Thrombolysis with tenecteplase in patients with wake-up stroke assessed by non-contrast CT (TWIST): a randomised, open-label trial with blinded endpoint assessment

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ABSTRACT

Background

Current evidence supports intravenous thrombolysis with alteplase in patients with wake-up stroke selected by magnetic resonance or perfusion imaging and is now recommended in clinical guidelines. Access to advanced imaging techniques is however often limited. We aimed to determine whether patients with wake-up stroke selected by non-contrast computed tomography (CT) benefit from thrombolytic treatment with tenecteplase.

Methods

Between June 2017 and Sept 2021, we conducted a multicentre, randomised, open trial with blinded endpoint-assessment of intravenous tenecteplase 0·25 mg/kg versus no thrombolysis in patients with ischaemic wake-up stroke assessed by non-contrast CT (EudraCT 2014-000096-80). The primary outcome was functional status at 90 days on modified Rankin Scale (mRS) scores (range, 0 [no disability] to 6 [death]).

Findings

The trial ended after 578 of the planned 600 patients had been enrolled, due to the Covid-19 pandemic and exhausted funding. The distribution of mRS scores showed no significant difference in functional outcome between treatment groups (adjusted OR 1·18, 95% CI 0·88-1·58; p=0·27). An mRS score of 0-1 occurred in 130 of 288 patients (45%) in the tenecteplase group and 111 of 290 patients (38%) in the control group. Symptomatic intracranial haemorrhage (sICH) occurred in 6 patients (2%) in the tenecteplase group and in 3 patients (1%) in the control group (adjusted OR 2·17, 95% CI 0·53-8·87; p=0·28).

Interpretation

In patients with wake-up stroke selected by non-contrast CT, there was no significant between-group difference in the primary functional outcome at 90 days. The number of sICH

was low in both treatment groups, similar to previous trials of thrombolysis in wake-up stroke patients selected by advanced imaging.

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Research in context

Evidence before this study

At the time we planned and started the study, no randomised controlled trials on thrombolytic treatment for wake-up stroke had been completed. A systematic review and meta-analysis of trials published before Sept 21, 2020 identified four randomised trials with 843 participants with stroke of unknown onset who were randomised to treatment with intravenous alteplase versus standard care or placebo. The patients were selected with perfusion-diffusion MRI, perfusion CT, or MRI with diffusion weighted imaging-fluid attenuated inversion recovery (DWI-FLAIR) mismatch. Intravenous alteplase resulted in higher rates of excellent functional outcome defined as a score of 0-1 on the modified Rankin Scale (mRS) at 90 days compared to control (47% vs. 39%), and a net benefit for all functional outcomes across the full range of the mRS. Based on these results, treatment with iv alteplase is now recommended in clinical guidelines for wake-up stroke patients with DWI/FLAIR or CT or MRI core/perfusion mismatch. In May 2021, we performed a Cochrane review where we searched the Cochrane Stroke Group Trials Register, the Cochrane Central Register of Controlled Trials, MEDLINE Ovid and Embase Ovid for randomised controlled trials of intravenous thrombolytic drugs versus control in people with acute ischaemic stroke presenting upon awakening from sleep. Strokes with unknown onset other than sleep were excluded. We identified five trials with 775 participants. Good functional outcome defined as mRS score 0-2 at 90 days follow-up was observed in 66% of participants randomised to thrombolytic treatment and 58% of participants randomised to control. Symptomatic intracranial haemorrhage occurred in 3% of participants in the thrombolysis group and 1% of participants in the control group. All trials were stopped early and had limited sample size, and all trials used advanced imaging for selection of patients. As access to advanced imaging is not universally available, treatment

decisions based on non-contrast CT criteria may be an alternative, but this has not been tested in randomised controlled trials. A previous systematic review and meta-analysis of controlled trials, observational cohort studies and single-arm safety studies conducted in 2021, showed no significant difference in functional or safety outcomes between studies that evaluated patients with wake-up stroke with non-contrast CT, MRI, or CT perfusion prior to treatment. Previous randomised studies of patients with known onset stroke found tenecteplase to be safe and at least as effective as alteplase, but the effect of tenecteplase in wake-up stroke has not been evaluated in randomised controlled trials.

Added value of this study

TWIST adds information to previous trials of thrombolytic treatment of wake-up stroke in that it differs with regards to both thrombolytic agent and imaging technique. It is the largest randomised controlled trial of thrombolytic treatment in patients with wake-up stroke. The participants were randomised to treatment with intravenous tenecteplase or standard care (no thrombolysis), based on findings on non-contrast CT. Treatment with tenecteplase within 4·5 hours of awakening was not significantly associated with better functional outcome at end of follow-up. Excellent functional outcome (mRS 0-1) was attained by 45% in the tenecteplase group and 38% in the control group. The risk of death and of symptomatic and any intracerebral haemorrhage was insignificantly higher in the tenecteplase group than in the control group and similar to previous meta-analyses of trials with selection based on advanced imaging.

Implications of all the available evidence

Treatment with tenecteplase in patients with wake-up stroke after screening with non-contrast CT was not found to be superior to standard care (no thrombolysis), although a numerically

higher proportion of patients achieved mRS 0-1. The safety profile of TWIST was similar to previous trials of treatment with alteplase in patients selected by advanced MRI or CT perfusion imaging.

Introduction

Results from recent trials support the use of intravenous thrombolysis with alteplase in patients with ischaemic stroke of unknown time of onset who presented with magnetic resonance imaging (MRI) findings of an ischaemic lesion on diffusion-weighted imaging (DWI) and absence of visible hyperintense signal in the corresponding region on fluid-attenuated inversion recovery (FLAIR) series (DWI/FLAIR mismatch) or findings indicating presence of salvageable tissue on CT or MRI perfusion imaging.[1-5]

Limited access to MRI or perfusion imaging in the emergency setting may prevent patients with stroke upon awakening, wake-up stroke, from receiving reperfusion treatments. Patients with an acute ischaemic lesion detected by DWI but not on FLAIR imaging are likely to be within a time window for which thrombolysis is safe and effective. However, such DWI/FLAIR mismatch was absent in 40% of patients with known stroke duration of less than three hours,[6] indicating that selection of patients based on this criterion could exclude patients with wake-up stroke who might benefit from thrombolysis. Approximately two thirds of patients who underwent screening for inclusion in the largest trial on thrombolytic treatment in wake-up stroke to date, the WAKE-UP trial (the Efficacy and Safety of MRI-Based Thrombolysis in Wake-Up Stroke), were excluded mainly due to lack of DWI/FLAIR mismatch criteria.[1] Non-contrast CT has low sensitivity for quantification of infarct core compared with DWI or CTP and may thus comprise safety if applied for selection of wake-up stroke patients to thrombolytic treatment.[7] Non-contrast CT was however found to be safe for selection for wake-up stroke patients to thrombolytic treatment in two prospective, single-armed open-label trials.[8,9] CT is widely available in stroke centres.

Tenecteplase has higher fibrin specificity, longer half-life and simpler single-bolus administration compared to alteplase.[10] Recent systematic reviews suggest that tenecteplase 0·25 mg/kg is non-inferior to alteplase with regard to functional outcome after acute ischaemic stroke,[10,11] and superior at increasing reperfusion rate.[12] We conducted the Tenecteplase in Wake-up Ischaemic Stroke Trial (TWIST) to determine whether thrombolytic treatment with intravenous tenecteplase given within 4.5 hours of awakening improves functional outcome in patients with ischaemic wake-up stroke selected by use of non-contrast CT.

Methods

Study design

TWIST was an investigator-initiated, multicentre, prospective, randomised, controlled, open-label trial of tenecteplase 0·25 mg/kg bodyweight in patients with acute ischaemic stroke upon awakening, with blinded end-point assessment. The trial was conducted at 77 centres in 10 countries from June 12, 2017 to September 30, 2021. Methods of the trial have been published previously.[13]

The trial protocol was approved by national regulatory authorities in each participating country and by national and/or local ethics committees and/or institutional review boards. Patients or their legal representatives provided written informed consent according to national and local regulations. Members of the trial coordinating centre and steering committee designed the trial and met on a regular basis to oversee the conduct of the trial. The TWIST Investigators collected the data (listed in the Supplementary Appendix). The trial was overseen by the trial steering committee and an independent data monitoring committee. The

authors vouch for the accuracy and completeness of the data and adverse event reporting and for the fidelity of the trial to the protocol. The trial was conducted in accordance with the MRC Guidelines for Good Clinical Practice in Clinical Trials, the Council of Europe's Convention on Human Rights and Biomedicine, the ICH Harmonized Tripartite Guideline for Good Clinical Practice and the Declaration of Helsinki. The trial is registered in the EudraCT (no. 2014-000096-80), ClinicalTrials.gov (no. NCT03181360) and ISRCTN (no. 10601890) databases.

The trial was monitored by monitors affiliated with the Norwegian clinical research infrastructure network and with the University of Leicester, with onsite or online visits at initiation, during and at the end of the trial. Visits included confirmation of the existence of each patient, that documentation of consent procedure, confirmation of diagnosis, source documentation for the primary efficacy and safety outcomes, and review of all serious adverse events. Complete review of all of source data was done in selected trial subjects at each site.

Patients

The trial was carried out at 77 hospitals (listed in the Supplementary Appendix) in Denmark, Estonia, Finland, Latvia, Lithuania, New Zealand, Norway, Sweden, Switzerland, and the United Kingdom. Patient eligibility was assessed by the treating physician and required that patients were 18 years of age or older, had stroke symptoms upon awakening that were not present before sleep, with limb weakness and a National Institutes of Health Stroke Scale (NIHSS) score ≥3, or aphasia, and could be treated with tenecteplase within 4·5 hours of awakening.

Patients with intracranial haemorrhage or infarct comprising hypoattenuation in more than 1/3 of the middle cerebral artery territory on acute non-contrast CT were excluded to avoid inclusion of patients with a large infarct core who are at higher risk of intracerebral haemorrhage and less likely to benefit from treatment. The safety of this criterion was tested and found to be good in two single-arm, prospective, open-label safety trials of thrombolysis with alteplase in wake-up stroke patients.[8,9] The criterion is commonly used for selection of patients with known-onset stroke to treatment and is a method that stroke physicians are well trained to apply. A complete list of the inclusion and exclusion criteria is provided in the Supplementary Appendix.

Randomisation and masking

Patients were randomly assigned to either intravenous tenecteplase or control in a 1:1 allocation ratio using a central web-based computer-generated randomisation schedule. The schedule employed a minimisation algorithm that balanced age (under vs. at or above 80 years), NIHSS severity (under vs. at or above 15 points) and time since wake-up (under vs. at or above 3 hours). The dose of tenecteplase was 0·25 mg per kg of body weight (maximum 25 mg), given as a single intravenous bolus and was based on results from previous trials of tenecteplase for stroke with known symptom onset.[14] Weight was assessed according to local routine practice for thrombolysis of stroke patients. Patients randomised to control were to receive standard care, but not tenecteplase or any other thrombolytic agent. Thrombectomy was allowed in both treatment groups.

Procedures

Clinical assessments were performed on day 1 (at baseline) and day 7 (or on the day of discharge, whichever occurred first). A non-contrast CT examination of the head was a prerequisite for inclusion into the trial and was repeated after 24 hours. CT angiography and/or perfusion was recommended, if possible, but was not mandatory, and if undertaken was to be repeated within 24 hours in patients with large-vessel occlusion upon admission. While not part of the study protocol, supplemental brain imaging (MRI or perfusion imaging) was allowed but discouraged if it delayed randomization for more than 20 minutes. Centralised, blinded reading of all available images was used to assess acute ischaemic changes at baseline and at 24 (± 6) hours according to the Alberta Stroke Project Early CT Changes Score (ASPECTS),[14] cerebral artery patency and intracranial haemorrhage.

