Efficacy and safety of sonothrombolysis for acute ischemic stroke: a multi-centre, double-blind, phase 3, randomised controlled trial

Andrei V. Alexandrov, MD[1], Martin Köhrmann, MD[2,3], Lauri Soinne, MD, PhD[4], Georgios Tsivgoulis, MD, PhD[1,5,6], Andrew D. Barreto, MD, MS[7], Andrew M. Demchuk, MD[8], Vijay K. Sharma, MD[9], Robert Mikulik, MD, PhD[6,10], Keith W. Muir, MD[11], Gordon Brandt, MD[12], John Alleman, BS, MBA[12], James C. Grotta, MD[13], Christopher R. Levi, MD[14], Carlos A. Molina, MD, PhD[15], Maher Saqqur, MD, MPH[16,17], Dimitris Mavridis, PhD[18,19], Theodora Psaltopoulou, MD, PhD[20], Milan Vosko, MD[21], Jochen B. Fiebach, MD, PhD[22], Pitchaiah Mandava, MD, PhD[23,24], Thomas A. Kent, MD[23,24], Anne W. Alexandrov, PhD[1,25], Peter D. Schellinger, MD[26], for the CLOTBUST-ER Trial Investigators

[1]Department of Neurology, University of Tennessee Health Science Center, Memphis, TN, USA

[2]Department of Neurology, Universitaetsklinikum Erlangen, Erlangen, Germany

[3]Department of Neurology, University Duisburg-Essen, Essen, Germany

[4]Department of Neurology, Helsinki University Hospital and Clinical Neurosciences, Neurology, University of Helsinki Finland, Helsinki, Finland

[5]Second Department of Neurology, “Attikon” University Hospital, School of Medicine, National and Kapodistrian University of Athens, Athens, Greece

[6]International Clinical Research Center and Department of Neurology, St. Anne's University Hospital in Brno, Brno, Czech Republic

[7]Department of Neurology, University of Texas Health Science Center at Houston, Houston, TX, USA

[8]Department of Clinical Neurosciences and Radiology, Hotchkiss Brain Institute, University of Calgary, Calgary, AB, Canada

[9]Yong Loo Lin School of medicine, National University of Singapore and Division of Neurology, Department of Medicine, National University Hospital, Singapore, Singapore

[10]Medical Faculty, Masaryk University, Brno, Czech Republic

[11]Institute of Neuroscience and Psychology, University of Glasgow, Queen Elizabeth University Hospital, Glasgow, United Kingdom

[12]Cerevast Therapeutics, Inc., Redmond, WA, USA

[13]Clinical Innovation and Research Institute, Memorial Hermann Hospital-Texas Medical Center, Houston, TX, USA

[14]Department of Neurology, John Hunter Hospital, University of Newcastle, Newcastle, Australia

[15]Stroke Unit, Department of Neurology, Vall d'Hebron University Hospital, Vall d'Hebron Research Institute, Autonomous University of Barcelona, Barcelona, Spain

[16]Department of Medicine (Neurology), University of Alberta, Edmonton, Alberta, Canada

[17]Neuroscience Institute, Hamad Medical Corporation, Doha, Qatar

[18]Department of Hygiene and Epidemiology, University of Ioannina School of Medicine, Ioannina, Greece

[19]Department of Primary Education, School of Education, University of Ioannina, Ioannina, Greece

[20]Department of Hygiene, Epidemiology and Medical Statistics, Medical School, National and Kapodistrian University of Athens, Athens, Greece

[21]Department of Neurology, Kepler University Clinic, General Hospital Linz (AKH), Linz, Austria

[22]Center for Stroke Research Berlin, Charité-University Medicine Berlin, Berlin, Germany

[23]Stroke Outcomes Laboratory, Department of Neurology, Baylor College of Medicine, , Houston, TX, USA

[24]Michael E. DeBakey VA Medical Center Stroke Program and Center for Translational Research on Inflammatory Diseases, Houston, TX, USA.

[25]Australian Catholic University, Sydney, Australia

[26]Departments of Neurology and Neurogeriatry, John Wesling Medical Center Minden, Ruhr University Bochum, Minden, Germany

Corresponding author:

Dr. Andrei V. Alexandrov

Department of Neurology, University of Tennessee Health Science Center, Memphis, TN, USA

855 Monroe Avenue, Suite 415, Memphis, TN, USA, 38163

Email: avalexandrov@att.net

Tel :(901) 4486199

Fax : (901) 4486740

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Research in context

Evidence before this study

We searched MEDLINE and SCOPUS databases up to November 17, 2018, **without language or any other restrictions**, for randomized controlled trials on the utility of ultrasonography in enhancing the thrombolytic activity of tPA and found six small scale (phase II) randomized clinical trials (RCTs) comparing sonothrombolysis to intravenous tissue plasminogen activator (tPA) or conventional treatment. However, no large scale (phase III) RCT using an operator-independent transcranial ultrasound device delivering sonothrombolysis has been performed to date.

Added value of the study

Exposure of acute ischemic stroke patients to low-power ultrasound using an operator-independent device was found to be feasible and likely safe, but with no three-month clinical benefit.

Implications of all available evidence

Sonothrombolysis with high-frequency ultrasound appears to be safe but offers no clinical benefit in acute ischemic stroke patients. The potential efficacy of sonothrombolysis may be further investigated in stroke centers that are dependent on patient transfer for endovascular reperfusion therapies, or in countries where these therapies cannot yet be offered as standard of care.

Abstract

Background: Pulsed-wave ultrasound increases exposure of intracranial thrombus to tissue-plasminogen-activator (tPA) thereby potentially facilitating early reperfusion. We sought to determine if a novel operator-independent transcranial ultrasound device delivering sonothrombolysis improves functional outcome after acute ischemic stroke (AIS).

