indicated that there was no difference in safety and efficacy outcomes after exclusion of patients with documentation of their 90-day functional status after the second interim analysis. Furthermore, we formally tested and verified the randomness of the missing follow-up data in exploratory analyses. Fourth, potential enrolment bias at some sites—arising from higher priority given to endovascular treatment options—might have led to enrolment of fewer large-vessel occlusions at those centres.

Our study has other limitations, including the non-significant difference in time from symptom onset to treatment in favour of the intervention group (117 min vs 126 min in the control group) and reliance on the investigator's ability to properly mount the device and gel pads without any further onsite validation being done. We should also highlight the paucity of prospectively gathered data for ischaemic stroke aetiological classification or anatomic localisation and, therefore, our inability to do additional subgroup analyses for patients with lacunar versus non-lacunar strokes and patients with anterior versus posterior circulation strokes. Further, only 38 patients were enrolled in the designed arterial recanalisation substudy (based on pretreatment and post-treatment CT angiography), and we were unable to assess

the effect of sonothrombolysis on recanalisation and functional outcome of patients with acute ischaemic stroke with large-vessel occlusions. The steering committee decided not to make vascular imaging mandatory for patients' inclusion because some participating centres did not have CT angiography available 24 h a day 7 days a week, and because round-the-clock availability of CT angiography was not part of standard-of-care treatment at the time of study design. Moreover, we decided to implement a similar approach to the IMS III trial,²⁴ to identify patients with large-vessel occlusions using a cutoff for the NIHSS score of 10 points or greater. Unfortunately, the negative results of IMS III could not be predicted during CLOTBUST-ER design and initiation.

In view of the positive results of recent thrombectomy trials (highlighting CT angiography as part of standard-of-care treatment), we have redesigned the operator-independent ultrasound device to target CT angiography-located large-vessel occlusions with only one set of transducers that will be placed either over the right or left temporal window or suboccipitally dependent on occlusion location seen on CT angiography. The redesigned device will also use novel coupling gel pads to achieve improved headframe fixation during insonation. This new device will be tested in the TRUST trial (NCT03519737), in which all patients with large-vessel occlusions who meet standard criteria for intravenous alteplase and who are being transferred from primary to comprehensive stroke centres will be randomised to either ultrasound or no ultrasound, with the primary endpoint being recanalisation at receiving hospitals on digital subtraction angiography before thrombectomy. Finally, it should be mentioned that the study was terminated by the funder, and no additional funding was available after completion of the follow-up of enrolled patients. The lengthy process of report preparation was the main reason for delaying publication of the study findings, which were presented in part at the European Stroke Organisation Conference in Barcelona in 2016.

Our experience in CLOTBUST-ER suggests that the increasing implementation of endovascular treatments across major academic stroke centres will present challenges for clinical trials aiming to test non-interventional or adjuvant reperfusion strategies. The potential effectiveness of sonothrombolysis might be further investigated in randomised controlled trials undertaken in stroke centres that are dependent on transfer of patients for endovascular reperfusion treatments or in countries where these therapies cannot yet be offered as standard of care.

Contributors

AVA and GT did the literature search and wrote the first draft. PM and GT prepared the figures. AVA, GB, and JA designed the study. DM, TAK, and PM contributed to data analysis. AVA, MK, LS, GT, ADB, AMD, VKS, RM, KWM, JCG, CRL, CAM, MS, MV, AWA, and PDS contributed to data interpretation. JBF contributed to adjudication of intracranial haemorrhage. MK, LS, ADB, AMD, VKS, RM, KWM, JCG, CRL, CAM, MS, TP, MV, AWA, and PDS contributed to critical review and revision of the report.

Declaration of interests

MK reports advisory board and speaker honoraria from Boehringer Ingelheim, Bayer, Bristol-Myers Squibb, Pfizer, Daichii Sankyo, Novartis, Amgen, Stryker, and Medtronic and an unrestricted research grant from Boehringer Ingelheim. GT reports advisory board and speaker honoraria from Boehringer Ingelheim, Bayer, Daichii Sankyo, Medtronic, Shire, CSL Behring, and Biogen and an unrestricted research grant from Medtronic. JBF reports consulting, lecture, and advisory board fees from BioClinica, Cerevast Therapeutics, Artemida, Brainomix, and Merck; a grant from the German Federal Ministry of Education and Research (01EO0801 and 01EO01301); funding from the European Union Seventh Framework Program (FP7/2007–2013; grant agreement no 278276 [WAKE-UP]); and holds European patent no 17179320.01-1906. AMD reports grants from Cerevast Therapeutics. RM reports grants from Ministry of Education, Youth and Sports of Czech Republic (project no LQ1605). KWM reports personal fees and non-financial support from Boehringer Ingelheim and non-financial support from Pulse Therapeutics. PDS reports personal fees and travel grants from Cerevast Therapeutics and personal fees from Boehringer Ingelheim. GB and JA were employees of Cerevast Therapeutics, during the conduct of the study. AVA reports consultant fees, travel reimbursement, and stock options from Cerevast Therapeutics and speakers' bureau and honoraria from Genentech. TAK was supported in part by the US National Institutes of Health (R01 NS094535) and the Welch Foundation (grant no BE-0048). LS, ADB, VKS, JCG, CRL, CAM, MS, DM, TP, MV, PM, and AWA declare no competing interests.

Data sharing statement

De-identified participant data will be made available from the corresponding author on reasonable request.

Acknowledgments

This study was supported by Cerevast Therapeutics. We thank Sean Condon (senior biostatistician, Dataphiles Programming, Durham, NC, USA) who served as the independent project statistician and facilitated the role of Data Safety Monitoring Board in all meetings on data interpretation; and Travis Rothlisberger (Cerevast Therapeutics) for database maintenance.