Outcomes

Outcome data at 90 days were collected through centralised standardised telephone interviews and was performed in each country by trained research personnel blinded to treatment allocation. The primary outcome was functional outcome assessed by the modified Rankin Scale (mRS) at 90 days (ordinal scale). The mRS ranges from 0 to 6, with 0 indicating no neurologic deficit, 1 no clinically significant disability (return to all usual activities), 2 slight disability (able to handle own affairs without assistance but unable to carry out all previous activities), 3 moderate disability requiring some help (e.g., with shopping, cleaning, and finances but able to walk unassisted), 4 moderately severe disability (unable to attend to bodily needs without assistance and unable to walk unassisted), 5 severe disability (requiring constant nursing care and attention), and 6 death. Secondary effect outcomes were excellent functional outcome defined as mRS score of 0-1, good functional outcome defined as mRS score of 0-2 and response to treatment according to neurological deficit at study entry defined as mRS score of 0 for patients with mild deficits (NIHSS <=7), 0-1 for patients with moderate

deficits (NIHSS 8-14) and 0-2 for patients with severe deficits (NIHSS >14]). Other secondary outcomes were EuroQol score (EQ-5D-VAS), mini-mental state examination (MMSE, telephone version) and Barthel Index score at 90 days.

Safety outcomes were death from all causes at 90 days and symptomatic intracranial haemorrhage (by SITS-MOST [Safe Implementation of Thrombolysis in Stroke Monitoring Study][15] and IST-3 [the Third International Stroke Trial][16] definitions), parenchymal haemorrhage type 2,[17] any intracranial haemorrhage, and major extracranial bleeding. An independent endpoint adjudication committee whose members were unaware of trial group assignment, adjudicated prespecified serious adverse events including secondary safety outcomes based on source data provided by the participating centres. Images were assessed with standardised case-report forms by an imaging committee whose members were unaware of clinical data except for date and time of image acquisition.

Statistical analysis

The original sample size estimation resulted in a target sample size of 500 patients and was based on the results of a Cochrane systematic review of the effect of rt-PA within 4.5 hours of stroke onset, which showed an absolute risk reduction of 9% in the thrombolysis group for the binary endpoint mRS 0-2 versus mRS 3-6.[18] As there were concerns about whether the assumptions for the sample size estimations would hold, the Trial Steering Committee in June 2020 decided to undertake a revised sample size calculation. As the primary endpoint in TWIST was mRS across the full ordinal scale (shift analysis), the revised sample size estimation was based on ordinal logistic regression analysis where the distribution of scores on the mRS in the control group of the WAKE-UP trial was used as reference.[1] We assumed a treatment effect with odds ratio (OR) of 1·50, corresponding to an absolute difference of

approximately 10% between the trial groups in the percentage of patients achieving a mRS score of 0 to 1 at 90 days. A sample size of 600 patients would provide 80% power to detect a true treatment effect at alpha level 0·05 (Supplementary Methods in Supplementary Appendix). A detailed statistical analysis plan was made publicly available before the database was locked.[19]

Baseline characteristics are presented for the tenecteplase and control groups. Discrete variables are summarised as frequencies and percentages. Unless otherwise indicated, percentages were calculated according to the number of patients for whom data are available. For variables with more than 5% missing values, the percentage with missing values is added as a footnote to the corresponding summary table. Continuous variables are summarised as mean and standard deviation, or median and interquartile range (IQR). Time intervals are summarised as median and IQR.

The primary analysis compared functional outcome between the study groups by means of ordinal logistic regression adjusted for age, stroke severity (baseline NIHSS score) and time from wake-up to randomisation in the intention-to-treat population. The primary effect was determined by the common OR with 95% confidence intervals (CI), for a shift in the direction of improved outcome on the mRS scale in the tenecteplase group. Assessment of proportionality with the approximate likelihood-ratio test of proportionality of odds was not significant.

Secondary and safety outcomes were compared between treatment groups by means of binary logistic regression for dichotomous outcomes to estimate OR with corresponding 95% CIs.

Cox proportional hazard regression was used to calculate hazard ratios (HR) and corresponding 95% CI for death during follow-up. The primary and secondary analyses were adjusted for age, baseline NIHSS score and time from wake-up to randomisation.

As all subgroup analyses are of exploratory nature, no adjustment for multiple comparisons was made. The 2-way interactions between treatment groups (tenecteplase or control) and the pre-defined demographic and clinical variables on the primary outcome were explored through multivariable ordinal logistic regression for the primary outcome, adjusted for age, baseline NIHSS score, and time from wake-up to randomisation. For each treatment-by-subgroup interaction a likelihood ratio test was used with appropriate degrees of freedom. If information about the mRS score at 90 days was missing, we used the level of function recorded on day 7 after randomisation or at discharge from hospital to impute functional status at 90 days.

Pre-specified sensitivity analyses were performed for the "per protocol" population, defined as those who actually received their allocated treatment (crossovers excluded), and in the "complete case" population, where patients with missing information on the primary endpoint were excluded (no imputation). Additional pre-specified sensitivity analyses of safety outcomes were undertaken in the "safety population", where patients in the control group who received tenecteplase were assigned to the tenecteplase group while other patients who did not receive their allocated treatment were excluded. A separate set of analyses was performed stratified by patients who received endovascular treatment and those who did not. We further present unadjusted analysis, as well as adjusted analysis taking clustering effects by country

and centre into account in mixed effect ordered logistic regression models. All analyses were performed using SAS software version 9·4 (SAS Institute).

Role of the funding sources

The funders of the trial had no role in the study design, data collection, data analysis, data interpretation, or writing of the report.

Results

From June 2017 through September 2021, 578 of the target of 600 patients were included. Further extension of the trial was not found feasible due to markedly reduced enrolment rate after the onset of the Covid-19 pandemic, a halt in the production of tenecteplase in 2021, and exhausted funding. Of the 578 patients, 288 were randomised to receive tenecteplase and 290 to the control group (Figure 1). Five patients allocated to tenecteplase did not receive the assigned treatment, and six patients allocated to the control group received thrombolysis (Table S1 in the Supplementary appendix). Thirteen of the included patients had ischemic lesions comprising more than 1/3 of the middle cerebral artery territory as judged by the centralised assessment of images, of whom 8 received tenecteplase. Sixteen patients who were alive at 90 days were lost to follow-up, eight (2·8%) in the tenecteplase group and eight (2·8%) in the control group.

Baseline and clinical characteristics were similar between groups, except for a higher proportion with atrial fibrillation in the tenecteplase group than in the control group (20·6% vs 11·4%). The proportion with intracranial large vessel occlusion was higher in the control group (36·7%) than in the tenecteplase group (29·9%), as were the proportion who were

treated with endovascular interventions (14·5% in the control group vs 6·3% in the tenecteplase group) (Table 1).

Treatment with tenecteplase was not associated with better functional outcome for the primary outcome assessed as a shift in the score on the modified Rankin scale at 90 days (adjusted common OR ratio 1·18, 95% CI 0·88-1·58; p=0·27) (Table 2). The median score on the mRS was 2 in both treatment groups. The proportion with an excellent clinical outcome, defined as mRS score 0 or 1, was 45·1% in the tenecteplase group and 38·3% in the control group (adjusted OR 1·34, 95% CI 0·95-1·88; p=0·09) (Figure 2, Table 2). There was no difference in good functional outcome between treatment groups, defined as mRS score 0 to 2 (adjusted OR 1·07; 95% CI 0·75 to 1·54; p=0·70). Response to treatment according to neurological deficit at study entry was attained by 24·3% in the tenecteplase group and 19·3% in the control group (adjusted OR 1·35, 95% CI 0·91-2·02; p=0·14). The median scores on the MMSE, Barthel Index and the EQ-5D-VAS at 90 days follow-up were similar in the treatment groups, as were the NIHSS score at 24 hours and 7 days and the median change in NIHSS from baseline to 24 hours and to 7 days (Table 3). Neurological deterioration from index ischemic stroke occurred in 17 patients (5·9%) in the tenecteplase group and 20 patients (6·9%) in the control group, while recurrent ischaemic stroke occurred in 4 (1·4%) and 2 (0·7%) patients in the tenecteplase and control group, respectively (Table 4).

Unadjusted analyses for the primary and secondary outcomes are presented in Table 2 and do not differ substantially from the prespecified primary multivariable adjusted analyses. Similar results were found in sensitivity analyses based on the “per protocol” (Table S2), “complete case” (Table S3), and “safety” populations (Table S4). In mixed effect ordered logistic regression models taking clustering effects by country into account, the adjusted OR for the

primary outcome was 1·25 (95% CI 0·93 to 1·68; p=0·14) and 1·41 (95% CI 0·99-2·01; p=0.054) for the excellent outcome. Similar results were found in models adjusted for clustering effects by centre. Subgroup analyses did not show any consistent interactions between trial group and subgroups, except for previous stroke, where the treatment effect tended to be better in patients without previous stroke (Figure S1 and Table S5 in the Supplementary Appendix). The point estimates for patients with large vessel occlusions treated with thrombectomy was lower than for patients not treated with thrombectomy, although without significant interaction.

There was no significant difference between treatment groups in the proportion of deaths within 90 days after treatment (9·7% in the tenecteplase group and 7·9% in the control group, adjusted HR 1·29, 95% CI 0·74-2·26; p=0·37) (Table 2, Figure 3). The proportion with poor functional outcome defined as mRS of 4 (moderately severe disability), 5 (severe disability) and 6 (dead) was 18·4% in the tenecteplase group and 20% in the control group (adjusted HR 0·90; 95% CI 0·56-1·43).

Symptomatic intracranial haemorrhage according to the SITS-MOST definition occurred in 6 patients treated with tenecteplase and in 3 controls (adjusted OR 2·17, 95% CI 0·53-8·87, p=0·28). The corresponding numbers for the IST-3 definition were 12 and 8 (adjusted OR 1·54, 95% CI 0·62-3·82; p=0·36). The results were similar in sensitivity analyses of the "safety population" (Table S4 in the Supplementary Appendix). Three of the 9 symptomatic intracranial haemorrhages as defined by the SITS-MOST criteria and 8 of the 20 defined according to the IST-3 definition occurred in patients treated with thrombectomy (Table S6 in the Supplementary Appendix). Three patients in each treatment group had fatal intracranial haemorrhage. None of the patients with ischemic lesions comprising >1/3 of the middle

cerebral artery territory who were treated with tenecteplase arm experienced symptomatic intracranial haemorrhage. Details of all serious adverse events are reported in the Supplementary Appendix, Table S7.

Discussion

Among patients who presented with acute ischaemic stroke upon awakening selected by non-contrast CT, ordinal analysis of the primary endpoint did not show significantly better functional outcome in patients treated with tenecteplase within 4·5 hours of awakening compared to control. Treatment with tenecteplase resulted in a non-significantly higher percentage with excellent functional outcome defined as a mRS score of 0-1 at 90 days.