Methods: We performed a double-blind, multicenter, phase III randomized-controlled clinical trial between August 2013 and April 2015, in 76 medical centers in North America, Europe and Australasia. We included patients with acute ischaemic stroke (National Institutes of Health Stroke Scale scores ≥10) who were eligible for intravenous thrombolysis within a 4.5-hour treatment window worldwide and within a 3-hour treatment window in North America. After receiving standard of care treatment, including full dose intravenous tPA, participants were randomly assigned 1:1, via an interactive web randomization system, to 2 h of 2-MHz pulsed-wave ultrasound (intervention group) or sham treatment (control group) using an operator-independent device, which had to be activated within 30 min of the tPA-bolus. **Participants, investigators, and those assessing outcomes were blinded to group assignment.** The primary outcome was the adjusted improvement in three-month modified Rankin Scale (mRS) scores analyzed using ordinal logistic regression as a common odds ratio (cOR, shift analysis) **in the intention-to-treat population**.

Findings: The trial was stopped early by the study sponsor after the second interim analysis due to futility. **We randomized 335 patients to the intervention group and 341 patients to the control group.** The adjusted cOR for 1-point improvement in mRS-score in the intervention group (compared to the control group) was 1.05 (95%CI: 0.77-1.45) for patients

treated with tPA within 0-3h. There was no strong evidence to indicate a difference between groups in the adjusted analyses of three-month mortality (OR=1.19, 95%CI: 0.74-1.92), sICH (OR=1.39, 95%CI: 0.51-3.95), brain herniation (OR=2.09, 95%CI: 0.73-6.87), ICH (OR=1.78, 95%CI: 0.98-3.31), or cerebral edema (OR=2.15, 95%CI: 0.93-5.40).

Interpretation: Exposure of AIS patients treated with tPA to low-power and high-frequency ultrasound using an operator-independent device was feasible and likely safe, but with no three-month clinical benefit. **The potential efficacy of sonothrombolysis may be further investigated in RCTs conducted in stroke centers that are dependent on patient transfer for endovascular reperfusion therapies, or in countries where these therapies cannot yet be offered as standard of care.**

Funding: Cerevast Therapeutics

Trial Registration: CLOTBUST-ER, NCT01098981,https://clinicaltrials.gov/ct2/show/NCT01098981

TEXT

Introduction

Intravenous recombinant tissue plasminogen activator (tPA) is the only approved medical reperfusion treatment for acute ischemic stroke (AIS),[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, due to the initial severity of ischemic insult and inadequate response to intravenous thrombolysis (IVT).[4,5] Therefore, amplification of tPA effectiveness in thrombus dissolution remains an important goal in future development of more effective medical stroke therapies even in the era of mechanical thrombectomy, since endovascular reperfusion therapies are not readily available in the majority of stroke centers across the world.[6]

A phase II randomized-controlled clinical trial[7] (RCT) of 2-MHz diagnostic ultrasound equipment (transcranial Doppler) and meta-analyses[8,9] of other similar studies showed that ultrasound aimed at the residual flow/thrombus interface can at least double the chance of early recanalization. Sonothrombolysis was also associated with a higher likelihood of favorable functional outcome in the subgroup of patients with pretreatment National Institutes of Health Stroke Scale (NIHSS) scores ≥10 points.[10] However, a major obstacle for emergency physicians, neurologists and health professionals limiting the use of diagnostic ultrasound epuipment in AIS is its operator dependency.[11] Therefore, we had previously developed a novel “hands-free” therapeutic device with operator-independent targeting of the intracranial vessels, tested it in early phase clinical studies, and have demonstrated the safety of this technology in humans.[12,13]

Based on our previous work, we undertook a phase III RCT of sonothrombolysis in AIS. Our objective was to determine the safety and therapeutic efficacy of our operator-independent device in combination with intravenous tPA to improve functional outcome, compared to intravenous tPA alone in patients with AIS presenting within **3 hours** (h) from symptom onset.

Methods

Study design

The Combined Lysis of Thrombus using Ultrasound and Systemic tPA for Emergent Revascularization (CLOTBUST-ER) was a multinational, double-blind, sham-controlled RCT. Details of the methods used in the trial have been published.[14] **In brief the study was performed in 76 institutions and 14 countries.** The study was conducted and reported with fidelity to the study protocol, available with the full text of this article as an online supplement. The trial was approved by the institutional review board at each site or national ethics committee, as required.

Participants

We enrolled AIS patients aged 18-80 years with baseline NIHSS scores of ≥10 points who received intravenous tPA within a 4.5-hour treatment window worldwide and within a 3-hour treatment window in North America as per national approval labels.[14] The cut-off of 10 points in NIHSS-score was selected based on sensitivity analysis of our earlier phase II trial indicating that the beneficial effect of sonothrombolysis was amplified in the subgroups of acute ischemic stroke patients with NIHSS-scores of ≥10 points.[10,14] All subjects were

independently functioning in the community immediately prior to their stroke [pre-morbid modified Rankin scale (mRS) score 0-1]. Patients were included irrespectively of the anticipated stroke localization (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 (including planned endovascular reperfusion procedures) criteria has been published[14] and are available in the Supplementary Appendix.

Randomization and masking

Subjects were randomized 1:1, using web-based central randomization[14] and random permuted blocks stratified by site (random block size of 2, 4, or 6), to active ultrasound+tPA (intervention group) or to sham ultrasound+tPA (control group). Each subject was assigned a unique, site-specific, identification number after signing the informed consent. Patients were randomized either prior or subsequent to administration of the tPA bolus with device to be activated within 30 min of tPA bolus. Headframes were programmed based on a randomization code that maintained blinding of treating physicians, patients and the sponsor to active vs. sham assignments. Randomization was performed (IWRS) provided through IT Clinical, Portugal. The system was audited and met all required good clinical practice compliance requirements. Blinding was ascertained through an algorithm that determined whether "A" setting delivers active insonation and "B" delivers Sham (placebo) insonation, or the reverse. The IWRS system was programmed to mask the A or B assignments, therefore no user could see which assignment subjects were given. The success of masking procedures was not assessed.