References

- 1 The NINDS rt-PA Stroke Study Group. Tissue plasminogen activator for acute ischemic stroke. *N Engl J Med* 1995; **333**: 1581–87.
- 2 Hacke W, Kaste M, Bluhmki E, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. *N Engl J Med* 2008; **359**: 1317–29.
- 3 Emberson J, Lees KR, Lyden P, et al. Effect of treatment delay, age, and stroke severity on the effects of intravenous thrombolysis with alteplase for acute ischaemic stroke: a meta-analysis of individual patient data from randomised trials. *Lancet* 2014; **384**: 1929–35.
- 4 Christou I, Burgin WS, Alexandrov AV, Grotta JC. Arterial status after intravenous TPA therapy for ischemic stroke: a need for further interventions. *Intl Angiology* 2001; **3**: 208–13.
- 5 Saqqur M, Uchino K, Demchuk AM, et al. Site of arterial occlusion identified by transcranial Doppler (TCD) predicts the response to intravenous thrombolysis for stroke. *Stroke* 2007; **38**: 948–54.
- 6 Barreto AD, Alexandrov AV. Adjunctive and alternative approaches to current reperfusion therapy. *Stroke* 2012; **43**: 591–98.
- 7 Alexandrov AV, Molina CA, Grotta JC, et al. Ultrasound-enhanced systemic thrombolysis for acute ischemic stroke. *N Engl J Med* 2004; **351**: 2170–78.
- 8 Tsigoulis G, Eggers J, Ribo M, et al. Safety and efficacy of ultrasound-enhanced thrombolysis: a comprehensive review and meta-analysis of randomized and nonrandomized studies. *Stroke* 2010; **41**: 280–87.
- 9 Ricci S, Dinia L, Del Sette M, et al. Sonothrombolysis for acute ischaemic stroke. *Cochrane Database Syst Rev* 2012; **10**: CD008348.
- 10 Barlinn K, Tsigoulis G, Barreto AD, et al. Outcomes following sonothrombolysis in severe acute ischemic stroke: subgroup analysis of the CLOTBUST trial. *Int J Stroke* 2014; **9**: 1006–10.
- 11 Alexandrov AV, Sloan MA, Tegeler CH, et al. Practice standards for transcranial Doppler (TCD) ultrasound: part II—clinical indications and expected outcomes. *J Neuroimaging* 2012; **22**: 215–24.
- 12 Barlinn K, Barreto AD, Sisson A, et al. CLOTBUST-hands free: initial safety testing of a novel operator-independent ultrasound device in stroke-free volunteers. *Stroke* 2013; **44**: 1641–46.

- 13 Barreto AD, Alexandrov AV, Shen L, et al. CLOTBUST-Hands Free: pilot safety study of a novel operator-independent ultrasound device in patients with acute ischemic stroke. *Stroke* 2013; **44**: 3376–81.
- 14 Schellinger PD, Alexandrov AV, Barreto AD, et al. Combined lysis of thrombus with ultrasound and systemic tissue plasminogen activator for emergent revascularization in acute ischemic stroke (CLOTBUST-ER): design and methodology of a multinational phase 3 trial. *Int J Stroke* 2015; **10**: 1141–48.
- 15 US Food and Drug Administration. Information for manufacturers seeking marketing clearance of diagnostic ultrasound systems and transducers. Sept 9, 2008. <https://www.fda.gov/downloads/UCM070911.pdf> (accessed Jan 21, 2019).
- 16 Quinn TJ, Lees KR, Hardemark HG, Dawson J, Walters MR. Initial experience of a digital training resource for modified Rankin Scale assessment in clinical trials. *Stroke* 2007; **38**: 2257–61.
- 17 Wahlgren N, Ahmed N, Dávalos A, et al. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. *Lancet* 2007; **369**: 275–82.
- 18 Stekhoven DJ, Bühlmann P. MissForest: non-parametric missing value imputation for mixed-type data. *Bioinformatics* 2012; **28**: 112–18.
- 19 Bland JM, Altman DG. The odds ratio. *BMJ* 2000; **320**: 1468.
- 20 Whiteley WN, Emberson J, Lees KR, et al. Risk of intracerebral haemorrhage with alteplase after acute ischaemic stroke: a secondary analysis of an individual patient data meta-analysis. *Lancet Neurol* 2016; **15**: 925–33.
- 21 Alexandrov AV, Mikulik R, Ribo M, et al. A pilot randomized clinical safety study of sonothrombolysis augmentation with ultrasound-activated perflutren-lipid microspheres for acute ischemic stroke. *Stroke* 2008; **39**: 1464–69.
- 22 Molina CA, Barreto AD, Tsivgoulis G, et al. Transcranial ultrasound in clinical sonothrombolysis (TUCSON) trial. *Ann Neurol* 2009; **66**: 28–38.
- 23 Schlegel DJ, Tanne D, Demchuk AM, et al. Prediction of hospital disposition after thrombolysis for acute ischemic stroke using the National Institutes of Health Stroke Scale. *Arch Neurol* 2004; **61**: 1061–64.
- 24 Broderick JP, Palesch YY, Demchuk AM, et al. Endovascular therapy after intravenous t-PA versus t-PA alone for stroke. *N Engl J Med* 2013; **368**: 893–903.
- 25 Tsivgoulis G, Alexandrov AV. Ultrasound-enhanced thrombolysis in acute ischemic stroke: potential, failures, and safety. *Neurotherapeutics* 2007; **4**: 420–27.
- 26 Tsivgoulis G, Culp WC, Alexandrov AV. Ultrasound enhanced thrombolysis in acute arterial ischemia. *Ultrasonics* 2008; **48**: 303–11.