The WAKE-UP trial was the first randomised controlled trial to show benefit from thrombolytic treatment with alteplase in patients with stroke of unknown time of onset. Imaging criteria in that trial included MRI findings of an ischaemic lesion on DWI sequences and absence of visible hyperintense signal in the corresponding region on FLAIR series (DWI/FLAIR mismatch).[1] Following the publication of the results from WAKE-UP, three ongoing trials were terminated early. While a positive effect in favour of thrombolysis was found in the EXTEND (Extending the Time for Thrombolysis in Emergency Neurological Deficits) trial,[2] THAWS (THrombolysis for Acute Wake-up and unclear- onset Strokes with alteplase at 0·6 mg/kg trial)[20] and ECASS-4 (European Cooperative Acute Stroke Study 4)[3] were neutral. The difference of 6·8% between patients achieving excellent outcome in the tenecteplase and control groups in our trial is similar to the observed difference between treatment groups seen in a meta-analysis of patients treated within 3 to 4·5 hours of known

symptom onset[21] and in an individual participant meta-analysis of WAKE-UP, THAWS, EXTEND and ECASS-4.[4]

There was no significant difference between treatment groups in the risk of death at 90 days. The overall percentage who died within 90 days was higher in our study (9·7% in the tenecteplase group and 7·9% in controls) than in the WAKE-UP trial (4·1% and 1·2% in the alteplase and placebo group, respectively) which may probably be explained by WAKE-UP patients being on average 8 years younger than TWIST patients. For comparison, in a meta-analysis of patients treated with alteplase within 4·5 hours of known symptom onset and mean age of 71 years, the overall mortality at 90 days was 18·8% in the treatment group and 18.0% in the control group.[21] There were numerically more symptomatic intracranial haemorrhages in the tenecteplase group than in controls, which is in line with results from previous trials of stroke patients with known symptom onset where thrombolysis with alteplase was associated with significantly increased risk of symptomatic intracranial haemorrhage and fatal intracranial haemorrhage within the first week.[21] The proportions of symptomatic haemorrhage and any intracranial haemorrhage were low in both treatment groups, similar to the combined results from WAKE-UP, THAWS, EXTEND and ECASS-4 (3% in the alteplase group versus 1% in the control group).[4]

Our trial adds information to previous trials as it differs with regards to both thrombolytic agent and imaging technique. Tenecteplase has several pharmacological advantages, including longer free plasma half-life, allowing easy and quick administration as one single intravenous bolus dose.[22] Previous studies found tenecteplase at dosage 0·25 mg/kg (to a maximum of 25 mg) to be safe and at least as effective as alteplase.[23-25] We chose non-contrast CT imaging as

a screening tool for selection of patients. Previous randomised controlled trials used either DWI/FLAIR mismatch techniques or CT or MR perfusion imaging for selection of patients, with the underlying assumption that these techniques are more likely to identify patients with salvageable tissue or short duration of ischaemia. However, knowledge on which imaging techniques that are most likely to identify patients who will benefit from treatment is limited. The sensitivity of MRI DWI/FLAIR mismatch for identifying patients with short duration of cerebral ischaemia in patients with known stroke duration of less than three hours is low,[6] selection of patients based on MRI DWI/FLAIR mismatch criteria may thus exclude a substantial proportion of patients who might benefit from thrombolysis. Lacunar infarcts, which are shown to benefit from thrombolysis,[26] will not be detected by perfusion imaging.[4] Furthermore, MRI or perfusion imaging are not universally available and access is often limited even in hospitals which have the necessary equipment. CT scanners are, in turn, more widely available and are used for acute stroke imaging in everyday practice. Limited observer agreement with regards to recognising and quantifying early ischemic changes on non-contrast CT is a concern when applying this imaging approach.[27] In the present trial, none of the eight patients with more extensive ischaemic lesion than 1/3 of the middle cerebral artery territory and who were treated with tenecteplase experienced symptomatic intracranial haemorrhage. Our trial adds evidence to a previous systematic review and meta-analysis of controlled trials, observational cohort studies and single-arm safety studies which showed no significant difference in functional or safety outcomes between studies that evaluated patients with non-contrast CT, MRI, or CT perfusion prior to treatment.[28]

Our trial has several limitations. Based on a revised power estimation, the recruitment target was increased from 500 to 600 patients. The revised estimation assumed a treatment effect of 10% absolute difference in a binary endpoint setting (mRS 0 to 1 versus 2 to 6) and a

distribution between mRS categories similar to that of the WAKE-UP trial. In light of the actual results, it is evident that the estimated treatment effect was too optimistic and that the trial as a consequence was underpowered. In addition, we did not reach our inclusion target, mainly due to a marked slowdown in recruitment after the onset of the Covid-19 pandemic and concurrent temporary halt in the production of tenecteplase in 2021. This limits the interpretation of our results. The limited number of participants precluded analyses stratified by sex.

The higher percentages in the control group of patients with large vessel occlusion and treated with thrombectomy might have further attenuated the results. Patients undergoing thrombectomy were not included in previous studies assessing the effect of thrombolytic treatment in wake-up stroke patients.[2] We found no treatment effect of tenecteplase in the sub-group of patients undergoing thrombectomy. This is not unexpected given the large recanalisation effect of thrombectomy and the relatively small additional effect of thrombolysis shown in recent studies.[29,30] The relatively long median door-to-needle time of 56 minutes was not optimal.

The results from WAKE-UP and other trials of thrombolytic treatment with alteplase in selected wake-up stroke patients with MRI DWI/FLAIR mismatch or perfusion imaging criteria were published during the trial period. Unfortunately, we do not have complete screening log information nor systematic information on access to advanced imaging in the participating hospitals to explore whether this has affected recruitment to the trial. We cannot exclude that increased use of MRI or perfusion imaging may have led to more patients being enrolled who did not have imaging signs indicative of salvageable tissue or short duration of symptoms, while those who fulfilled such criteria were treated outside the trial. The

publication of trials showing benefit of thrombectomy in the extended time window may have led to a lower recruitment rate of patients with more severe strokes.

In conclusion, treatment with tenecteplase was not significantly associated with better functional outcome at 90 days of follow-up. The number of symptomatic intracranial haemorrhages was numerically higher in the tenecteplase group, in line with results from previous trials of stroke patients with known symptom onset and with wake-up stroke.[4,17] However, compared to previous trials, the number of symptomatic haemorrhages and any intracranial haemorrhages was low in both treatment groups, indicating that selection by NCCT did not lead to inclusion of stroke patients with increased risk of haemorrhage.

Contributors

TW did the statistical analysis, with input from AE and EBM. MBR, AE and EBM wrote the first draft of the article. All authors contributed to the collection of data and to the writing of the manuscript, had full access to all the data in the study, and had final responsibility for the decision to submit for publication. TW, AE and EBM have accessed and verified the data.

Declaration of interests

MBR reports a grant from the Norwegian National Association for Public Health. HC reports grants from the Velux-foundation, Helse-fonden and Tværs-fonden, personal speaker honoraria from Bayer and BMS, and personal fees from American Heart Association. SE reports advisory board compensation from Boehringer Ingelheim and Bayer, and grants from Daiichy-Sankyo. JK reports personal fees and non-financial support from Boehringer Ingelheim, Servier and Pfizer. JP reports grants from the Swedish ALF and the Southern Healthcare Region. JPu reports personal fees from Boehringer Ingelheim, Portola, Herantis

Pharma and Terve Media, personal fees, speakers honorary, advisory board, and research grant from BMS-Pfizer, Bayer and Abbott/St. Jude Medical, research collaboration, and stock ownership from Vital Signum, and grants from Business Finland and Amgen. DJW reports speaking honoraria from Bayer, Portola, and NovoNordisk; and consultancy fees from Alnylam, Portola, Alexion, and NovoNordisk. EBM reports grants from the Norwegian Clinical Therapy Research in the Specialist Health Services Research Programme and from the Northern Norway Regional Health Authority.

Data sharing

De-identified data collected for the study and presented in this manuscript, including individual participant data and a data dictionary defining each field in the set, will be made available to others upon reasonable request upon publication of this article, provided approval by regulatory authorities. Data can be requested by sending an e-mail to the corresponding author. Study protocol and statistical analysis plan are available on the trial's website.

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We are thankful and indebted to Professor Eivind Berge who had a central role in the initiation, planning and implementation of the trial as trial co-coordinating investigator until his death in February 2020.

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Table 1. Characteristics of patients at baseline (intention-to-treat population)*

	Tenecteplase (n=288)	Control (n=290)
Age, years		
Mean ± SD	72·7 ± 11·3	72·9 ± 11·6
Median (IQR)	73·9 (66·4 - 80·8)	73·3 (65·8 - 82·0)
Age groups, no.(%)		
<60 years	44 (15·3)	40 (13·8)
60-80 years	164 (56·9)	168 (57·9)
>80 years	80 (27·8)	82 (28·3)
Male sex, no. (%)	164 (56·9)	168 (57·9)
Country, no. (%)		
Norway	75 (26·0)	82 (28·3)
Sweden	22 (7·6)	26 (9·0)
Denmark	18 (6·3)	15 (5·2)
Finland	18 (6·3)	14 (4·8)
Estonia	8 (2·8)	12 (4·1)
Latvia	6 (2·1)	5 (1·7)
Lithuania	45 (15·6)	29 (10·0)
United Kingdom	82 (28·5)	83 (28·6)
Switzerland	8 (2·8)	16 (5·5)
New Zealand	6 (2·1)	8 (2·8)
Final diagnosis at discharge, no. (%)		
Definite ischaemic stroke	258 (89·6)	260 (89·7)
Probable ischaemic stroke	18 (6·3)	14 (4·8)
Other diagnosis (stroke mimic)	12 (4·2)	16 (5·5)
Stroke risk factors and medical history, no. (%)		
Hypertension	176 (63·8)	177 (63·4)
Diabetes mellitus	55 (19·8)	52 (18·5)
Atrial fibrillation	55 (20·6)	31 (11·4)
Active smoker	51 (21·3)	46 (20·1)
Previous stroke or TIA	75 (27·1)	60 (21·9)
Coronary artery disease	43 (16·6)	43 (15·8)
Current use of an anticoagulant agent	11 (3·9)	10 (3·5)
Current use of an antiplatelet agent	101 (36·3)	88 (31·0)
Pre-morbid modified Rankin Scale – no. (%)		
0	188 (65·3)	191 (65·9)
1	63 (21·9)	57 (19·7)
2	37 (12·8)	42 (14·5)
Median NIHSS† score (IQR)	6 (5 – 11)	6 (5 – 10)
NIHSS† score, no. (%)		
Mild (0-7)	171 (59·4)	176 (60·7)
Moderate (8–14)	80 (27·8)	77 (26·6)
Severe (≥15)	37 (12·8)	37 (12·8)
Median ASPECT‡ score (IQR)	10 (10 - 10)	10 (9 - 10)
ASPECT‡ score, n (%)		
10	220 (77·4)	195 (69·6)
8-9	47 (16·6)	59 (21·1)
6-7	10 (3·5)	22 (7·9)
0-5	7 (2·5)	4 (1·4)
Large vessel occlusion§	69 (29·9)	83 (36·7)
Endovascular treatment, no. (%)	18 (6·3)	42 (14·5)
Median time from last known to be well to randomisation (IQR), min	652 (553 - 774)	653 (524 - 755)
Median time from wake-up to hospital admission (IQR), min	112 (75 - 160)	110 (80 - 150)
Median time from wake-up to randomisation (IQR), min	173 (126 - 217)	175 (126 - 220)
Median time from hospital arrival to start of thrombolysis (IQR), min	56·0 (43·0 - 80·0)	NA

*Plus-minus values are means ±SD. IQR denotes interquartile range. NA; not applicable. There were more than 5% missing values for the following variables: Coronary heart disease 8%, atrial fibrillation 7%, smoking 19%, large vessel occlusion 21%.

†NIHSS=National Institutes of Health Stroke Scale

‡ASPECT score=The Alberta Stroke Project Early CT Changes (ASPECT) score

§Large-vessel occlusion was defined as occlusion of the internal carotid artery, first division of the middle cerebral artery (M1), and proximal portion of the second division of the middle cerebral artery (M2). The diagnosis was based on CT angiography in 455 patients and on MR angiography in 2 of the 457 patients where this information was available.