Procedures

All eligible subjects received standard of care treatment including full dose intravenous tPA (0.9 mg/kg; 90 mg maximum; 10% bolus followed by 90% IV infusion over 60 minutes).

The headframe had to be placed on all subjects prior to or shortly after tPA-bolus (in order not to delay IVT administration), and had to be activated within 30 minutes of tPA-bolus to achieve maximum overlap between exposure to the device and tPA infusion. All subjects regardless of device activation time were required to wear the headframe for a total of 120 minutes. Devices were equipped with a timer showing completion of 120 min exposure, and a pause button in case the patient had to have repeat CT scan performed as standard of care. Interruption time to deliver standard of care procedures requiring temporary device removal could not exceed 15 min. A training video was created prior to study initiation. All site investigators watched training video and practiced in assembling and placement of devices under direct supervision of trained clinical monitors for each country. All sites were trained prior to site initiation. In addition, all new investigators were required to undergo similar training during the trial. Finally, all global and local investigators meetings had training sessions for new and existing sites.

In accordance with parameters mandated by the FDA for currently approved and marketed transcranial Doppler (TCD) diagnostic ultrasound devices,[15] the intervention group received standard of care 2 MHz pulsed-wave transcranial ultrasound for 120 minutes (total average power 32Mw; maximum Spatial Peak Temporal Average Intensity: 207Mw/cm^2; PRF: 8.3kHz;

Pulse duration: 5μS). The control group received sham (inactive) ultrasound for 120 minutes. A detailed description of the operator-independent device, vessel targeting without imaging or Doppler echo-location, safety testing and phase II functional outcomes data has been previously published.[12,13]

Investigators obtained NIHSS scores pre-treatment, 2h, 24h, on day 7 or at discharge (from an acute facility to home), and at day 90; mRS-scores were recorded at day 7 or at discharge (if the patient was discharged earlier than day 7) and at day 90. Significant neurological worsening, defined as a total NIHSS score increase by ≥ 4 points from the best score at any time during the first 24h post tPA-bolus, required a non-contrast CT to rule out symptomatic intracranial hemorrhage (sICH). Routine post-stroke imaging was not mandatory but was conducted at all participating centers as standard of care for AIS management.

Outcomes

The primary outcome is the cumulative ordinal logistic regression analysis of mRS-scores at 90±10 days from randomization, for all participants enrolled within 3 h of onset of stroke symptoms (according to the US Food & Drug Administration regulatory requirements). This analysis was repeated as a secondary analysis for all patients who were enrolled within 4.5h (Global outcome).[14]

Other secondary efficacy endpoints included dichotomous mRS 0–1 and mRS 0–2 rates at day 90, Dramatic Clinical Recovery at 2h, Clinical Recovery at 24h, Clinical Recovery at day 90, Neurological Improvement at 24h, Neurologic Worsening at 24h, Length of Stay,

Sliding Dichotomy Independent Functional Outcome at Day 90, NIHSS at 2h, NIHSS at 24h, NIHSS at day 7, NIHSS at day 90 and mRS at day 7.[14] Dramatic clinical recovery assessed at 120±15 min after headframe activation included a reduction of 10 or more points in NIHSS compared with pretreatment, or a total NIHSS score of 3 or less.[14] Clinical recovery assessed at 24±2 h after headframe activation included a reduction of 10 or more points on NIHSS compared with pretreatment, or a total NIHSS score of 3 or less.[14] Neurological improvement assessed at 24±2 h after headframe activation required a reduction of 5 or more points on NIHSS compared with the pretreatment score.[14] Neurological worsening assessed at 24±2 h after headframe activation required an increase of 4 or more points on NIHSS compared with the pretreatment score.[14] Independent functional outcome adjusting for pretreatment NIHSS assessed at 90±10 days included mRS score 0–1 for subjects with pretreatment NIHSS 10–14, and mRS score 0–2 for subjects with pretreatment NIHSS>14.[14]

Safety outcomes included the proportion of subjects in the intervention vs. control group experiencing sICH within 24h of tPA-bolus and an overall analysis of adverse events as previously described.[14] Symptomatic ICH per study protocol (online supplement) was defined as neurological deterioration (≥4 points worsening on the NIHSS compared with the best prior examination) within 24h after tPA bolus with documented parenchymal hemorrhage type 2 or remote parenchymal hemorrhage type 2.[14] All intracranial bleeds within 24h which were associated with neurological deterioration as defined above were sent to a central imaging core lab for independent adjudication. To allow comparison of sICH rates to the recently adopted 36h time window,[17] the above mentioned sICH definition and adjudication process were also applied to all neurological deteriorations reported within 36h post tPA

bolus.[1] Three sICH cases were diagnosed by the local investigators without central adjudication due to early trial termination by the sponsor. However, we included these cases in the final sICH group assuming the worst case scenario. All intracranial hemorrhages which were not associated with neurological deterioration of 4 or more points on the NIHSS scale were subsequently classified as asymptomatic intracranial hemorrhages.

