

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

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1 **ABSTRACT**

2 **Background**

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

8 **Methods**

9 We conducted a multicentre, randomised, open-label trial at 77 centres in 10 countries where
10 patients aged 18 years or older with ischaemic wake-up stroke who were assessed by non-
11 contrast CT were randomly allocated by a central web-based computer-generated
12 randomisation schedule to treatment with intravenous tenecteplase 0.25 mg/kg versus no
13 thrombolysis. The primary outcome was functional status at 90 days on modified Rankin
14 Scale (mRS) scores (range, 0 [no disability] to 6 [death]), assessed in the intention-to-treat
15 population. The endpoint assessment was blinded to random allocation. This trial is registered
16 with EudraCT-number 2014-000096-80. We planned to recruit 600 patients, but the trial was
17 stopped early due to the Covid-19 pandemic and exhausted funding

18 **Findings**

19 Between June 2017 and Sept 2021, we recruited 578 of the planned 600 patients to the study,
20 of whom 288 were allocated tenecteplase and 290 were allocated no thrombolysis. The
21 distribution of mRS scores showed no significant difference in functional outcome between
22 treatment groups (adjusted OR 1.18, 95% CI 0.88-1.58; $p=0.27$). The number of deaths at 90
23 days was 28 (10%) in the tenecteplase group and 23 (8%) in the control group (adjusted OR
24 1.29 (0.74-2.26; $p=0.37$). Symptomatic intracranial haemorrhage (sICH) occurred in 6
25 patients (2%) in the tenecteplase group and in 3 patients (1%) in the control group (adjusted

1 OR 2·17, 95% CI 0·53-8·87; p=0·28), while any intracranial haemorrhage occurred in 33
2 (11%) and 30 (10%), respectively (adjusted OR 1·14 (0·67-1·94; 0·64) .

3 **Interpretation**

4 In patients with wake-up stroke selected by non-contrast CT, the primary functional outcome
5 did not differ between groups at 90 days. The number of patients with sICH was low in both
6 treatment groups, similar to previous trials of thrombolysis in wake-up stroke patients selected
7 by advanced imaging. Current evidence does not support treatment with tenecteplase in
8 patients selected by non-contrast CT.

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10 Specialist Health Services, British Heart Foundation, Swiss Heart Foundation, and Norwegian
11 National Association for Public Health. The cost of tenecteplase was covered by Boehringer
12 Ingelheim Norway.

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14

1 **Research in context**

2

3 **Evidence before this study**

4 It is well established that intravenous thrombolysis with alteplase improves outcome in
5 patients with acute ischaemic stroke within 4.5 hours of symptom onset. It has also been
6 found to improve outcome in patients with wake-up stroke and is now recommended in
7 clinical guidelines for patients with wake-up stroke selected by magnetic resonance or
8 perfusion imaging. Tenecteplase is a genetically modified variant of alteplase with greater
9 fibrin specificity, longer halflife and simpler single-bolus administration compared to
10 alteplase. We searched MEDLINE for published randomised controlled trials for of
11 intravenous thrombolytic drugs versus control in patients with wake-up stroke up till
12 November 2022 without language restrictions. The search terms used were (“wake-up stroke”,
13 “thrombolysis” or “thrombolytic treatment” and “randomised trial/study”. We identified five
14 trials with 775 participants. Thrombolysis with alteplase resulted in better functional outcome
15 compared to control in two of the trials. All trials were stopped early and had limited sample
16 size, and all trials used advanced imaging for selection of patients. A systematic review and
17 meta-analysis of controlled trials, observational cohort studies and single-arm safety studies
18 conducted in 2021, showed no significant difference in functional or safety outcomes between
19 studies that evaluated patients with wake-up stroke with non-contrast CT, MRI, or CT
20 perfusion prior to treatment. As access to advanced imaging is not universally available,
21 treatment decisions based on non-contrast CT criteria may be an alternative, but this has not
22 been tested in randomised controlled trials. We also searched MEDLINE up to November
23 2022 for published randomised controlled trials for thrombolytic treatment with tenecteplase
24 for ischaemic stroke without language restrictions. The search terms used were “stroke”,
25 “tenecteplase” and “randomised trial/study”. The search results identified 9 trials.

1 Tenecteplase 0.25 mg/kg was found to be non-inferior to alteplase, while a dose of 0.4 mg/kg
2 resulted in higher risk of intracranial haemorrhage. The effect of tenecteplase in wake-up
3 stroke has not been evaluated in randomised controlled trials.

4

5 **Added value of this study**

6 TWIST adds information to previous trials of thrombolytic treatment of wake-up stroke in
7 that it differs with regards to both thrombolytic agent and imaging technique. It is the largest
8 randomised controlled trial of thrombolytic treatment in patients with wake-up stroke. The
9 participants were randomised to treatment with intravenous tenecteplase 0.25 mg/kg or
10 standard care (no thrombolysis), based on findings on non-contrast CT. Treatment with
11 tenecteplase within 4.5 hours of awakening was not significantly associated with better
12 functional outcome at end of follow-up. Excellent functional outcome (mRS 0-1) was attained
13 by 45% in the tenecteplase group and 38% in the control group. The risk of death and of
14 symptomatic and any intracerebral haemorrhage was insignificantly higher in the tenecteplase
15 group than in the control group and similar to previous meta-analyses of trials with selection
16 based on advanced imaging.

17

18 **Implications of all the available evidence**

19 Treatment with tenecteplase in patients with wake-up stroke after screening with non-contrast
20 CT was not found to be superior to standard care (no thrombolysis), although a numerically
21 higher proportion of patients achieved mRS 0-1. The safety profile of TWIST was similar to
22 previous trials of treatment with alteplase in patients selected by advanced MRI or CT
23 perfusion imaging. Current evidence does not support treatment with tenecteplase in patients
24 selected by non-contrast CT.