Table 2. Efficacy and safety outcomes (intention-to-treat population)*

Outcome	Tenecteplase (n=288)	Control (n=290)	Unadjusted Effect Size (95% CI)†	P Value	Adjusted Effect Size (95% CI)†	P Value
Primary efficacy outcome						
Score on the modified Rankin scale at 90 days						
0	40 (13·9)	32 (11·0)				
1	90 (31·3)	79 (27·2)				
2	47 (16·3)	62 (21·4)				
3	58 (20·1)	59 (20·3)				
4	19 (6·6)	27 (9·3)				
5	6 (2·1)	8 (2·8)				
6	28 (9·7)	23 (7·9)				
Functional improvement‡			1·18 (0·89, 1·58)	0·26	1·18 (0·88, 1·58)	0·27
Secondary efficacy outcomes						
Excellent functional outcome at 90 days§	130 (45·1)	111 (38·3)	1·33 (0·95, 1·85)	0·10	1·34 (0·95, 1·88)	0·09
Good functional outcome¶	177 (61·5)	173 (59·7)	1·08 (0·77, 1·51)	0·66	1·07 (0·75, 1·54)	0·70
Response to treatment according to baseline neurological deficit**	70 (24·3)	56 (19·3)	1·34 (0·90, 2·00)	0·15	1·35 (0·91, 2·02)	0·14
Safety outcomes						
Death within 90 days after intervention	28 (9·7)	23 (7·9)	1·29 (0·74, 2·26)	0·37	1·29 (0·74, 2·26)	0·37
Symptomatic intracranial haemorrhage						
- as defined by SITS-MOST††	6 (2·1)	3 (1·0)	2·04 (0·50, 8·22)	0·09	2·17 (0·53, 8·87)	0·28
- as defined by IST-3‡‡	12 (4·2)	8 (2·8)	1·53 (0·62, 3·81)	0·36	1·54 (0·62, 3·82)	0·36
Parenchymal haemorrhage type 2§§	7 (2·4)	5 (1·7)	1·42 (0·45, 4·53)	0·55	1·47 (0·46, 4·73)	0·51
Any intracranial haemorrhage	33 (11·5)	30 (10·3)	1·12 (0·66, 1·89)	0·67	1·14 (0·67, 1·94)	0·64
Poor functional outcome or death¶¶	53 (18·4)	58 (20·0)	0·90 (0·60, 1·37)	0·63	0·90 (0·56, 1·43)	0·64

* Adjusted analyses included age, baseline NIHSS score and time since wake-up as covariates.

† Effect sizes are assessed as odds ratios, except for death within 90 days assessed as hazard ratios. The 95% confidence intervals for the secondary outcomes were not adjusted for multiple comparisons.

‡ Functional improvement was defined as an improvement of at least 1 point on the mRS at 90 days and was assessed as a common odds ratio in an ordinal logistic regression analysis.

§ Excellent functional outcome was defined as a score of 0 to 1 on the mRS at 90 days.

¶ Good functional outcome was defined as a score of 0 to 2 on the mRS at 90 days.

** Response to treatment is defined as mRS 0 for patients with mild neurological deficits at study entry (National Institute of Health Stroke Scale score [NIHSS] <=7), mRS 0-1 for patients with moderate deficits (NIHSS 8-14), and mRS 0-2 for patients with severe deficits (NIHSS >14).

†† Symptomatic intracranial haemorrhage defined according to the Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS-MOST) was local or remote parenchymal haematoma type 2 on the imaging scan obtained 22 to 36 hours after treatment, plus neurologic deterioration, as indicated by a score on the NIHSS that was higher by 4 points or more than the baseline value or the lowest value between baseline and 24 hours, or haemorrhage leading to death.

‡‡ Symptomatic intracranial haemorrhage defined according to the Third International Stroke Trial (IST-3) was clinically significant deterioration (neurological deterioration, new headache, new acute hypertension, new nausea or vomiting, or sudden decrease in conscious level) or death within the first 7 days of treatment with evidence of either significant brain parenchymal haemorrhage (local or distant from the infarct) or significant haemorrhagic transformation of an infarct on brain imaging.

§§ Parenchymal haemorrhage type 2 was defined as an intracerebral haemorrhage that involved more than 30% of the infarcted area with a substantial space-occupying effect or that was remote from the original infarcted area.

¶¶ Poor functional outcome or death was defined as patients with a mRS score of 4 (moderately severe disability), 5 (severe disability) or 6 (death) at 90 days of follow-up.

Table 3. Secondary efficacy outcomes (intention-to-treat population)

	Tenecteplase (n=288)	Control (n=290)
Barthel index*		
>= 61, no. (%)	223 (93.7)	217 (91.2)
Median (IQR)	100 (90.0-100.0)	100 (90.0-100.0)
MSE†		
Median (IQR)	20.0 (18.0- 22.0)	20.0 (18.0- 21.0)
EQ-5D-VAS‡		
Median (IQR)	75 (60-85)	70 (50-85)
NIHSS at 24 hours §		
Median NIHSS score (IQR)	3.0 (1.0 - 7.0)	4.0 (2.0 - 7.0)
NIHSS score,no. (%)		
Mild (0-day seven)	219 (76.0)	221 (76.2)
Moderate (8–14)	40 (13.9)	43 (14.8)
Severe (≥15)	29 (10.1)	26 (9.0)
Median difference from baseline NIHSS score (IQR)†	-3.0 (-5.0 to -1.0)	-2.0 (-5.0 to 0.0)
NIHSS at 7 days		
Median NIHSS score (IQR)	2.0 (1.0 - 5.0)	2.0 (1.0 - 6.0)
NIHSS score – no. (%)		
Mild (0-day seven)	244 (84.7)	233 (80.3)
Moderate (8–14)	30 (10.4)	40 (13.8)
Severe (≥15)	14 (4.9)	17 (5.9)
Median difference from baseline NIHSS score (IQR)†	-4.0 (-6.0 to -2.0)	-4.0 (6.0 to -1.0)

IQR=interquartile range.

* 61% of all patients with non-missing values had a Barthel index equal to 100.

† Scores on the MMSE (Mini Mental State Examination) telephone version range from 0 to 22.

‡ EQ5-D-VAS range is 0-100.

§ NIHSS=National Institutes of Health Stroke Scale.

The number of missing values was 102 for Barthel index, 188 for MMSE and 141 for EQ5-D-VAS.

Table 4. Cumulative summary tabulations of serious adverse events

Event	Total	Tenecteplase	Control
Recurrent ischemic stroke after index stroke*	6	4	2
Neurological deterioration from initial/index ischemic stroke*	37	17	20
Any intracranial hemorrhage*	63	34	29
Symptomatic intracranial hemorrhage*			
- IST-3 definition	20	12	8
- SITS-MOST definition	9	6	3
Fatal symptomatic intracranial hemorrhage*	6	3	3
Major systemic bleeding*	1	1	0
Minor systemic bleeding*	9	8	1
Hypotension	11	4	7
Angioedema	4	3	1
Renal failure	7	5	2
Myocardial infarction*	4	2	2
Venous thromboembolism	8	4	4

***** These events were adjudicated by the Endpoint Adjudication Committee, as specified in the protocol.

Symptomatic intracranial hemorrhage defined according to the Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS–MOST) was local or remote parenchymal hematoma type 2 on the imaging scan obtained 22 to 36 hours after treatment, plus neurologic deterioration, as indicated by a score on the NIHSS that was higher by 4 points or more than the baseline value or the lowest value between baseline and 24 hours, or hemorrhage leading to death.

Symptomatic intracranial hemorrhage defined according to the Third International Stroke Trial (IST-3) was clinically significant deterioration (neurological deterioration, new headache, new acute hypertension, new nausea or vomiting, or sudden decrease in conscious level) or death within the first 7 days of treatment with evidence of either significant brain parenchymal hemorrhage (local or distant from the infarct) or significant hemorrhagic transformation of an infarct on brain imaging.

Figure legends

Figure 1. Trial profile

Figure 2. Bar chart showing the distribution of modified Rankin Scale scores in each treatment group at 90 days follow-up (intention-to-treat-analysis).

Scores on the modified Rankin scale range from 0 to 6, with 0 indicating no neurologic deficit, 1 no clinically significant disability (return to all usual activities), 2 slight disability (able to handle own affairs without assistance but unable to carry out all previous activities), 3 moderate disability requiring some help (e.g., with shopping, cleaning, and finances but able to walk unassisted), 4 moderately severe disability (unable to attend to bodily needs without assistance and unable to walk unassisted), 5 severe disability (requiring constant nursing care and attention), and 6 death. Percentages may not total 100 because of rounding.

Figure 3. Kaplan Meier survival plot of cumulative risk of death in patients treated with tenecteplase versus controls.

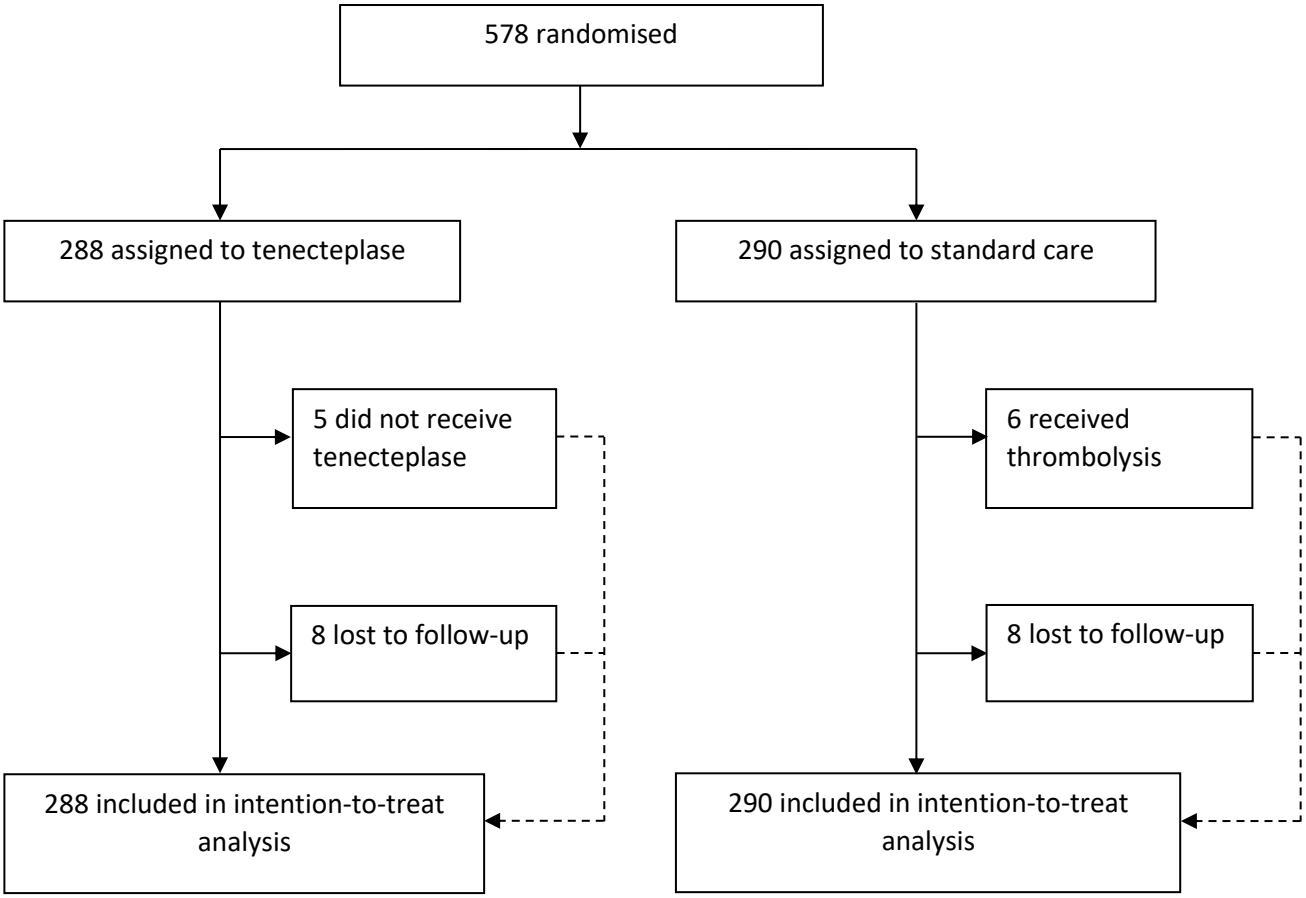

578 randomised
288 assigned to tenecteplase
290 assigned to standard care
5 did not receive tenecteplase
6 received thrombolysis
8 lost to follow-up
8 lost to follow-up
288 included in intention-to-treat analysis
290 included in intention-to-treat analysis

Figure 1.