All cases of pre-specified adverse events were reported by the blinded clinical investigators of the participating centers. These events were reviewed and adjudicated by a blinded independent adjudication panel within the DSMB. In the event of a discrepancy between the adjudication panel and the clinical investigator, the adjudication panel determination was final. Brain herniation, cerebral oedema and midline shift were not pre-specified adverse events of our study and there were not centrally adjudicated. Information on these adverse events was collected based on the onsite clinical and radiology reports. There was no standardized definition for these adverse events. All adverse events were coded and tabulated by MedDRA System Organ Class and presented in descending frequency. Adverse events were also tabulated by severity and relationship to the investigational device.[14] Death from any cause within 90 days of treatment and the proportion of subjects who died due to adverse events were also summarized by treatment group.[14]

Statistical analysis

Details on our pre-planned statistical analysis plan, power estimations and planned interim analyses have been published previously,[14] and are also available in the Appendix. Interim analyses assessing the primary outcome between treatment groups were scheduled

after approximately one-third and two-thirds of 90-day mRS outcomes becoming available. Using O'Brien-Fleming boundaries for the group sequential design with 90% power and testing at approximately one-third and two-thirds of the subjects imply critical values of P = 0.0003525 and P = 0.0120085 at the first and second interim analyses, respectively, and P = 0.0462386 at the final analysis. In addition, a conditional power futility analysis was scheduled to be performed at each of the interim analysis point by the DSMB, where the study would stop should the conditional power fall below 15%.

Analyses reported here were performed in the intention-to-treat population (by PM and TAK) using a program written in Matlab© (version R2018b) and a single 'Master' data file was generated. All further statistical analyses were performed in R (version 3.4) running under an R Studio environment and primary outcomes were cross-checked in Matlab environment. The plans of all statistical analyses were performed prior to the unblinding of the data.

Primary outcome was specified as the proportional odds logistic regression (*polr* command in R) over the 90-day mRS distribution after collapsing grades 5 and 6.[14] By doing univariate logistic regressions for each of the five groupings we observed that the odds ratios bump around one with negligible differences attributed to random variation, giving credit to the hypothesis of proportional odds across the groupings of the mRS. Additionally, we conducted two imputation analyses on the primary endpoint (US outcome). The missing mRS-score values were estimated using multiple imputation methodology[18] in the first analysis based on the strongest predictors of 90 day mRS-score as pre-specified in our statistical analysis protocol[14] (baseline NIHSS, 24 hour NIHSS and day 7/discharge mRS along

with assignment to treatment or control). The missing mRS-score values were imputed to the worst case (e.g. mRS-score of 6) in the second analysis.

Unadjusted and adjusted analyses are reported separately. Both unadjusted and adjusted statistical analyses for secondary endpoints were pre-specified. The unadjusted approach was the primary analytic approach, while the adjusted approach served as a secondary analysis. Pre-specified secondary outcomes[14] were tested in the unadjusted analyses with Fisher's two-sided test of proportion and confidence intervals were provided according to the methodology of Bland and Altman.[19] Pre-specified safety outcomes[14] were also tested using Fisher's two-sided test of proportions. Adjustment was done in terms of baseline NIHSS, age, baseline serum glucose, and time to tPA-bolus. These factors were chosen post-hoc by the steering committee prior to unblinding of the data. Adjustment for these factors was applied uniformly for all efficacy and safety outcomes. In all analyses no allowance for multiplicity was made. **To allow for the interim analyses** alpha spend adjustment was not done while calculating the p-values in all analyses. **Also,** the point estimates were naïve and **not bias adjusted for the interim analyses**.

Role of the funding source

The trial was funded by Cerevast Therapeutics and designed and led by a Steering Committee that included academic investigators and representatives of the sponsor. The site investigators gathered the data, with monitoring and database maintenance performed by the sponsor. The first and subsequent drafts of the manuscript were written by AVA and GT incorporating input from all the authors. The academic authors had unrestricted access to

the data, performed the data analysis with the primary and the independent statisticians, and attest to the integrity of the trial and the completeness and accuracy of the reported data. The trial was monitored by an independent Data and Safety Monitoring Board (Supplementary Appendix). The study sponsor had no involvement in the manuscript preparation, including data analysis and text drafting. **The steering committee of CLOTBUST-ER had the final responsibility for reaching the decision to submit for publication.**

Results

A total of 676 participants underwent randomization (335 to the intervention group and 341 to the control group). Patients were enrolled at 76 medical centers between August 2013 and April 2015 in North America (n=30), Europe (n=39) and Australasia (n=7). Details regarding the individual centers that enrolled patients in CLOTBUST-ER are available in the Supplementary Appendix. Intervention and control groups of the intention to treat population did not differ in any of the baseline characteristics (Table 1). The median elapsed time from tPA bolus to headframe activation was similar in the intervention (20min; interquartile range: 13-27) and control group (20 min; interquartile range: 13-25). Values on mean systolic blood pressure levels before tPA-bolus were missing in 9 and 13 patients in the intervention and control groups, respectively, while values on mean diastolic blood pressure before tPA-bolus were unavailable in 8 and 13 patients in the intervention and control groups, respectively. The CONSORT flow diagram is presented in Figure 1.

Primary outcome

CLOTBUST-ER was stopped early for futility after the per protocol defined second

interim analysis, having the two-thirds of 90-day mRS outcomes available, from the DSMB according to pre-specified stopping rules. The results of the first and second interim analysis on the primary outcome of interest are available in eFigure 1, appendix. Subjects who were enrolled in the study at the time of the futility determination were followed until 90 days post-tPA administration by the site investigators despite discontinuation of the study by the study sponsor. We therefore describe the results in the total sample of patients randomized in CLOTBUST-ER.