25

1 **Introduction**

2 Results from recent trials support the use of intravenous thrombolysis with alteplase in
3 patients with ischaemic stroke of unknown time of onset who presented with magnetic
4 resonance imaging (MRI) findings of an ischaemic lesion on diffusion-weighted imaging
5 (DWI) and absence of visible hyperintense signal in the corresponding region on fluid-
6 attenuated inversion recovery (FLAIR) series (DWI/FLAIR mismatch) or findings indicating
7 presence of salvageable tissue on CT or MRI perfusion imaging.¹⁻⁵ Treatment with
8 intravenous alteplase is now recommended in clinical guidelines for wake-up stroke patients
9 with DWI/FLAIR or CT or MRI core/perfusion mismatch.

10

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

1
2 Tenecteplase has higher fibrin specificity, longer half-life and simpler single-bolus
3 administration compared to alteplase.¹⁰ Recent systematic reviews suggest that tenecteplase
4 0.25 mg/kg is non-inferior to alteplase with regard to functional outcome after acute
5 ischaemic stroke,^{10,11} and superior at increasing reperfusion rate,¹² A higher dose of 0.40
6 mg/kg was recently stopped early due to increased rate of symptomatic intracranial
7 haemorrhage and worse functional outcome.¹³ We conducted the Tenecteplase in Wake-up
8 Ischaemic Stroke Trial (TWIST) to determine whether thrombolytic treatment with
9 intravenous tenecteplase 0.25 mg/kg given within 4.5 hours of awakening improves
10 functional outcome in patients with ischaemic wake-up stroke selected by use of non-contrast
11 CT.

12

13 **Methods**

14 **Study design**

15 TWIST was an investigator-initiated, multicentre, prospective, randomised, controlled, open-
16 label trial of tenecteplase 0.25 mg/kg bodyweight in patients with acute ischaemic stroke
17 upon awakening, with blinded end-point assessment. The trial was conducted at 77 centres in
18 10 countries from June 12, 2017 to September 30, 2021. Methods of the trial have been
19 published previously.¹⁴

20

21 The trial protocol was approved by national regulatory authorities in each participating
22 country and by national and/or local ethics committees and/or institutional review boards.
23 Patients or their legal representatives provided written informed consent according to national
24 and local regulations. Members of the trial coordinating centre and steering committee

1 designed the trial and met on a regular basis to oversee the conduct of the trial. The TWIST
2 Investigators collected the data (listed in the Supplementary Appendix). The trial was
3 overseen by the trial steering committee and an independent data monitoring committee
4 (DMC). The data monitoring committee reviewed data relating to treatment efficacy, patient
5 safety and quality of trial conduct, and were provided open and closed data reports by an
6 independent statistician (for details, see Supplementary Appendix, page xx). The trial steering
7 committee and the investigators remained blinded to results throughout the course of the trial.
8 The authors vouch for the accuracy and completeness of the data and adverse event reporting
9 and for the fidelity of the trial to the protocol. The trial was conducted in accordance with the
10 MRC Guidelines for Good Clinical Practice in Clinical Trials, the Council of Europe's
11 Convention on Human Rights and Biomedicine, the ICH Harmonized Tripartite Guideline for
12 Good Clinical Practice and the Declaration of Helsinki. The trial is registered in the EudraCT
13 (no. 2014-000096-80), ClinicalTrials.gov (no. NCT03181360) and ISRCTN (no. 10601890)
14 databases.

15

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

22

23 After the onset of the Covid-19 pandemic, there was a marked drop in recruitment in April
24 2020 and onwards. In addition, there was a concurrent shortage tenecteplase. As a

1 consequence of this, Trial Steering Committee decided to extend the inclusion period from the
2 planned end of inclusion on December 31, 2020, to September 30, 2021. In the months to
3 follow, the inclusion rate continued to be lower than prior to the pandemic. Due to these
4 extenuating circumstances, including exhausted funding, the inclusion period was not
5 extended any further.

6

7 **Patients**

8 The trial was carried out at 77 hospitals (listed in the Supplementary Appendix) in Denmark,
9 Estonia, Finland, Latvia, Lithuania, New Zealand, Norway, Sweden, Switzerland, and the
10 United Kingdom. Patient eligibility was assessed by the treating physician and required that
11 patients were 18 years of age or older, had stroke symptoms upon awakening that were not
12 present before sleep, with limb weakness and a National Institutes of Health Stroke Scale
13 (NIHSS) score ≥ 3 , or aphasia, and could be treated with tenecteplase within 4.5 hours of
14 awakening.

15

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

1

2 **Randomisation and masking**

3 Patients were randomly assigned to either intravenous tenecteplase or control in a 1:1
4 allocation ratio using a central web-based computer-generated randomisation schedule. The
5 schedule employed a minimisation algorithm that balanced age (under vs. at or above 80
6 years), NIHSS severity (under vs. at or above 15 points) and time since wake-up (under vs. at
7 or above 3 hours). The dose of tenecteplase was 0.25 mg per kg of body weight (maximum 25
8 mg), given as a single intravenous bolus and was based on results from previous trials of
9 tenecteplase for stroke with known symptom onset selected by non-contrast CT and on
10 patients with minor stroke and intracranial arterial occlusion selected by non-contrast CT and
11 CT angiography or by CT perfusion.¹⁰ Body weight was assessed according to local routine
12 practice for thrombolysis of stroke patients. Patients randomised to control were not to receive
13 tenecteplase or any other thrombolytic agent. Thrombectomy was allowed in both treatment
14 groups.