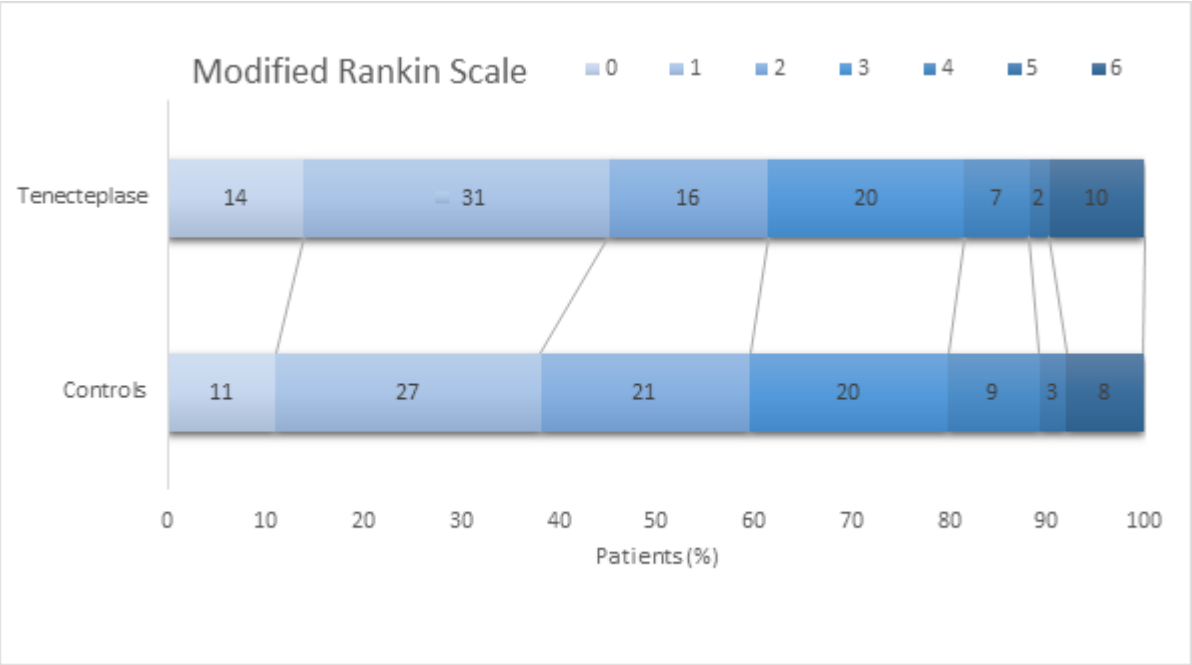
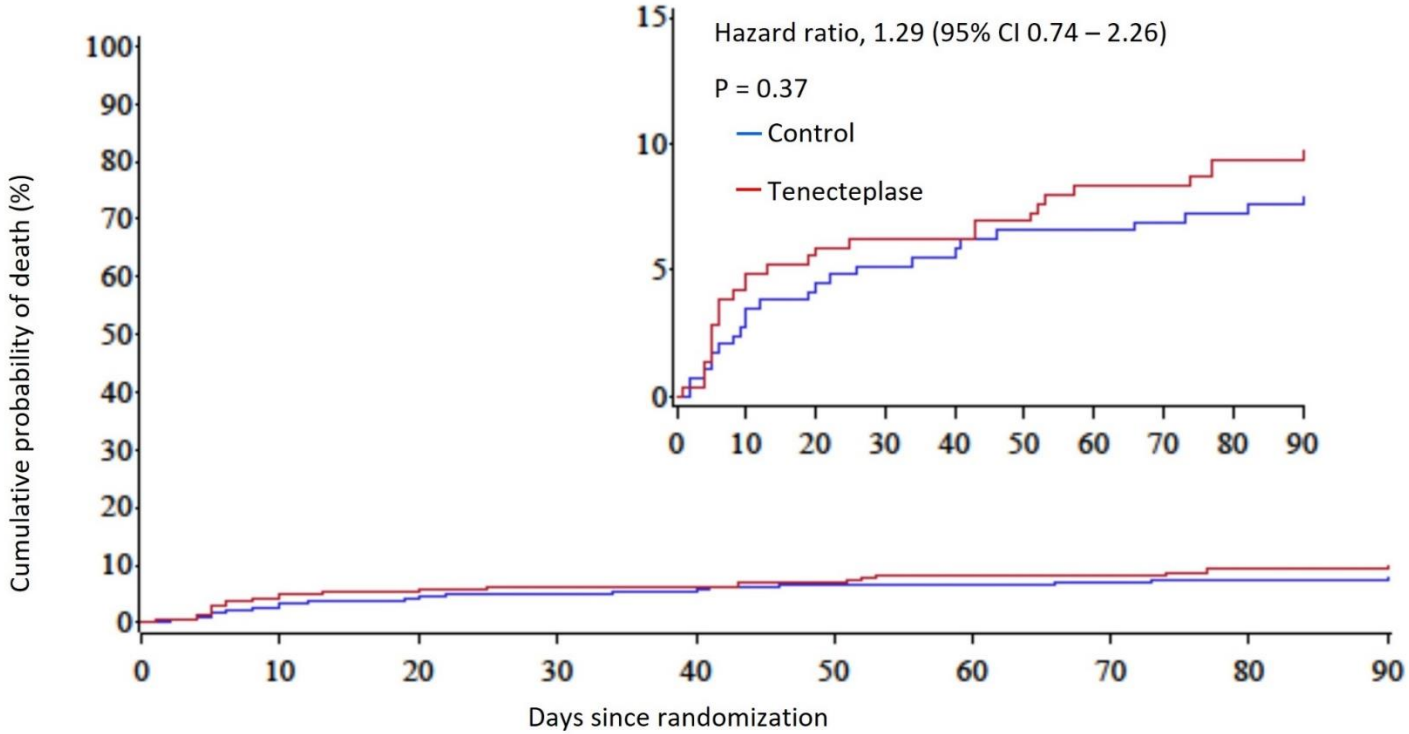

Modified Rankin Scale
0
1
2
3
4
5
6
Tenecteplase
14
31
16
20
7
2
10
Controls
11
27
21
20
9
3
8
0
10
20
30
40
50
60
70
80
90
100
Patients (%)

Figure 2.



No at risk										
Tenecteplase	288	276	272	270	270	268	264	264	261	261
Controls	290	282	278	275	274	271	271	270	269	268

Figure 3

Supplementary Material

Supplementary Appendix

Table of Contents

List of centers (number of patients enrolled) and investigators

Enrolling centers

Denmark: National Coordinator H.K. Christensen
Bispebjerg Hospital (30), L. Christensen (PI), K. Ægidius (PI), H.K. Christensen, T. Pihl, C. Fassel-Larsen, A. Hansen, N. Preisler, M. Folke, L. Wassvik. **Odense University Hospital** (3), K. Ægidius (PI), S. Gharehbagh.

Estonia: National Coordinator J. Kõrv
Pärnu Hospital (1), K. Antsov (PI). **Tartu University Hospital** (19), J. Kõrv (PI), S. Mallene, M. Lill, M. Herodes, R. Vibo, A. Rakitin.

Finland: National Coordinator J. Putaala
Central Hospital in Vaasa (3), J. Saarinen (PI). **Helsinki University Hospital** (20), J. Putaala (PI), M. Tiainen, O. Tumpula, T. Noppari, S. Räty, G. Sibolt, J. Nieminen. **North Karelia Central Hospital** (1), J. Sipilä (PI). **Satakunta Central Hospital** (8), J. Puustinen (PI), T-M. Haula.

Latvia: National Coordinator G. Karelis
Riga East Clinic University Hospital (11), PI G. Karelis (PI), I. Haritoncenko.

Lithuania: National Coordinator D. Jatužis
Klaipeda Seamen's Hospital (27), B. Viesulaite (PI), S. Taroza. **Lithuanian University of Health Sciences Kauno Klinikos** (23), D. Rastenyte (PI), V. Matijosaitis. **Republican Vilnius University Hospital** (9), A. Vilionskis (PI), V. Lukosaitis. **Vilnius University Hospital Santaros Klinikos** (15), D. Jatužis (PI), R. Masiliunas, A. Ekkert, P. Chmeliauskas.

New Zealand: National Coordinator T. Wu
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Finland
South Karelia Central Hospital, T. Tapiola (PI).

Lithuania
Alytus S. Kudirkos hospital, J. Kestutis (PI).

Norway
Drammen Hospital, K-F Amtor (PI). Lofoten Hospital, B. Heermann (PI). Helgeland Hospital Mosjøen, V. Ottesen (PI). Kirkenes Hospital, T. Melum (PI). Stavanger University Hospital, M. Kurz (PI).

Sweden
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UK

Watford General Hospital, S. Sundayi (PI). Northumbria Specialist Emergency Care Hospital, M. Garside (PI). Aberdeen Royal Infirmary, M-J. Macleod (PI). Royal Liverpool University Hospital, A. Manoj (PI). Royal Bournemouth and Christchurch Hospital, O. Hopper (PI).

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Independent Data Monitoring Committee: Terje Pedersen (Chair), Hans Wedel and Peter Sandercock.

Responsible Statistician: Tom Wilsgaard

Event Adjudication Committee: Stein-Harald Johnsen (Chair), Michael Mazya and Thomas Christensen.

Patient Advisory Board: Arne Hagen (the Norwegian Association for Stroke Survivors) and Anne Heimdal (LHL Stroke).

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Medical Monitors

NorCRIN (Norwegian clinical research infrastructure network) partner Gunn-Janne Paulsen.

In the United Kingdom: University of Leicester: Alice Durham and Athesam Ebraimo.