There were 28 and 35 patients with missing data on three-month mRS-scores in the intervention and the control arms respectively. Patients with missing follow-up data were censored from the analyses of the primary endpoint and the secondary endpoints that were evaluated at 90 days following symptom onset. The two groups did not differ in terms of the primary US outcome [adjusted common odds ratio (cOR): 1.05; 95%CI: 0.77-1.45; Table 2 & Figure 2]. Additionally, the primary Global outcome did not differ between the two groups (adjusted cOR: 1.06; 95%CI: 0.80-1.42; Table 2 & eFigure 2, appendix). We also detected no difference between groups on the primary outcome of interest (primary US outcome), after adjusting for the per-protocol defined covariates (site, baseline NIHSS, pre-morbid mRS and age) in the statistical analysis plan (adjusted cOR: 0.93, 95%CI: 0.69-1.24). In addition, there was no difference in the adjusted analyses on the primary outcome of interest (primary US outcome) using either multiple imputation methodology (unadjusted OR: 0.98, 95%CI: 0.73-1.31; adjusted OR: 0.99, 95%CI: 0.74-1.34; eTable 1) or imputation to the worst case (unadjusted OR: 1.08, 95%CI: 0.80-1.45; adjusted OR: 1.14, 95%CI: 0.84-1.54; eTable 2).

<u>Secondary efficacy outcomes</u>

All secondary outcomes are shown in Table 2. The two groups did not differ in any of the secondary outcomes. The adjusted ORs for patients randomized within 3 hours were 1.27 (95%CI: 0.85-1.89) for functional independence, 0.99 (95%CI:0.65-1.52) for dramatic clinical recovery at 2 h, 0.79 (95%CI:0.54-1.15) for clinical recovery at 24 h, 1.04 (95%CI:0.73-1.49) for neurological improvement at 24h and 1.37 (95%CI:0.70-2.71) for neurologic deterioration at 24h.

Safety outcomes

The safety outcomes in the safety population are shown in Table 3. The rates of death (16.7% vs. 13.4%, **OR: 1.23, 95%CI: 0.79-1.90**) and serious adverse events (26.2% vs. 24.0%, **OR: 1.12, 95%CI: 0.79-1.60**) were similar in the two groups. The rates of sICH were 2.8% and 2.1% in intervention and control groups respectively (**OR: 1.34; 95%CI: 0.49-3.65**). The two groups did not differ in any of the safety outcomes with the exception of asymptomatic hemorrhage (10.7% vs 6.1%; **OR: 1.86, 95%CI: 1.04-3.30**); this association did not retain statistical significance in adjusted analyses (OR for asymptomatic intracranial hemorrhage: 1.78, 95%CI: 0.98-3.31). The only adverse event that differed between the two groups was atrial fibrillation (8.8% in intervention vs. 4.2% in control groups, **OR: 2.18, 95%CI: 1.12-4.22**). However, after excluding patients with atrial fibrillation at baseline assessment, this difference did not retain statistical significance (7.3% vs. 4.0%; **OR: 1.90 0.95-3.82**). The occurrence of partial seizures was 0% (n=0) and 0.6% (n=2) in the control and intervention groups respectively (**OR: 5.22, 95%CI: 0.25-109.20**).

Subgroup and sensitivity analyses

We did not detect any significant differences (p value for interaction ≥0.1) in the

effect of sonothrombolysis in pre-specified subgroup analyses by sex, age, baseline stroke severity and onset to treatment time (Figure 3). Sensitivity analyses failed to detect any difference in primary and secondary efficacy outcomes, mortality and sICH after removing subjects with ascertainment of three-month mRS-scores following the completion of the second interim analysis (47 & 52 in the intervention and control groups respectively). Further details on sensitivity analyses are available in the appendix. The analyses of efficacy outcomes in the per-protocol and safety populations (eTable 3 & eTable 4, appendix) yielded similar results to the respective analyses in the intention-to-treat population (Table 2 & eFigure 3). Similarly, the analyses of safety outcomes yielded almost identical results in the intention-to-treat (eTable 5), per protocol (eTable 6) and safety (Table 3) populations.

Discussion

CLOTBUST-ER was stopped early due to futility, according to pre-specified rules and failed to show an additional benefit in functional outcome with sonothrombolysis using a novel operator-independent ultrasound device as compared with the standard therapy of IVT alone. However, the results of our trial indicate the potential feasibility and safety of exposure of AIS patients treated with IVT to high-frequency (low-power) ultrasound using an operator-independent device.

Our findings regarding sonothrombolysis safety corroborate the conclusions of two independent meta-analyses suggesting the potential safety of high-frequency ultrasound coupled with IVT as an investigational reperfusion therapy for AIS.[8,9] The sICH rate (2.8%) in the intervention group of CLOTBUST-ER is less than the pooled sICH rate of previous smaller

RCTs of sonothrombolysis (3.8%).[8] It is also comparable to the rate of European Cooperative Acute Stroke Study III[2] (2.4%) and the Safe Implementation of Thrombolysis in Stroke-Monitoring Study[16] (1.7%), while being lower than the sICH rate (3.7%) reported in an individual patient data meta-analysis of 9 IVT trials[20], despite the fact that pre-treatment stroke severity was higher in our trial.

A potential safety concern that needs to be addressed is the higher rate of cerebral edema, brain herniation and asymptomatic ICH detected in the sonothrombolysis group in the unadjusted analyses. These adverse events were reported on the basis of radiology reports by local investigators without being subjected to central adjudication. Previous RCTs failed to detect any association between ultrasound-enhanced thrombolysis and risk of cerebral edema.[7,21,22] Likewise, contrary to sICH asymptomatic ICH is not related to clinical outcome in patients treated with intravenous thrombolysis.[23] Moreover, the rates of midline shift were practically identical in the two groups of CLOTBUST-ER (2.7% & 2.6%), while no difference was noted in the rates of neurological deterioration at 24h. Finally, the associations of sonothrombolysis with cerebral edema, brain herniation or asymptomatic ICH were not significant after adjustment for pre-specified confounders. Nevertheless, the potential relationship between 2-MHz frequency sonothrombolysis and cerebral edema deserves further exploration in future RCTs with central adjudication of brain herniation.