15

16 **Procedures**

17 Clinical assessments were performed on day 1 (at baseline) and day 7 (or on the day of
18 discharge, whichever occurred first). A non-contrast CT examination of the head was a
19 prerequisite for inclusion into the trial and was repeated after 24 hours. CT angiography
20 and/or perfusion was recommended, if possible, but was not mandatory, and if undertaken
21 was to be repeated within 24 hours in patients with large-vessel occlusion upon admission.
22 While not part of the study protocol, supplemental brain imaging (MRI or perfusion imaging)
23 was allowed but discouraged if it delayed randomisation for more than 20 minutes.
24 Centralised, blinded reading of all available images was used to assess acute ischaemic

1 changes at baseline and at 24 (\pm 6) hours according to the Alberta Stroke Project Early CT
2 Changes Score (ASPECTS),¹⁵ cerebral artery patency and intracranial haemorrhage.

3

4 **Outcomes**

5 Outcome data at 90 days were collected through centralised standardised telephone interviews
6 and was performed in each country by trained research personnel blinded to treatment
7 allocation. The primary outcome was functional outcome assessed by the modified Rankin
8 Scale (mRS) at 90 days (ordinal scale). The mRS ranges from 0 to 6, with 0 indicating no
9 neurologic deficit, 1 no clinically significant disability (return to all usual activities), 2 slight
10 disability (able to handle own affairs without assistance but unable to carry out all previous
11 activities), 3 moderate disability requiring some help (e.g., with shopping, cleaning, and
12 finances but able to walk unassisted), 4 moderately severe disability (unable to attend to
13 bodily needs without assistance and unable to walk unassisted), 5 severe disability (requiring
14 constant nursing care and attention), and 6 death. Secondary effect outcomes were excellent
15 functional outcome defined as mRS score of 0-1, good functional outcome defined as mRS
16 score of 0-2 and response to treatment according to neurological deficit at study entry defined
17 as mRS score of 0 for patients with mild deficits (NIHSS \leq 7), 0-1 for patients with moderate
18 deficits (NIHSS 8-14) and 0-2 for patients with severe deficits (NIHSS $>$ 14]). Other
19 secondary outcomes were EuroQol score (EQ-5D-VAS), mini-mental state examination
20 (MMSE, telephone version) and Barthel Index score at 90 days.

21

22 Safety outcomes were death from all causes at 90 days and symptomatic intracranial
23 haemorrhage at day 7 (by SITS-MOST [Safe Implementation of Thrombolysis in Stroke
24 Monitoring Study]¹⁶ and IST-3 [the Third International Stroke Trial]¹⁷ definitions),
25 parenchymal haemorrhage type 2,¹⁸ any intracranial haemorrhage, and major extracranial

1 bleeding. An independent endpoint adjudication committee whose members were unaware of
2 trial group assignment, adjudicated prespecified serious adverse events including secondary
3 safety outcomes based on source data provided by the participating centres. Images were
4 assessed with standardised case-report forms by an imaging committee whose members were
5 unaware of clinical data except for date and time of image acquisition.

6

7 **Statistical analysis**

8 The original sample size estimation resulted in a target sample size of 500 patients and was
9 based on the results of a Cochrane systematic review of the effect of rt-PA within 4.5 hours of
10 stroke onset, which showed an absolute risk reduction of 9% in the thrombolysis group for the
11 binary endpoint mRS 0-2 versus mRS 3-6.¹⁹ As there were concerns about whether the
12 assumptions for the sample size estimations would hold, the Trial Steering Committee in June
13 2020 decided to undertake a revised sample size calculation. The results from WAKE-UP
14 trial, published during the course of the trial, showed a difference between thrombolysed and
15 non-thrombolysed patients of 11.5% for a favourable outcome defined as mRS 0-1.¹ The same
16 difference of 11.5% was also found in a meta-analysis of six observational studies on patients
17 with unknown stroke onset time, where favourable outcome was defined as mRS 0-2.²⁰ As the
18 primary endpoint in TWIST was mRS across the full ordinal scale (shift analysis), the revised
19 sample size estimation was based on ordinal logistic regression analysis.¹ We assumed a
20 clinically relevant treatment effect with odds ratio (OR) of 1.50, corresponding to an absolute
21 difference of approximately 10% between the trial groups in the percentage of patients
22 achieving a mRS score of 0 to 1 at 90 days, with a favourable outcome of 42% in the non-
23 thrombolysed group versus 52% in the thrombolysis group. We further assumed a similar
24 distribution of mRS scores in the control group as in the WAKE-UP trial.¹ A sample size of
25 600 patients would provide 80% power to detect a true treatment effect at alpha level 0.05

1 (Supplementary Methods in Supplementary Appendix). A detailed statistical analysis plan
2 was made publicly available before the database was locked.²¹

3

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

11

12 Differences in all outcomes between the two treatment groups were tested independently at
13 the two-tailed 0.05 level of significance. All estimates of treatment effects are presented with
14 95% confidence intervals (CIs).

15

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

23

1 Secondary and safety outcomes were compared between treatment groups by means of binary
2 logistic regression for dichotomous outcomes to estimate OR with corresponding 95% CIs.
3 Cox proportional hazard regression was used to calculate hazard ratios (HR) and
4 corresponding 95% CI for death during follow-up. The primary and secondary analyses were
5 adjusted for age, baseline NIHSS score and time from wake-up to randomisation.