Supplementary Methods

Inclusion criteria

- Stroke symptoms on awakening that were not present before sleep
- Clinical diagnosis of stroke with limb weakness with NIHSS score ≥3, or dysphasia
- Treatment with tenecteplase is possible within 4.5 hours of awakening
- Written consent from the patient, non-written consent from the patient (witnessed by non-participating health care personnel), or written consent from the nearest family member

Exclusion criteria

- Age <18 years
- NIHSS score >25 or NIHSS consciousness score >2, or seizures during stroke onset
- Findings on plain CT that indicate that the patient is unlikely to benefit from treatment:
    - Infarction comprising more than >1/3 of the middle cerebral artery territory on non-contrast CT or CT perfusion
    - Intracranial hemorrhage, structural brain lesions which can mimic stroke (e.g. cerebral tumor)
- Active internal bleeding of high risk of bleeding, e.g.:
    - Major surgery, trauma or gastrointestinal or urinary tract hemorrhage within the previous 21 days, or arterial puncture at a non-compressible site within the previous 7 days
    - Any known defect in coagulation, e.g., current use of vitamin K antagonist with an INR >1.7 or prothrombin time >15 seconds, or use of direct thrombin inhibitors or direct factor Xa inhibitors during the last 24 hours (unless reversal of effect can be achieved by agents such as idarusizumab) or with elevated sensitive laboratory tests (such as aPTT, INR, platelet count, eucarin clotting time, TT, or appropriate factor Xa activity assays), or heparins during the last 24 hours or with an elevated aPTT greater than the upper limit of normal
    - Known defect of clotting or platelet function or platelet count below 100,000/mm^3 (but patients on antiplatelet agents can be included)
    - Ischemic stroke or myocardial infarction in previous 3 months, previous intracranial hemorrhage, severe traumatic brain injury or intracranial or intraspinal operation in previous 3 months, or known intracranial neoplasm, arteriovenous malformation or aneurysm
- Contraindications to tenecteplase, e.g., acute bacterial endocarditis or pericarditis; acute pancreatitis; severe hepatic dysfunction, including hepatic failure, cirrhosis, portal hypertension; active hepatitis; systemic cancer with increased bleeding risk; hemostatic defect including secondary to severe hepatic, renal disease; organ biopsy; prolonged cardiopulmonary resuscitation > 2 min (within 2 weeks)
- Persistent blood pressure elevation (systolic ≥185 mmHg or diastolic ≥110 mmHg), despite blood pressure lowering treatment
- Blood glucose <2.7 or >20.0 mmol/L (use of finger-stick measurement devices is acceptable)
- Pregnancy, positive pregnancy test, childbirth during last 10 days, or breastfeeding. In any woman of childbearing potential, a pregnancy test must be performed and the result assessed before trial entry
- Other serious or life-threatening disease before the stroke: severe mental or physical disability (e.g. Mini Mental Status score <20, or modified Rankin Scale score ≥3), or life expectancy less than 12 months
- Patient unavailability for follow-up (e.g. no fixed address)

Protocol amendments

There have been two major amendments; changes to the inclusion and exclusion criteria (Protocol amendment July 4, 2018) and revision of the sample size estimation (Protocol amendment Sept 17, 2020). In Protocol amendment July 4, 2018, the cutoff for NIHSS score in the main inclusion criterion was changed from NIHSS ≥5 to ≥3 (Clinical diagnosis of stroke with limb weakness with NIHSS score ≥3, or dysphasia). The rationale for this was that many wake-up stroke patients with mild strokes (low NIHSS score) have clinically relevant deficits which could benefit from treatment.[1] Furthermore, we allowed inclusion of patients who were to be treated with intra-arterial interventions for proximal cerebral artery occlusion.

The Protocol amendment of July 4, 2018 concerned increase in target sample size. The primary endpoint in TWIST is modified Rankin Scale (mRS) score across the full ordinal scale (shift analysis). We originally based our sample size estimation on the results of a systematic review of the effect of rt-PA within 4.5 hours of stroke onset, assessed as a binary endpoint (favourable outcome mRS 0-2 versus mRS 3-6).[2] As sample size estimation based on ordinal logistic regression analysis is more appropriate, we re-estimated the sample size in June 2020. The revised sample size estimation was based on observations from recent studies on thrombolytic treatment in patients with wake-up stroke. In the largest randomised controlled trial of wake-up stroke, WAKE-UP, the difference between thrombolysed and non-thrombolysed patients was 11.5% for a favorable outcome defined as mRS 0-1.[1] A difference of 11.5% was also found in a recent meta-analysis of six observational studies on patients with unknown stroke onset time,[3] where favourable outcome was defined as mRS 0-2. The MRI-based inclusion criteria in WAKE-UP compared to the CT-based inclusion in TWIST could lead to a smaller treatment effect in TWIST. We assumed a treatment effect of 10% absolute difference in a binary endpoint setting (mRS 0-1 versus mRS 2-6) and a distribution between mRS categories similar to that of the WAKE-UP trial anticipating 42% with favourable outcome in the non-thrombolysed group vs 52% in the thrombolysed group, which corresponds to an odds ratio of 1.50, and mRS distribution in the control group in six levels (categories 5 and 6 merged) as 15%, 27%, 23%,

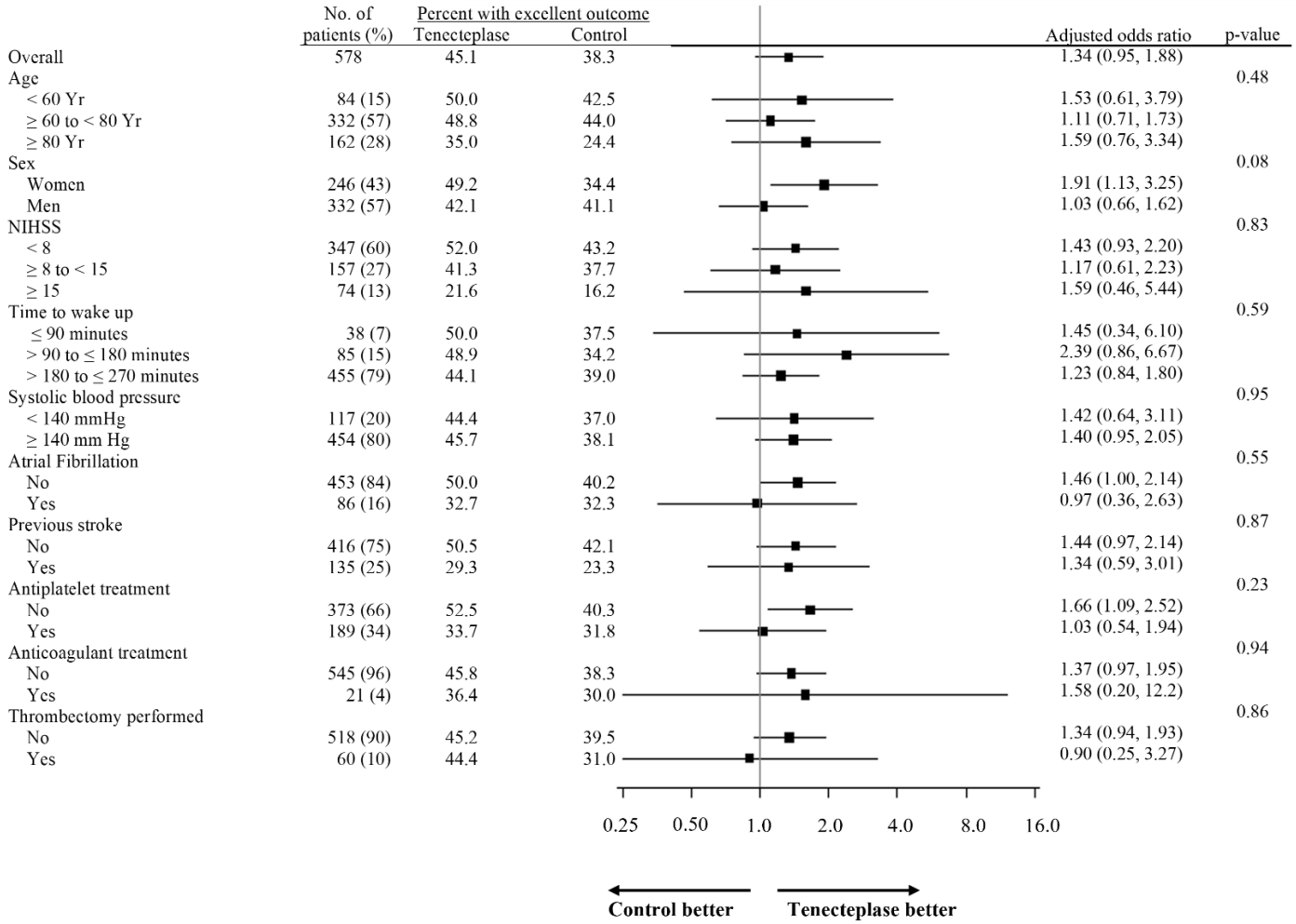
17%, 13%, 5%. With a power of 80%, a two-sided significance level of 5%, and an effect size specified as an odds ratio of 1.50 from an ordinal logistic regression model for the ordinal outcome in the control group, the estimated sample size is 600. The Trial Steering Committee therefore decided to increase the inclusion target from 500 to 600 patients, i.e. 300 patients in each treatment arm.

A complete list of amendments is available in the trial protocol (*https://twist.uit.no)*

References

1. Thomalla G, Simonsen CZ, Boutitie F, et al. MRI-guided thrombolysis for stroke with unknown time of onset. N Engl J Med 2018;379:611-622
2. Wardlaw JM, Murray V, Berge E, et al. Recombinant tissue plasminogen activator for acute ischaemic stroke: An updated systematic review and meta-analysis. Lancet 2012;379:2364-2372
3. Zhu RL, Xu J, Xie CJ, et al. Efficacy and safety of thrombolytic therapy for stroke with unknown time of onset: A meta-analysis of observational studies. J Stroke Cerebrovasc Diseases 2020;29:104742

Figure 1. Forest plot of odds ratios (95% CI) for excellent outcome* for tenecteplase vs control according to sub-groups



*Excellent functional outcome was defined as a score of 0 to 1 on the modified Rankin scale at 90 days.
P-value for test of interaction between the treatment and any subgroup variable.

Table S1. Details of patients who did not receive the allocated treatment (crossover patients)

Age	Sex	Reason given for protocol deviation	Mode of administration, generic name, dose
Tenecteplase group			
84	F	NIHSS fell spontaneously from 8 to 1 right before treatment was to be given	NA
75	M	Patient had taken dabigatran less than 12 hours prior to randomization	NA
53	M	Normalization after inclusion, no longer any symptoms	NA
53	M	Diagnose revised to alcohol intoxication	NA
59	M	Blood pressure too high after randomization	NA
Control group			
86	M	Thrombolysed according to the local PI's judgment	Intravenous tenecteplase, bolus, dose not known
82	F	Failed to recanalize during thrombectomy, therefore intraarterial thrombolysis after reversal of dabigatran	Intraarterial alteplase 10 mg
79	M	Deteriorated 30-40 minutes after randomization, MRI showed DWI/FLAIR mismatch in pons	Intravenous alteplase 7 mg bolus + 65 mg infusion/1 hour
47	M	Intraarterial thrombolysis during thrombectomy	Intraarterial alteplase 5 mg
67	F	Intraarterial thrombolysis during thrombectomy	Intraarterial alteplase 2.5 mg
74	F	Thrombolysis prior to thrombectomy	Intravenous alteplase, dose not known

Table S2. Efficacy and safety outcomes in the per protocol population*

Outcome	Tenecteplase (n=283)	Control (n=284)	Unadjusted Effect Size (95% CI)†	P Value	Adjusted Effect Size (95% CI)†	P Value
Primary efficacy outcome						
Score on the modified Rankin scale at 90 days						
0	39 (13.8)	30 (10.6)				
1	90 (31.8)	78 (27.5)				
2	44 (15.5)	62 (21.8)				
3	57 (20.1)	57 (20.1)				
4	19 (6.7)	27 (9.5)				
5	6 (2.1)	8 (2.8)				
6	28 (9.9)	22 (7.7)				
Functional improvement‡			1.19 (0.89, 1.59)	0.25	1.21 (0.90, 1.62)	0.20
Secondary efficacy outcomes						
Excellent functional outcome at 90 days§	129 (45.6)	108 (38.0)	1.37 (0.98, 1.91)	0.069	1.40 (0.99, 1.97)	0.057
Good functional outcome¶	173 (61.1)	170 (59.9)	1.05 (0.75, 1.48)	0.76	1.07 (0.74, 1.54)	0.72
Response to treatment according to baseline neurological deficit**		53 (18.7)	1.41 (0.94, 2.10)	0.098	1.41 (0.94, 2.12)	0.10
Safety outcomes						
Deaths within 90 days after intervention	28 (9.9)	22 (7.7)	1.35 (0.77, 2.38)	0.30	1.33 (0.75, 2.34)	0.33
Symptomatic intracranial hemorrhage						
As defined by SITS- MOST††	6 (2.1)	3 (1.1)	2.03 (0.50, 8.19)	0.32	2.17 (0.53, 8.88)	0.28
As defined by IST-3‡‡	12 (4.2)	7 (2.5)	1.75 (0.68, 4.52)	0.25	1.74 (0.67, 4.50)	0.25
Parenchymal hemorrhage type 2§§	7 (2.5)	4 (1.4)	1.78 (0.51, 6.13)	0.36	1.85 (0.53, 6.46)	0.33
Any intracranial hemorrhage	33 (11.7)	28 (9.9)	1.21 (0.71, 2.06)	0.49	1.22 (0.71, 2.10)	0.48
Poor functional outcome or death¶¶	53 (18.7)	57 (20.1)	0.92 (0.61, 1.39)	0.69	0.89 (0.55, 1.42)	0.61

* Adjusted analyses included age, baseline NIHSS score and time since wake-up as covariates.