Sonothrombolysis did not improve functional outcome in CLOTBUST-ER. This may be partially explained by certain design features and study limitations. First, unlike previous studies of ultrasound-enhanced thrombolysis requiring imaging documentation of proximal intracranial occlusions,[7,21,22] stroke severity was used as the surrogate measure of large

vessel occlusion and vascular imaging was not mandatory in our trial. Consequently, some of our patients might not have had a proximal occlusion within the target area of our operator-independent device. We speculate that our findings parallel the results of the Interventional Management of Stroke III (IMS III) trial[24] that confirmed the need to select patients with proximal arterial occlusions using vessel imaging to test acute reperfusion therapies (instead of enrolling those with severe stroke as surrogate for an occlusion).

Second, compared to a hand-held device as used in previous positive studies,[7,8] it is possible that our operator-independent device provided less direct thrombus exposure to ultrasound as a result of multi-transducer headframe design.[25,26] Third, data on functional outcome at three months were unavailable in 63 patients (9% of the study population) due to the early discontinuation of CLOTBUST-ER following the second interim analysis by the study sponsor. After the study termination, three-month follow-up evaluations were completed in the majority of cases due to the tremendous efforts of onsite investigators who were asked to complete the trial at their own time and efforts. Nevertheless, it should be noted that our sensitivity analysis indicated that there was no difference in efficacy and safety outcomes after exclusion of subjects with documentation of their three-month functional status following the second interim analysis. Furthermore, we formally tested and verified the randomness of the missing follow-up data in exploratory analyses. Fourth, potential enrollment bias at certain sites arising from higher priority given to endovascular treatment options might have led to enrollment of fewer large vessel occlusions at those centers.

Our study has limitations such as lack of pre-treatment visualization of a proximal

intracranial arterial occlusion, substantial number of incomplete three-month follow up evaluations (9% of enrolled patients), non-significant difference in onset-to-treatment times in favor of the intervention group (117 vs. 126 min) and reliance on investigator ability to properly mount the device and gel pads, without any further on-site validation being carried out. We should also highlight the lack of prospectively collected data on the ischemic stroke etiologic classification or anatomic localization, and therefore the inability to perform additional subgroup analyses for patients with lacunar vs. non-lacunar strokes and patients with anterior vs. posterior circulation strokes.

Moreover, only a limited number of patients (n=38) was enrolled in the designed arterial recanalization substudy [based on pre- and post-treatment CT angiography (CTA)] and we were unable evaluate the effect of sonothrombolysis on recanalization and functional outcomes of AIS patients with large vessel occlusions. The steering committee decided not to make vascular imaging mandatory for patient inclusion given the participation of centers with unavailable CT angiography on a 24/7 basis and since 24/7 CTA was not standard of care at the time of study design. Moreover, we decided to implement a similar approach to IMS III Trial[24] to identify patients with large vessel occlusions using a cut-off of 10 points or greater in NIHSS-score. Unfortunately, the negative results of IMS III could not be predicted during CLOTBUST-ER design and initiation. However, we acknowledge the lack of pre-treatment visualization of a proximal intracranial arterial occlusion in the vast majority of our population as a major study limitation.

After taking also into account the positive results of recent thrombectomy trials (highlighting CT angiography as standard of care), we have re-designed the operator-

independent ultrasound device to target CTA-located large vessel occlusions with only one set of transducers that will be placed over the right or left temporal window or suboccipitally dependently on occlusion location seen on CTA. The re-designed device will also use novel coupling gel pads to achieve improved headframe fixation during insonation. This new device will be tested in the recently launched TRUST trial (NCT 03519737),[27] in which all patients with large vessel occlusions who meet standard tPA criteria and are being transferred from primary to comprehensive stroke centers ("drip-n-ship") will be randomized to ultrasound or no ultrasound with primary end-point being recanalization at receiving hospitals on digital subtraction angiography prior to thrombectomy. Finally, it should be mentioned that the study was terminated by the sponsor and no additional funding was available beyond completion of follow-ups of enrolled patients. The lengthy process of manuscript preparation was the main reason for delaying publication of the study findings that were partially presented in European Stroke Organization Conference 2016 in Barcelona.

In conclusion, exposure of stroke patients treated with tPA to low-power ultrasound delivered by a novel operator-independent device was feasible and likely safe with no overall significant clinical benefit at 90 days. Our experience in CLOTBUST-ER indicates that the increasing implementation of endovascular therapies across major academic stroke centers raises significant challenges for clinical trials aiming to test non-interventional or adjuvant reperfusion strategies. The potential efficacy of sonothrombolysis may be further investigated in RCTs conducted in stroke centers that are dependent on patient transfer for endovascular reperfusion therapies, or in countries where these therapies cannot yet be offered as standard of care. Given that a more targeted approach of sonothrombolysis based

on pre-treatment CTA may have a potential therapeutic effect and utility in the "drip and ship paradigm", a newly designed ultrasound device to deliver ultrasound to primary region of occlusion will be assessed in a forthcoming phase 3 RCT.[27]

Data sharing statement

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

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Author contributions

Literature search: AVA, GT

Figures preparation: PM, GT

Study design: AVA, GB, JA

Data analysis: DM, TAK, PM

Data interpretation: AVA, MK, LS, GT, ADB, AMD, VKS, RM, KWM, JCG, CRL, CAM, MS, MV, AWA, PDS

Writing the first manuscript draft: AVA, GT

ICH adjudication: JBF

Critical review and revisions: MK, LS, ADB, AMD, VKS, RM, KWM, JCG, CRL, CAM, MS, TP, MV, AWA, PDS