6

7 As all subgroup analyses are of exploratory nature, no adjustment for multiple comparisons
8 was made. The 2-way interactions between treatment groups (tenecteplase or control) and the
9 pre-defined demographic and clinical variables on the primary outcome were explored
10 through multivariable ordinal logistic regression for the primary outcome, adjusted for age,
11 baseline NIHSS score, and time from wake-up to randomisation. For each treatment-by-
12 subgroup interaction a likelihood ratio test was used with appropriate degrees of freedom.

13

14 Information on age, geographical region, time of randomisation after wake-up and NIHSS
15 score at baseline were complete as this was assured by the web-based randomisation
16 procedure. Information on safety endpoints (intracranial haemorrhage and death) was also
17 complete. When information about the mRS score at 90 days was missing (n=16), we used the
18 level of function recorded on day 7 after randomisation or at discharge, whichever came first,
19 to impute functional status at 90 days. No imputation was made for secondary efficacy
20 outcomes.

21

22 Pre-specified sensitivity analyses were performed for the “per protocol” population, defined
23 as those who actually received their allocated treatment (crossovers excluded), and in the
24 “complete case” population, where patients with missing information on the primary endpoint

1 were excluded (no imputation). Additional pre-specified sensitivity analyses of safety
2 outcomes were undertaken in the “safety population”, where patients in the control group who
3 received tenecteplase were assigned to the tenecteplase group while other patients who did not
4 receive their allocated treatment were excluded. A separate set of analyses was performed
5 stratified by patients who received endovascular treatment and those who did not. We further
6 present unadjusted analysis, as well as adjusted analysis taking clustering effects by country
7 and centre into account in mixed effect ordered logistic regression models. All analyses were
8 performed using SAS software version 9.4 (SAS Institute).

9

10 **Role of the funding sources**

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

13

14 **Results**

15 From June 2017 through September 2021, 578 of the target of 600 patients were included.

16 Further extension of the trial was not found feasible due to markedly reduced enrolment rate

17 after the onset of the Covid-19 pandemic, a shortage of tenecteplase in 2021, and exhausted

18 funding. Of the 578 patients, 288 were randomised to receive tenecteplase and 290 to the

19 control group (Figure 1). Five patients allocated to tenecteplase did not receive the assigned

20 treatment, and six patients allocated to the control group received thrombolysis (Table S1 in

21 the Supplementary appendix). Thirteen of the included patients had ischaemic lesions

22 comprising more than 1/3 of the middle cerebral artery territory as judged by the centralised

23 assessment of images, of whom 8 received tenecteplase. Sixteen patients who were alive at 90

1 days were lost to follow-up, eight (3%) in the tenecteplase group and eight (3%) in the control
2 group.

3
4 Baseline and clinical characteristics were similar between groups, except for a higher
5 proportion with atrial fibrillation in the tenecteplase group than in the control group (21% vs
6 11%). The proportion with intracranial large vessel occlusion was higher in the control group
7 (37%) than in the tenecteplase group (30%), as were the proportion who were treated with
8 endovascular interventions (15% in the control group vs 6% in the tenecteplase group) (Table
9 1).

10
11 Treatment with tenecteplase was not associated with better functional outcome for the
12 primary outcome assessed as a shift in the score on the modified Rankin scale at 90 days
13 (adjusted common OR ratio 1·18, 95% CI 0·88-1·58; $p=0\cdot27$) (Table 2). The median score on
14 the mRS was 2 in both treatment groups. The proportion with an excellent clinical outcome,
15 defined as mRS score 0 or 1, was 45% in the tenecteplase group and 38% in the control group
16 (adjusted OR 1·34, 95% CI 0·95-1·88) (Figure 2, Table 2). There was no difference in good
17 functional outcome between treatment groups, defined as mRS score 0 to 2 (adjusted OR
18 1·07; 95% CI 0·75 to 1·54). Response to treatment according to neurological deficit at study
19 entry was attained by 24% in the tenecteplase group and 19% in the control group (adjusted
20 OR 1·35, 95% CI 0·91-2·02). The median scores on the MMSE, Barthel Index and the EQ-
21 5D-VAS at 90 days follow-up were similar in the treatment groups, as were the NIHSS score
22 at 24 hours and 7 days and the median change in NIHSS from baseline to 24 hours and to 7
23 days (Table S2 in the Supplementary Appendix). Neurological deterioration from index
24 ischaemic stroke occurred in 17 patients (6%) in the tenecteplase group and 20 patients (7%)

1 in the control group, while recurrent ischaemic stroke occurred in 4 (1%) and 2 (1%) patients
2 in the tenecteplase and control group, respectively (Table 3).

3
4 Unadjusted analyses for the primary and secondary outcomes are presented in Table 2 and do
5 not differ substantially from the prespecified primary multivariable adjusted analyses. Similar
6 results were found in sensitivity analyses based on the “per protocol” (Table S3), “complete
7 case” (Table S4), and “safety” populations (Table S5). In mixed effect ordered logistic
8 regression models taking clustering effects by country into account, the adjusted OR for the
9 primary outcome was 1.25 (95% CI 0.93 to 1.68; $p=0.14$). Similar results were found in
10 models adjusted for clustering effects by centre. Exploratory subgroup analyses are presented
11 in the appendix (Figure S1 and Table S6).

12
13 There was no significant difference between treatment groups in the proportion of deaths
14 within 90 days after treatment (10% in the tenecteplase group and 8% in the control group,
15 adjusted HR 1.29, 95% CI 0.74-2.26; $p=0.37$) (Table 2, Figure 3). The proportion with poor
16 functional outcome defined as mRS of 4 (moderately severe disability), 5 (severe disability)
17 and 6 (dead) was 18% in the tenecteplase group and 20% in the control group (adjusted HR
18 0.90; 95% CI 0.56-1.43).