† Effect sizes are assessed as odds ratios, except for death within 90 days assessed as hazard ratios. The 95% confidence intervals for the secondary outcomes were not adjusted for multiple comparisons.

‡ Functional improvement was defined as an improvement of at least 1 point on the modified Rankin scale at 90 days and was assessed as a common odds ratio in an ordinal logistic regression analysis.

§ Excellent functional outcome was defined as a score of 0 to 1 on the modified Rankin scale at 90 days.

¶ Good functional outcome was defined as a score of 0 to 2 on the modified Rankin scale at 90 days.
** Response to treatment is defined as mRS 0 for patients with mild neurological deficits at study entry (National Institute of Health Stroke Scale score [NIHSS] <=7), mRS 0-1 for patients with moderate deficits (NIHSS 8-14), and mRS 0-2 for patients with severe deficits (NIHSS >14).
†† Symptomatic intracranial hemorrhage defined according to the Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS–MOST) was local or remote parenchymal hematoma type 2 on the imaging scan obtained 22 to 36 hours after treatment, plus neurologic deterioration, as indicated by a score on the NIHSS that was higher by 4 points or more than the baseline value or the lowest value between baseline and 24 hours, or hemorrhage leading to death.
‡‡ Symptomatic intracranial hemorrhage defined according to the Third International Stroke Trial (IST-3) was clinically significant deterioration (neurological deterioration, new headache, new acute hypertension, new nausea or vomiting, or sudden decrease in conscious level) or death within the first 7 days of treatment with evidence of either significant brain parenchymal hemorrhage (local or distant from the infarct) or significant hemorrhagic transformation of an infarct on brain imaging.
§§ Parenchymal hemorrhage type 2 was defined as an intracerebral hemorrhage that involved more than 30% of the infarcted area with a substantial space-occupying effect or that was remote from the original infarcted area.
¶¶ Poor functional outcome or death was defined as patients with a modified Rankin Scale score of 4 (moderately severe disability), 5 (severe disability) or 6 (death) at 90 days of follow-up.

Table S3 Efficacy and safety outcomes in the complete case population*

Outcome	Tenecteplase (n=280)	Control (n=282)	Unadjusted Effect Size (95% CI)†	P Value	Adjusted Effect Size (95% CI)†	P Value
Primary efficacy outcome						
Score on the modified Rankin scale at 90 days						
0	39 (13.9)	32 (11.3)				
1	88 (31.4)	77 (27.3)				
2	46 (16.4)	60 (21.3)				
3	56 (20.0)	58 (20.6)				
4	17 (6.1)	25 (8.9)				
5	6 (2.1)	7 (2.5)				
6	28 (10.0)	23 (8.2)				
Functional improvement‡			1.17 (0.87, 1.57)	0.29	1.17 (0.87, 1.57)	0.30
Secondary efficacy outcomes						
Excellent functional outcome at 90 days§	127 (45.4)	109 (38.7)	1.32 (0.94, 1.84)	0.11	1.32 (0.94, 1.87)	0.11
Good functional outcome¶	173 (61.8)	169 (59.9)	1.08 (0.77, 1.52)	0.65	1.08 (0.75, 1.56)	0.67
Response to treatment according to baseline neurological deficit**	69 (24.6)	56 (19.9)	1.32 (0.89, 1.97)	0.17	1.33 (0.89, 1.99)	0.16
Safety outcomes						
Deaths within 90 days after intervention	28 (10.0)	23 (8.2)	1.29 (0.74, 2.26)	0.37	1.29 (0.74, 2.25)	0.38
Symptomatic intracranial hemorrhage						
As defined by SITS- MOST††	6 (2.1)	3 (1.1)	2.04 (0.50, 8.22)	0.32	2.15 (0.53, 8.77)	0.29
As defined by IST-3‡‡	12 (4.3)	8 (2.8)	1.53 (0.62, 3.81)	0.36	1.54 (0.62, 3.82)	0.36
Parenchymal hemorrhage type 2§§	7 (2.5)	5 (1.8)	1.42 (0.45, 4.53)	0.55	1.46 (0.46, 4.69)	0.52
Any intracranial hemorrhage	33 (11.8)	29 (10.3)	1.17 (0.69, 1.98)	0.57	1.18 (0.69, 2.03)	0.54
Poor functional outcome or death¶¶	51 (18.2)	55 (19.5)	0.92 (0.60, 1.40)	0.70	0.91 (0.57, 1.47)	0.70

* Adjusted analyses included age, baseline NIHSS score and time since wake-up as covariates.

† Effect sizes are assessed as odds ratios, except for death within 90 days assessed as hazard ratios. The 95% confidence intervals for the secondary outcomes were not adjusted for multiple comparisons.

‡ Functional improvement was defined as an improvement of at least 1 point on the modified Rankin scale at 90 days and was assessed as a common odds ratio in an ordinal logistic regression analysis.

§ Excellent functional outcome was defined as a score of 0 to 1 on the modified Rankin scale at 90 days.

¶ Good functional outcome was defined as a score of 0 to 2 on the modified Rankin scale at 90 days.
** Response to treatment is defined as mRS 0 for patients with mild neurological deficits at study entry (National Institute of Health Stroke Scale score [NIHSS] <=7), mRS 0-1 for patients with moderate deficits (NIHSS 8-14), and mRS 0-2 for patients with severe deficits (NIHSS >14).
†† Symptomatic intracranial hemorrhage defined according to the Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS–MOST) was local or remote parenchymal hematoma type 2 on the imaging scan obtained 22 to 36 hours after treatment, plus neurologic deterioration, as indicated by a score on the NIHSS that was higher by 4 points or more than the baseline value or the lowest value between baseline and 24 hours, or hemorrhage leading to death.
‡‡ Symptomatic intracranial hemorrhage defined according to the Third International Stroke Trial (IST-3) was clinically significant deterioration (neurological deterioration, new headache, new acute hypertension, new nausea or vomiting, or sudden decrease in conscious level) or death within the first 7 days of treatment with evidence of either significant brain parenchymal hemorrhage (local or distant from the infarct) or significant hemorrhagic transformation of an infarct on brain imaging.
§§ Parenchymal hemorrhage type 2 was defined as an intracerebral hemorrhage that involved more than 30% of the infarcted area with a substantial space-occupying effect or that was remote from the original infarcted area.
¶¶ Poor functional outcome or death was defined as patients with a modified Rankin Scale score of 4 (moderately severe disability), 5 (severe disability) or 6 (death) at 90 days of follow-up.

Table S4. Efficacy and safety outcomes in the safety population*

Outcome	Tenecteplase (n=284)	Control (n=284)	Unadjusted Effect Size (95% CI)†	P Value	Adjusted Effect Size (95% CI)†	P Value
Primary efficacy outcome						
Score on the modified Rankin scale at 90 days						
0	39 (13.7)	30 (10.6)				
1	90 (31.7)	78 (27.5)				
2	44 (15.5)	62 (21.8)				
3	57 (20.1)	57 (20.1)				
4	19 (6.7)	27 (9.5)				
5	6 (2.1)	8 (2.8)				
6	29 (10.2)	22 (7.7)				
Functional improvement‡			1.17 (0.88, 1.57)	0.28	1.20 (0.89, 1.60)	0.23
Secondary efficacy outcomes						
Excellent functional outcome at 90 days§	129 (45.4)	108 (38.0)	1.36 (0.97, 1.90)	0.074	1.39 (0.98, 1.95)	0.063
Good functional outcome¶	173 (60.9)	170 (59.9)	1.05 (0.75, 1.46)	0.80	1.06 (0.74, 1.52)	0.76
Response to treatment according to baseline neurological deficit**	69 (24.3)	53 (18.7)	1.40 (0.93, 2.09)	0.10	1.40 (0.94, 2.11)	0.10
Safety outcomes						
Deaths within 90 days after intervention	29 (10.2)	22 (7.7)	1.40 (0.80, 2.45)	0.24	1.37 (0.78, 2.41)	0.27
Symptomatic intracranial hemorrhage						
As defined by SITS- MOST††	6 (2.1)	3 (1.1)	2.02 (0.50, 8.16)	0.32	2.15 (0.53, 8.81)	0.29
As defined by IST-3‡‡	13 (4.6)	7 (2.5)	1.90 (0.75, 4.83)	0.18	1.89 (0.74, 4.82)	0.18
Parenchymal hemorrhage type 2§§	7 (2.5)	4 (1.4)	1.77 (0.51, 6.11)	0.37	1.84 (0.53, 6.41)	0.34
Any intracranial hemorrhage	34 (12.0)	28 (9.9)	1.24 (0.73, 2.11)	0.42	1.26 (0.73, 2.16)	0.41
Poor functional outcome or death¶¶	54 (19.0)	57 (20.1)	0.94 (0.62, 1.42)	0.75	0.91 (0.57, 1.45)	0.69

* Adjusted analyses included age, baseline NIHSS score and time since wake-up as covariates.

† Effect sizes are assessed as odds ratios, except for death within 90 days assessed as hazard ratios. The 95% confidence intervals for the secondary outcomes were not adjusted for multiple comparisons.

‡ Functional improvement was defined as an improvement of at least 1 point on the modified Rankin scale at 90 days and was assessed as a common odds ratio in an ordinal logistic regression analysis.

§ Excellent functional outcome was defined as a score of 0 to 1 on the modified Rankin scale at 90 days.

¶ Good functional outcome was defined as a score of 0 to 2 on the modified Rankin scale at 90 days.
** Response to treatment is defined as mRS 0 for patients with mild neurological deficits at study entry (National Institute of Health Stroke Scale score [NIHSS] <=7), mRS 0-1 for patients with moderate deficits (NIHSS 8-14), and mRS 0-2 for patients with severe deficits (NIHSS >14).
†† Symptomatic intracranial hemorrhage defined according to the Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS–MOST) was local or remote parenchymal hematoma type 2 on the imaging scan obtained 22 to 36 hours after treatment, plus neurologic deterioration, as indicated by a score on the NIHSS that was higher by 4 points or more than the baseline value or the lowest value between baseline and 24 hours, or hemorrhage leading to death.
‡‡ Symptomatic intracranial hemorrhage defined according to the Third International Stroke Trial (IST-3) was clinically significant deterioration (neurological deterioration, new headache, new acute hypertension, new nausea or vomiting, or sudden decrease in conscious level) or death within the first 7 days of treatment with evidence of either significant brain parenchymal hemorrhage (local or distant from the infarct) or significant hemorrhagic transformation of an infarct on brain imaging.
§§ Parenchymal hemorrhage type 2 was defined as an intracerebral hemorrhage that involved more than 30% of the infarcted area with a substantial space-occupying effect or that was remote from the original infarcted area.
¶¶ Poor functional outcome or death was defined as patients with a modified Rankin Scale score of 4 (moderately severe disability), 5 (severe disability) or 6 (death) at 90 days of follow-up.

Table S5. Odds ratios of lower modified Rankin Scale scores for tenecteplase vs control according to subgroups.