Conflicts of Interest

Dr. Kohrmann reports advisory board, and speaker honoraria from Boehringer Ingelheim, Bayer, BMS/Pfitzer, Daichii Sankyo, Novartis, Amgen, Stryker, Medtronic and unrestricted research grant from Boeheringer Ingelheim, unrelated to current work. Dr. Tsivgoulis reports advisory board, and speaker honoraria from Boehringer Ingelheim, Bayer, Daichii Sankyo, Medtronic, Shire, CSL Behring, Biogen and unrestricted research grant from Medtronic unrelated to current work. Dr. Fiebach has received consulting, lecture, and advisory board fees from BioClinica, Cerevast, Artemida, Brainomix, and Merck as well as a grant from the German Federal Ministry of Education and Research (01EO0801 and 01EO01301). As PI he receives funding from the European Union Seventh Framework Program [FP7/2007–2013] under grant agreement no. 278276 (WAKE-UP). JBF is holding European Patent No 17179320.01-1906. Dr. Demchuk reports grants from Cerevast during the conduct of the study. Dr. Mikulik report grants from Project no. LQ1605 during the conduct of the study. Dr.

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Figure 1: Trial profile

Figure 2: Modified Rankin Scale Scores at 90 Days in patients treated with intravenous thrombolysis within 3 h

Analysis is in the 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.

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 analyzed according to ordinal logistic regression after collapsing mRS scores 5 and 6 and adjusting for age, NIHSS score at baseline; time from stroke onset to tPA (tissue plasminogen activator) bolus and baseline serum glucose across the different prespecified subgroups. The thresholds for age and National Institutes of Health Stroke Scale (NIHSS) score (range, 0 to 42, with higher scores indicating more severe neurologic deficits) were chosen at the median. The threshold for time from stroke onset to tPA (tissue plasminogen activator) bolus was pre-specified.

Tables

Table 1. Baseline characteristics of the study population (intention to treat analysis).

Variables	Intervention (n=335)	Control (n=341)
Mean age±SD, years	67.0±10.6	66.7±10.6
Male sex, no (%)	187 (55.8%)	206 (60.4%)
Median NIHSS-score (IQR), points	15 (11-18)	14 (11-18)
Hypertension, no (%)	196 (58.5%)	213 (62.5%)
Diabetes mellitus, no (%)	75 (22.4%)	80 (23.5%)
Atrial fibrillation, no (%)	62 (18.5%)	54 (15.8%)
Pre-stroke modified Rankin Scale score 0-1, no (%)	334 (99.7)	339 (99.4)
Mean systolic blood pressure before tPA-bolus±SD, mmHg*	150.3±20.2	150.3+20.4
Mean diastolic blood pressure before tPA-bolus±SD, mmHg**	81.7±13.2	81.8±13.2
Mean serum glucose before tPA-bolus±SD, mg/dL	139.6±53.0	137.5±53.4
Median time from symptom onset to tPA bolus (IQR), min	117 (95-156)	126 (96-165)
Time from symptom onset to tPA bolus within 3 h, no (%)	279 (83.3%)	285 (83.6%)
Median time from symptom onset to headframe activation (IQR), min	136 (117-175)	148 (115-185.5)
Mean Time from IV rtPA bolus to Head Frame Activation ± SD, min	20.6 ±9.7	19.7±10.3
Median Time from IV rtPA bolus to Head Frame Activation (IQR) min	20 (13-27)	20 (13-25)
Race		
White	261	270
Black/African-American	18	17
Hispanic-Latino	37	33
Asian	12	13
South-Asian/Indian	0	1
Filipino	0	1
American-Indian/Alaskan Native	0	2
Unknown	7	4

Table 2. Primary and secondary efficacy outcomes in the intention-to-treat population (335 & 341 patients in the intervention & control groups). A total of 297 and 296 patients from the intervention and the control groups were included in the analysis of US primary outcome.

Variables	Intervention (n=335)	Control (n=341)	Unadjusted OR (95% CI)	p	Adjusted OR (95%CI)	p
Primary outcome: mRS-score at 90 days (median, IQR)						
US Primary outcome	3.0 (1.0-4.0)	3.0 (1.0-4.0)	1.03 (0.76-1.40)	0.8440	1.05 (0.77-1.45)	0.7414
Global Primary outcome	3.0 (1.0-4.0)	3.0 (1.0-4.0)	1.00 (0.76-1.32)	0.9889	1.06 (0.80-1.42)	0.6732
Secondary outcomes						
mRS-Score at 7 days or Discharge US	3.0 (2.0-4.0)	4.0 (1.0-5.0)	1.03 (0.76-1.40)	0.8311	1.09 (0.80-1.50)	0.5791
mRS-Score at 7 days or Discharge Global	3.0 (2.0-4.0)	4.0 (1.0-5.0)	0.99 (0.75-1.31)	0.9698	1.10 (0.82-1.47)	0.5145
mRS-score at 90 days 0-1; US, no (%)	82 (32.2%)	78 (30.7%)	1.07 (0.73-1.55)	0.7747	1.16 (0.77-1.75)	0.4804
mRS-score at 90 days 0-1; Global, no (%)	96 (31.3%)	98 (32.0%)	0.97 (0.69-1.36)	0.8624	1.05 (0.73-1.52)	0.7867
mRS-score at 90 days 0-2; US, no (%)	127 (49.8%)	118 (46.5%)	1.14 (0.81-1.62)	0.4783	1.27 (0.85-1.89)	0.2404
mRS-score at 90 days 0-2; Global, no (%)	149 (48.5%)	142 (46.4%)	1.09 (0.79-1.50)	0.6278	1.25 (0.87-1.79)	0.2237
Independent functional outcome at 90 days; US, no (%)	96 (37.6%)	93 (36.6%)	1.04 (0.73-1.50)	0.8545	1.11 (0.76-1.63)	0.5768
Independent functional outcome at 90 days; Global, no (%)	113 (36.8%)	114 (37.2%)	0.98 (0.71-1.36)	0.9334	1.07 (0.75-1.51)	0.7178
Dramatic clinical recovery at 2 h; US, no (%)	58 (21.6%)	60 (21.7%)	0.99 (0.66-1.49)	>0.9999	0.99 (0.65-1.52)	0.9735
Dramatic clinical recovery at 2 h; Global, no (%)	60 (18.6%)	65 (19.7%)	0.93 (0.63-1.37)	0.7656	0.95 (0.63-1.43)	0.8012
Clinical recovery at 24 h; US, no (%)	83 (31.8%)	102 (37.6%)	0.77 (0.54-1.10)	0.1723	0.79 (0.54-1.15)	0.2222
Clinical recovery at 24 h; Global, no (%)	100 (31.9%)	116 (36.0%)	0.83 (0.60-1.16)	0.3148	0.88 (0.63-1.24)	0.4649
Neurological improvement at 24 h; US, no (%)	148 (56.7%)	154 (56.8%)	0.99 (0.71-1.40)	>0.9999	1.04 (0.73-1.49)	0.8339
Neurological improvement at 24 h; Global, no (%)	176 (56.2%)	180 (55.9%)	1.01 (0.74-1.39)	0.9365	1.08 (0.78-1.49)	0.6613
Neurological deterioration at 24 h; US, no (%)	23 (8.8%)	17 (6.3%)	1.44 (0.75-2.77)	0.3242	1.37 (0.70-2.71)	0.3627
Neurological deterioration at 24 h; Global, no (%)	29 (9.0%)	19 (6.2%)	1.63 (0.89-2.97)	0.1330	1.47 (0.80-2.75)	0.2162
NIHSS at Day 7 US (median, IQR)	5 (1-12)	6 (1-12)		0.7951		
NIHSS at Day 7 Global (median,	5 (1-12)	6 (1-12)		0.8167		