19
20 Symptomatic intracranial haemorrhage according to the SITS-MOST definition occurred in 6
21 patients treated with tenecteplase and in 3 controls (adjusted OR 2.17, 95% CI 0.53-8.87,
22 $p=0.28$). The corresponding numbers for the IST-3 definition were 12 and 8 (adjusted OR
23 1.54, 95% CI 0.62-3.82; $p=0.36$). The results were similar in sensitivity analyses of the
24 “safety population” (Table S4 in the Supplementary Appendix). Three of the 9 symptomatic
25 intracranial haemorrhages as defined by the SITS-MOST criteria and 8 of the 20 defined

1 according to the IST-3 definition occurred in patients treated with thrombectomy (Table S6 in
2 the Supplementary Appendix). Three patients in each treatment group had fatal intracranial
3 haemorrhage. None of the patients with ischaemic lesions comprising >1/3 of the middle
4 cerebral artery territory who were treated with tenecteplase arm experienced symptomatic
5 intracranial haemorrhage. Details of all deaths are reported in the Supplementary Appendix,
6 Table S8.

7

8 **Discussion**

9 Among patients who presented with acute ischaemic stroke upon awakening selected by non-
10 contrast CT, ordinal analysis of the primary endpoint did not show significantly better
11 functional outcome in patients treated with tenecteplase within 4.5 hours of awakening
12 compared to control.

13

14 The WAKE-UP trial was the first randomised controlled trial to show benefit from
15 thrombolytic treatment with alteplase in patients with stroke of unknown time of onset.
16 Imaging criteria in that trial included MRI findings of an ischaemic lesion on DWI sequences
17 and absence of visible hyperintense signal in the corresponding region on FLAIR series
18 (DWI/FLAIR mismatch).¹ Following the publication of the results from WAKE-UP, three
19 ongoing trials were terminated early. While a positive effect in favour of thrombolysis was
20 found in the EXTEND (Extending the Time for Thrombolysis in Emergency Neurological
21 Deficits) trial,² THAWS (THrombolysis for Acute Wake-up and unclear-onset Strokes with
22 alteplase at 0.6 mg/kg trial)²² and ECASS-4 (European Cooperative Acute Stroke Study 4)³
23 were neutral. The difference of 7% between patients achieving excellent outcome in the
24 tenecteplase and control groups in our trial is similar to the observed difference between

1 treatment groups seen in a meta-analysis of patients treated within 3 to 4.5 hours of known
2 symptom onset²³ and in an individual participant meta-analysis of WAKE-UP, THAWS,
3 EXTEND and ECASS-4.⁴

4

5 There was no significant difference between treatment groups in the risk of death at 90 days.
6 The overall percentage who died within 90 days was higher in our study (10% in the
7 tenecteplase group and 8% in controls) than in the WAKE-UP trial (4% and 1% in the
8 alteplase and placebo group, respectively) which may probably be explained by WAKE-UP
9 patients being on average 8 years younger than TWIST patients. For comparison, in a meta-
10 analysis of patients treated with alteplase within 4.5 hours of known symptom onset and mean
11 age of 71 years, the overall mortality at 90 days was 19% in the treatment group and 18% in
12 the control group.²³ There were numerically more symptomatic intracranial haemorrhages in
13 the tenecteplase group than in controls, which is in line with results from previous trials of
14 stroke patients with known symptom onset where thrombolysis with alteplase was associated
15 with significantly increased risk of symptomatic intracranial haemorrhage and fatal
16 intracranial haemorrhage within the first week.²³ As could be expected in patients with mainly
17 mild strokes, the proportions of symptomatic intracranial haemorrhage and any intracranial
18 haemorrhage were low in both treatment groups, similar to the combined results from
19 WAKE-UP, THAWS, EXTEND and ECASS-4 (3% in the alteplase group versus 1% in the
20 control group).⁴

21

22 Our trial adds information to previous trials as it differs with regards to both thrombolytic
23 agent and imaging technique. Tenecteplase has several pharmacological advantages, including
24 longer free plasma half-life, allowing easy and quick administration as one single intravenous

1 bolus dose.²³ Previous studies found tenecteplase at dosage 0.25 mg/kg (to a maximum of 25
2 mg) to be safe and at least as effective as alteplase,^{14,24,25} We chose non-contrast CT imaging
3 as a screening tool for selection of patients. Previous randomised controlled trials used either
4 DWI/FLAIR mismatch techniques or CT or MR perfusion imaging for selection of patients,
5 with the underlying assumption that these techniques are more likely to identify patients with
6 salvageable tissue or short duration of ischaemia. The relationship between time from
7 symptom onset and the FLAIR lesion intensity is dependent on the severity of hypoperfusion.
8 MRI DWI/FLAIR mismatch and perfusion imaging applied in previous studies in the field
9 provide insight into tissue viability, not only time since onset.²⁶ However, knowledge on
10 which imaging techniques that are most likely to identify patients who will benefit from
11 treatment is limited. The sensitivity and specificity of MRI DWI/FLAIR mismatch vary
12 between studies with sensitivity ranging between 40% and 80% and specificity between 78%
13 and 89%, respectively,²⁷ which can imply that selection of patients based on MRI
14 DWI/FLAIR mismatch criteria may exclude a substantial proportion of patients who might
15 benefit from thrombolysis. Lacunar infarcts, which have been shown to benefit from
16 thrombolysis,²⁸ will not be detected by perfusion imaging.⁴ Furthermore, MRI or perfusion
17 imaging are not universally available and access is often limited even in hospitals which have
18 the necessary equipment. CT scanners are, in turn, more widely available and are used for
19 acute stroke imaging in everyday practice. Limited observer agreement with regards to
20 recognising and quantifying early ischaemic changes on non-contrast CT has been reported
21 and may be a concern when applying this imaging approach.²⁹ There is also a limited potential
22 for quantification of ischemic core and viable penumbra with non-contrast CT. In the present
23 trial, none of the eight patients with more extensive ischaemic lesion than 1/3 of the middle
24 cerebral artery territory and who were treated with tenecteplase experienced symptomatic
25 intracranial haemorrhage. Our trial adds evidence to a previous systematic review and meta-