	No. of patients (%)	Odds Ratios (95% CI)	Percent with excellent outcome		p-value*
			Tenecteplase	Control	
Overall	578	1.18 (0.88, 1.58)	45.1	38.3	
Age, years					0.06
< 65	132 (23)	2.50 (1.31, 4.74)	58.1	37.1	
≥ 65	446 (77)	0.98 (0.71, 1.37)	41.6	38.6	
Age, years					0.41
< 60	84 (15)	1.83 (0.84, 4.01)	50.0	42.5	
≥ 60 to < 80	332 (57)	0.97 (0.66, 1.43)	48.8	44.0	
≥ 80	162 (28)	1.20 (0.68, 2.14)	35.0	24.4	
Sex					0.07
Women	246 (43)	1.61 (1.02, 2.52)	49.2	34.4	
Men	332 (57)	0.93 (0.63, 1.36)	42.1	41.1	
NIHSS					0.80
< 8	347 (60)	1.29 (0.89, 1.89)	52.0	43.2	
≥ 8 to < 15	157 (27)	1.05 (0.60, 1.83)	41.3	37.7	
≥ 15	74 (13)	0.98 (0.43, 2.24)	21.6	16.2	
Time to wake up, minutes					0.84
≤ 90	38 (7)	0.97 (0.29, 3.21)	50.0	37.5	
> 90 to ≤ 180	85 (15)	1.55 (0.70, 3.44)	48.9	34.2	
> 180 to ≤ 270	455 (79)	1.15 (0.83, 1.60)	44.1	39.0	
Country					0.68
Norway	157 (27)	0.93 (0.53, 1.64)	37.3	41.5	
Sweden	48 (8)	1.47 (0.51, 4.22)	63.6	46.2	
Denmark	33 (6)	1.21 (0.33, 4.46)	55.6	40.0	
Finland	32 (6)	2.00 (0.50, 8.04)	55.6	21.4	
Estonia	20 (3)	2.95 (0.45, 19.3)	37.5	8.3	
Latvia	11 (2)	1.10 (0.08, 14.4)	33.3	40.0	
Lithuania	74 (13)	2.04 (0.86, 4.85)	22.2	17.2	
United Kingdom	165 (29)	1.22 (0.70, 2.12)	53.7	45.8	
Switzerland	24 (4)	6.75 (0.80, 57.4)	50.0	37.5	
New Zealand	14 (2)	1.32 (0.16, 11.3)	83.3	50.0	
Systolic blood pressure, mmHg					
< 140	117 (20)	1.12 (0.58, 2.14)	44.4	37.0	
≥ 140	454 (80)	1.24 (0.89, 1.73)	45.7	38.1	
Atrial fibrillation					0.75
No	453 (84)	1.23 (0.88, 1.71)	50.0	40.2	
Yes	86 (16)	1.19 (0.53, 2.66)	32.7	32.3	
Previous stroke					0.03
No	416 (75)	1.46 (1.03, 2.06)	50.5	42.1	
Yes	135 (25)	0.70 (0.38, 1.28)	29.3	23.3	
Antiplatelet treatment					0.05
No	373 (66)	1.53 (1.06, 2.21)	52.5	40.3	
Yes	189 (34)	0.79 (0.47, 1.31)	33.7	31.8	
Anticoagulant treatment					0.19
No	545 (96)	1.15 (0.86, 1.56)	45.8	38.3	
Yes	21 (4)	4.16 (0.75, 23.1)	36.4	30.0	
Thrombectomy performed					0.48

No	518 (90)	1.23 (0.90, 1.67)	45.2	39.5
Yes	60 (10)	0.79 (0.27, 2.32)	44.4	31.0

*P-value for test of interaction between the treatment and any subgroup variable.

Table S6. Efficacy and safety outcomes (intention-to-treat population)* stratified by patients not treated and treated with intraarterial interventions

Outcome	Patients not treated with thrombectomy				Patients treated with thrombectomy			
	Tenecteplase (n=270)	Control (n=248)	Adjusted Effect Size† (95% CI)	P Value	Tenecteplase (n=18)	Control (n=42)	Adjusted Effect Size† (95% CI)	P Value
Primary efficacy outcome								
Score on the modified Rankin scale at 90 days								
0	38 (14.1)	28 (11.3)			2 (11.1)	4 (9.5)		
1	84 (31.1)	70 (28.2)			6 (33.3)	9 (21.4)		
2	45 (16.7)	56 (22.6)			2 (11.1)	6 (14.3)		
3	55 (20.4)	52 (21.0)			3 (16.7)	7 (16.7)		
4	16 (5.9)	21 (8.5)			3 (16.7)	6 (14.3)		
5	6 (2.2)	4 (1.6)			0 (0.0)	4 (9.5)		
6	26 (9.6)	17 (6.9)			2 (11.1)	6 (14.3)		
Functional improvement‡			1.23 (0.90, 1.67)	0.19			0.79 (0.27, 2.32)	0.67
Secondary efficacy outcomes								
Excellent functional outcome at 90 days§	122 (45.2)	98 (39.5)	1.34 (0.94, 1.93)	0.11	8 (44.4)	13 (31.0)	0.90 (0.25, 3.27)	0.82
Good functional outcome¶	167 (61.9)	154 (62.1)	1.10 (0.75, 1.61)	0.63	10 (55.6)	19 (45.2)	0.82 (0.23, 2.92)	0.76
Response to treatment**	63 (23.3)	45 (18.1)	1.33 (0.86, 2.05)	0.19	7 (38.9)	11 (26.2)	1.32 (0.34, 5.04)	0.69
Safety outcomes								
Deaths within 90 days after intervention	26 (9.6)	17 (6.9)	1.25 (0.66, 2.35)	0.49	2 (11.1)	6 (14.3)	0.95 (0.17, 5.39)	0.95
Symptomatic intracranial hemorrhage								
As defined by SITS-MOST††	5 (1.9)	1 (0.4)	5.87 (0.66, 52.4)	0.11	1 (5.6)	2 (4.8)	1.35 (0.09, 21.3)	0.83
As defined by IST-3‡‡	10 (3.7)	2 (0.8)	4.71 (1.02, 21.8)	0.047	2 (11.1)	6 (14.3)	0.92 (0.14, 6.00)	0.93
Parenchymal hemorrhage type 2§§	5 (1.9)	1 (0.4)	5.87 (0.66, 52.4)	0.11	2 (11.1)	4 (9.5)	2.25 (0.27, 19.0)	0.46
Any intracranial hemorrhage	27 (10.0)	13 (5.2)	1.95 (0.97, 3.89)	0.059	6 (33.3)	17 (40.5)	1.08 (0.28, 4.14)	0.91
Poor functional outcome or death¶¶	48 (17.8)	42 (16.9)	0.87 (0.52, 1.46)	0.61	5 (27.8)	16 (38.1)	1.29 (0.32, 5.20)	0.72

* Adjusted analyses included age, baseline NIHSS score and time since wake-up as covariates.

† Effect sizes are assessed as odds ratios, except for death within 90 days assessed as hazard ratios. The 95% confidence intervals for the secondary outcomes were not adjusted for multiple comparisons.

‡ Functional improvement was defined as an improvement of at least 1 point on the modified Rankin scale at 90 days and was assessed as a common odds ratio in an ordinal logistic regression analysis.

§ Excellent functional outcome was defined as a score of 0 to 1 on the modified Rankin scale at 90 days.

¶ Good functional outcome was defined as a score of 0 to 2 on the modified Rankin scale at 90 days.

** Response to treatment is defined as mRS 0 for patients with mild neurological deficits at study entry (National Institute of Health Stroke Scale score [NIHSS] <=7), mRS 0-1 for patients with moderate deficits (NIHSS 8-14), and mRS 0-2 for patients with severe deficits (NIHSS >14).
†† Symptomatic intracranial hemorrhage defined according to the Safe Implementation of Thrombolysis in Stroke Monitoring Study (SITS–MOST) was local or remote parenchymal hematoma type 2 on the imaging scan obtained 22 to 36 hours after treatment, plus neurologic deterioration, as indicated by a score on the NIHSS that was higher by 4 points or more than the baseline value or the lowest value between baseline and 24 hours, or hemorrhage leading to death.
‡‡ Symptomatic intracranial hemorrhage defined according to the Third International Stroke Trial (IST-3) was clinically significant deterioration (neurological deterioration, new headache, new acute hypertension, new nausea or vomiting, or sudden decrease in conscious level) or death within the first 7 days of treatment with evidence of either significant brain parenchymal hemorrhage (local or distant from the infarct) or significant hemorrhagic transformation of an infarct on brain imaging.
§§ Parenchymal hemorrhage type 2 was defined as an intracerebral hemorrhage that involved more than 30% of the infarcted area with a substantial space-occupying effect or that was remote from the original infarcted area.
¶¶ Poor functional outcome or death was defined as patients with a modified Rankin Scale score of 4 (moderately severe disability), 5 (severe disability) or 6 (death) at 90 days of follow-up.

Table S7. Details of all deaths during follow-up

Age	Sex	Baseline NIHSS	Treatment group	Intra-arterial intevention	Cause of death*
70	M	14	Control	N	Index stroke
85	M	21	Tenecteplase	N	Index stroke
83	M	4	Tenecteplase	N	Index stroke
73	F	17	Control	N	Other vascular cause
94	M	23	Tenecteplase	N	Other non-vascular cause
79	M	23	Tenecteplase	N	Index stroke
86	F	8	Tenecteplase	N	Recurrent stroke
93	F	17	Control	Y	Index stroke
92	F	21	Control	N	Index stroke
84	M	6	Control	N	Index stroke
57	M	20	Control	N	Index stroke
88	M	23	Tenecteplase	N	Index stroke
81	F	10	Tenecteplase	N	Index stroke
92	M	5	Control	Y	Index stroke
85	M	3	Tenecteplase	N	Index stroke
85	F	11	Tenecteplase	N	Index stroke
75	M	6	Tenecteplase	Y	Index stroke
74	F	4	Tenecteplase	N	Recurrent stroke
76	M	11	Tenecteplase	N	Recurrent stroke
68	M	10	Tenecteplase	Y	Index stroke
86	M	5	Tenecteplase	N	Index stroke
60	M	14	Control	Y	Index stroke
86	F	12	Control	N	Infection
92	F	4	Control	Y	Recurrent stroke
79	F	24	Tenecteplase	N	Recurrent ischemic stroke†
76	F	17	Tenecteplase	N	Recurrent ischemic stroke†
64	F	23	Control	N	Other non-vascular†
90	M	18	Control	N	Index stroke†
76	F	7	Tenecteplase	N	Index stroke†
84	F	8	Control	N	Index stroke†
89	F	12	Control	N	Index stroke†
70	M	18	Tenecteplase	N	Other vascular†
91	M	8	Tenecteplase	N	Other non-vascular†
91	F	19	Control	N	Pneumonia†
82	M	25	Tenecteplase	N	Index stroke†
86	M	5	Control	N	Other vascular†
41	M	11	Tenecteplase	N	Recurrent ischemic stroke†
69	M	11	Control	Y	Myocardial infarction†
69	M	4	Tenecteplase	N	Pneumonia†
80	M	8	Control	N	Other non-vascular†
72	M	12	Control	N	Recurrent ischemic stroke†
88	F	18	Tenecteplase	N	Index stroke†
86	M	5	Control‡	N	Other vascular†
74	M	14	Tenecteplase	Y	Other non-vascular†
69	M	5	Control	N	Unknown
72	M	20	Control	Y	Unknown

96	F	6	Tenecteplase	N	Myocardial infarction†
77	M	8	Tenecteplase	N	Index stroke†
83	F	9	Tenecteplase	N	Other non-vascular†
87	F	19	Control	N	Other non-vascular†
85	M	25	Tenecteplase	N	Other non-vascular†

* Cause of death was adjudicated by the Endpoint Adjudication Committee in deaths that occurred before discharge from hospital

† Cause of death occurred after discharge from hospital and was not adjudicated

‡ Crossover patient (the patient was allocated to the control group, but received tenecteplase)