IQR)						
NIHSS at Day 90 US (median, IQR)	2 (0-6)	2 (0-5)		0.8413		
NIHSS at Day 90 Global (median, IQR)	2 (1-6)	2 (1-5)		0.6834		
Duration of hospital stay until discharge; US, days (median, IQR)	7 (5-12)	7 (4-11)		0.6033		
Duration of hospital stay until discharge; Global, days (median, IQR)	7 (5-12)	7 (4-11)		0.4772		

Table 3. Safety outcomes and serious adverse events within 90 days after randomization in the safety population (317 & 329 patients in the intervention & control groups).

Variables	Intervention (n=317)	Control (n=329)	OR (95% CI)	P	Adjusted OR (95%CI)	P
Death, no (%)	51 (16.7%)	44 (13.4%)	1.23 (0.79-1.90)	0.3726	1.19 (0.74-1.92)	0.4810
Death due to serious adverse event, no (%)	34 (10.7%)	34 (10.3%)	1.04 (0.63-1.72)	0.8985	1.00 (0.58-1.73)	0.9962
Serious adverse events, no (%)	83 (26.2%)	79 (24.0%)	1.12 (0.79-1.60)	0.5268	1.08 (0.74-1.57)	0.6890
Symptomatic intracranial hemorrhage at 24 h, no (%)	8 (2.5%)	6 (1.8%)	1.39 (0.48-5.06)	0.5974	1.43 (0.49-4.44)	0.5091
Symptomatic intracranial hemorrhage at 36 h, no (%)	9 (2.8%)	7 (2.1%)	1.34 (0.49-3.65)	0.6192	1.39 (0.51-3.95)	0.5227
Asymptomatic intracranial hemorrhage at 24 h, no (%)	34 (10.7%)	20 (6.1%)	1.86 (1.04-3.30)	0.0457	1.78 (0.98-3.31)	0.0609
Cerebral Edema, no (%)	17 (5.8%)	8 (2.4%)	2.27 (0.97-5.35)	0.0660	2.15 (0.93-5.40)	0.0839
Brain Herniation	11 (3.5%)	5 (1.5%)	2.33 (0.80-6.78)	0.1324	2.09 (0.73-6.87)	0.1877
Midline Shift	9 (2.8%)	9 2.7%)	1.04 (0.41-2.65)	>0.9999	0.98 (0.35-2.72)	0.9664
Study discontinuation due to adverse events, no (%)	21 (6.6%)	22 (6.7%)	0.99 (0.53-1.84)	>0.9999	1.01 (0.53-1.96)	0.9642
First most common adverse event (Headache), n (%)	57 (18.0%)	50 (15.2%)	1.22 (0.81-1.85)	0.3972	1.30 (0.85-2.00)	0.2275
Second most common adverse event (Pyrexia), n (%)	30 (9.5%)	37 (11.2%)	0.82 (0.50-1.37)	0.5192	0.81 (0.48-1.36)	0.4325
Third most common adverse event (Nausea), n (%)	33 (10.4%)	27 (8.2%)	1.30 (0.76-2.22)	0.3461	1.32 (0.77-2.29)	0.3126
Fourth common adverse event (Pneumonia/Aspiration Pneumonia), n (%)	34 (10.7%)	27 (8.2%)	1.34 (0.79-2.28)	0.2848	1.33 (0.76-2.36)	0.3159
Fifth most common adverse event (Constipation), n (%)	24 (7.6%)	33 (10.0%)	0.73 (0.42-1.27)	0.3315	0.69 (0.39-1.20)	0.1927
Atrial Fibrillation as adverse event, n (%)	28 (8.8%)	14 (4.3%)	2.18 (1.12-4.22)	0.0245	2.25 (1.17-4.52)	0.0181
Atrial Fibrillation as adverse event after exclusion of patients with atrial fibrillation at baseline, n (%)	23 (7.3%)	13 (4.0%)	1.90 (0.95-3.82)	0.0855	1.91 (0.96-3.97)	0.0722