1 analysis of controlled trials, observational cohort studies and single-arm safety studies which
2 showed no significant difference in functional or safety outcomes between studies that
3 evaluated patients with non-contrast CT, MRI, or CT perfusion prior to treatment.³⁰

4

5 Our trial has several limitations. Based on a revised power estimation, the recruitment target
6 was increased from 500 to 600 patients. The revised estimation assumed a treatment effect of
7 10% absolute difference in a binary endpoint setting (mRS 0 to 1 versus 2 to 6) and a
8 distribution between mRS categories similar to that of the WAKE-UP trial. In light of the
9 actual results, it is evident that the estimated treatment effect was too optimistic and that the
10 trial as a consequence was underpowered. In addition, we did not reach our inclusion target,
11 mainly due to a marked slowdown in recruitment after the onset of the Covid-19 pandemic
12 and concurrent shortage of tenecteplase in 2021. This limits the interpretation of our results.
13 Most of the patients included had mild or moderate strokes, limiting inferences with regards
14 to the safety of treatment with tenecteplase in patients selected by non-contrast CT. The
15 limited number of participants precluded analyses stratified by sex.

16 The higher percentages in the control group of patients with large vessel occlusion and treated
17 with thrombectomy might have further attenuated the results. Patients undergoing
18 thrombectomy were not included in previous studies assessing the effect of thrombolytic
19 treatment in wake-up stroke patients.⁴ We found no treatment effect of tenecteplase in the
20 sub-group of patients undergoing thrombectomy. This is not unexpected given the relatively
21 small additional effect of thrombolysis shown in recent studies.^{31,32} In addition, the relatively
22 long median door-to-needle time of 56 minutes in the present study was not optimal.

23

1 The results from WAKE-UP and other trials of thrombolytic treatment with alteplase in
2 selected wake-up stroke patients with MRI DWI/FLAIR mismatch or perfusion imaging
3 criteria were published during the trial period. Unfortunately, we do not have complete
4 screening log information nor systematic information on access to advanced imaging in the
5 participating hospitals to explore whether this has affected recruitment to the trial. We cannot
6 exclude that increased use of MRI or perfusion imaging may have led to more patients being
7 enrolled who did not have imaging signs indicative of salvageable tissue or short duration of
8 symptoms, while those who fulfilled such criteria were treated outside the trial. The
9 publication of trials showing benefit of thrombectomy in the extended time window may have
10 led to a lower recruitment rate of patients with more severe strokes.

11

12 In conclusion, treatment with tenecteplase was not significantly associated with better
13 functional outcome at 90 days of follow-up. The number of symptomatic intracranial
14 haemorrhages was numerically higher in the tenecteplase group, in line with results from
15 previous trials of stroke patients with known symptom onset and with wake-up stroke.^{4,17}
16 However, compared to previous trials, the number of symptomatic haemorrhages and any
17 intracranial haemorrhages was low in both treatment groups.

18

19 **Contributors**

20 HC, STE, BI, DJ, JK, EL, JPe, JPu, AT, DW, GMDM, TGR and EBM contributed to study
21 design, protocol development and selection of participating centres. GMDM, TGR and EBM
22 contributed to acquisition of funding and grants. TW did the statistical analysis, with input
23 from AE and EBM. MBR, AE and EBM wrote the first draft of the article. All authors
24 contributed to the collection of data and to the writing of the manuscript, had full access to all

1 the data in the study, and had final responsibility for the decision to submit for publication.

2 TW, AE and EBM have accessed and verified the data.

3

4 **Declaration of interests**

5 MBR reports a grant from the Norwegian National Association for Public Health. HC reports

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19

20 **Data sharing**

21 De-identified data collected for the study and presented in this manuscript, including

22 individual participant data and a data dictionary defining each field in the set, will be made

23 available to others upon reasonable request upon publication of this article, provided approval

24 by regulatory authorities. Data can be requested by sending an e-mail to the corresponding

25 author. Study protocol and statistical analysis plan are available on the trial's website.

1

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10

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1 **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)	40 (14)
60-80 years	164 (57)	168 (58)
>80 years	80 (28)	82 (28)
Male sex, no. (%)		
	164 (57)	168 (58)
Country, no. (%)		
Norway	75 (26)	82 (28)
Sweden	22 (8)	26 (9)
Denmark	18 (6)	15 (5)
Finland	18 (6)	14 (5)
Estonia	8 (3)	12 (4)
Latvia	6 (2)	5 (2)
Lithuania	45 (16)	29 (10)
United Kingdom	82 (29)	83 (29)
Switzerland	8 (3)	16 (6)
New Zealand	6 (2)	8 (3)
Final diagnosis at discharge, no. (%)		
Definite ischaemic stroke	258 (90)	260 (90)
Probable ischaemic stroke	18 (6)	14 (5)
Other diagnosis (stroke mimic)	12 (4)	16 (6)
Stroke risk factors and medical history, no. (%)		
Hypertension	176 (64)	177 (63)
Diabetes mellitus	55 (20)	52 (19)
Atrial fibrillation	55 (21)	31 (11)
Active smoker	51 (21)	46 (20)
Previous stroke or TIA	75 (27)	60 (22)
Coronary artery disease	43 (17)	43 (16)

Current use of an anticoagulant agent	11 (4)	10 (4)
Current use of an antiplatelet agent	101 (37)	88 (31)
Pre-morbid modified Rankin Scale – no. (%)		
0	188 (65)	191 (66)
1	63 (22)	57 (20)
2	37 (13)	42 (15)
Median NIHSS† score (IQR)	6 (5 – 11)	6 (5 – 10)
NIHSS† score, no. (%)		
Mild (0-7)	171 (60)	176 (61)
Moderate (8–14)	80 (28)	77 (27)
Severe (≥15)	37 (13)	37 (13)
Median ASPECT‡ score (IQR)	10 (10 - 10)	10 (9 - 10)
ASPECT‡ score, n (%)		
10	220 (78)	195 (70)
8-9	47 (17)	59 (21)
6-7	10 (4)	22 (8)
0-5	7 (3)	4 (1)
Large vessel occlusion§	69 (30)	83 (37)
Endovascular treatment, no. (%)	18 (6)	42 (15)
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

1

2 *Plus-minus values are means ±SD. IQR denotes interquartile range. NA; not applicable. There were
3 more than 5% missing values for the following variables: Coronary heart disease 8%, atrial fibrillation
4 7%, smoking 19%, large vessel occlusion 21%.

5 †NIHSS=National Institutes of Health Stroke Scale

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

7 §Large-vessel occlusion was defined as occlusion of the internal carotid artery, first division of the
8 middle cerebral artery (M1), and proximal portion of the second division of the middle cerebral

1 artery (M2). The diagnosis was based on CT angiography in 455 patients and on MR angiography in 2
2 of the 457 patients where this information was available.

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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 (14)	32 (11)				
1	90 (31)	79 (27)				
2	47 (16)	62 (21)				
3	58 (20)	59 (20)				
4	19 (7)	27 (9)				
5	6 (2)	8 (3)				
6	28 (10)	23 (8)				
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)	111 (39)	1.33 (0.95, 1.85)	0.10	1.34 (0.95, 1.88)	0.09
Good functional outcome¶	177 (62)	173 (60)	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)	56 (19)	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 (10)	23 (8)	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)	3 (1)	2.04 (0.50, 8.22)	0.09	2.17 (0.53, 8.87)	0.28
- as defined by IST-3‡‡	12 (4)	8 (2)	1.53 (0.62, 3.81)	0.36	1.54 (0.62, 3.82)	0.36
Parenchymal haemorrhage type 2§§	7 (2)	5 (1)	1.42 (0.45, 4.53)	0.55	1.47 (0.46, 4.73)	0.51
Any intracranial haemorrhage	33 (11)	30 (10)	1.12 (0.66, 1.89)	0.67	1.14 (0.67, 1.94)	0.64
Poor functional outcome or death¶¶	53 (18)	58 (20)	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. Number (%) of serious adverse events

Event	Total	Tenecteplase	Control
Deaths*	51 (9)	28 (10)	23 (8)
Serious adverse events*†	63 (11)	34 (12)	29 (10)
Treatment-related serious adverse events*†‡	-	18 (6)	-
All adverse events†	167 (29)	97 (34)	70 (24)
Recurrent ischaemic stroke after index stroke*§	6 (1)	4 (1)	2 (1)
Neurological deterioration from initial/index ischaemic stroke*§	37 (6)	17 (6)	20 (7)
Any intracranial haemorrhage*§	63 (11)	34 (12)	29 (10)
Symptomatic intracranial haemorrhage*§			
- IST-3 definition	20 (4)	12 (4)	8 (3)
- SITS-MOST definition	9 (2)	6 (2)	3 (1)
Fatal symptomatic intracranial haemorrhage*§	6 (1)	3 (1)	3 (1)
Major systemic bleeding*§	1 (0)	1 (0)	0 (0)
Minor systemic bleeding*§	9 (2)	8 (3)	1 (0)
Hypotension§	11 (2)	4 (1)	7 (2)
Angioedema§	4 (1)	3 (1)	1 (0)
Renal failure§	7 (1)	5 (2)	2 (1)
Myocardial infarction*§	4 (1)	2 (1)	2 (1)
Venous thromboembolism§	8 (1)	4 (1)	4 (1)

*Calculated as number of patients with one or more events (%)

†Expected serious adverse events.

‡The number includes one patients in the control group who was treated with tenecteplase.

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

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.

Figure legends

Figure 1. Trial profile

ITT= Intention to treat. Screening logs were not systematically collected

* included in safety population

† excluded from complete case analysis

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.

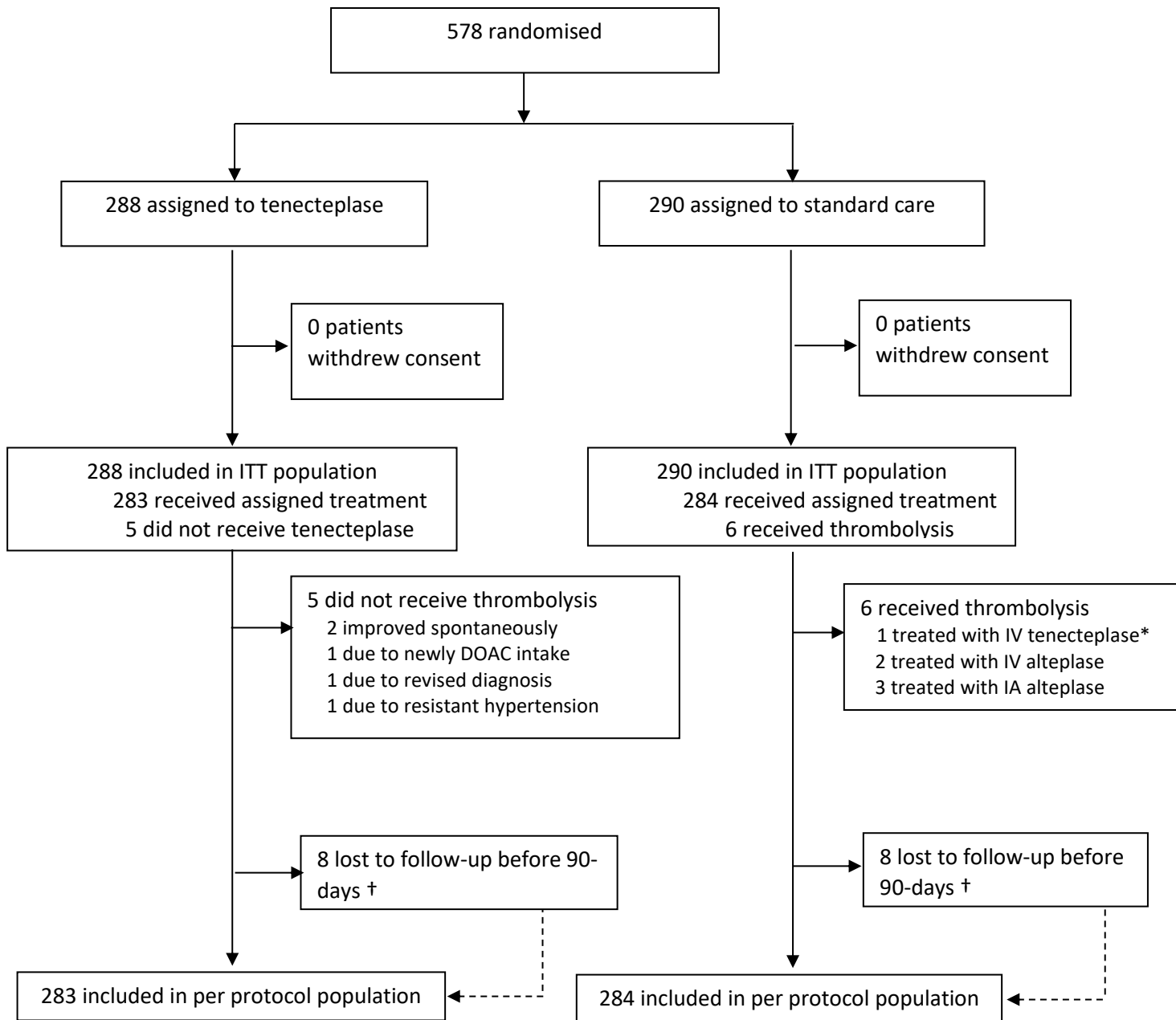


Figure 1.

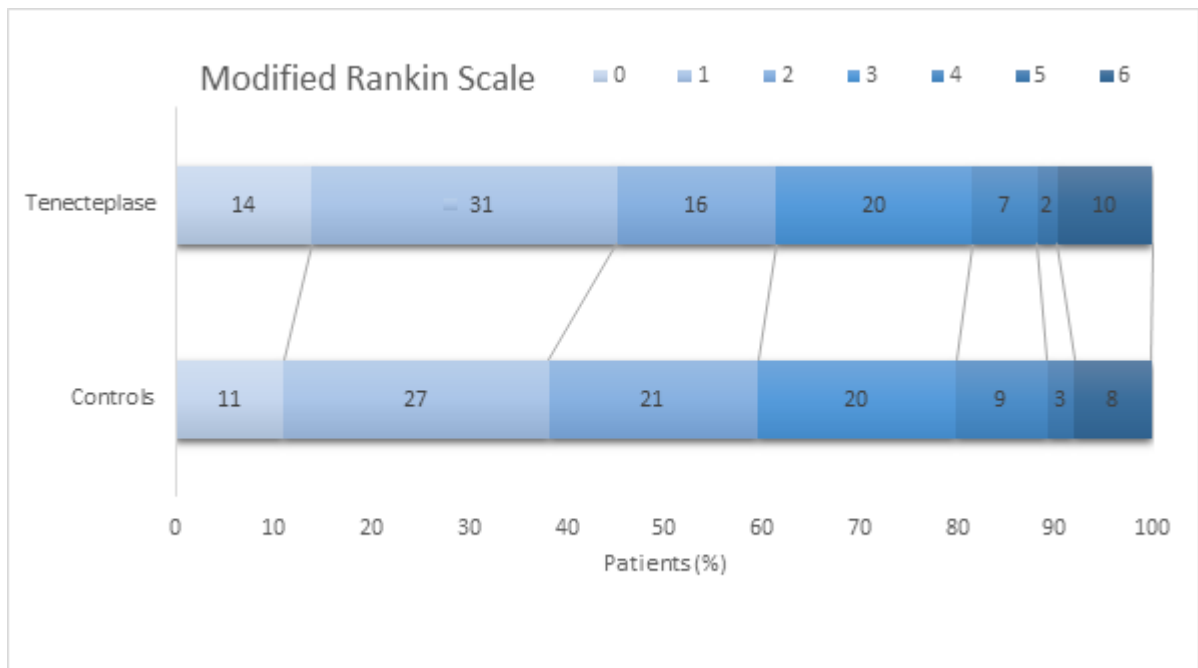
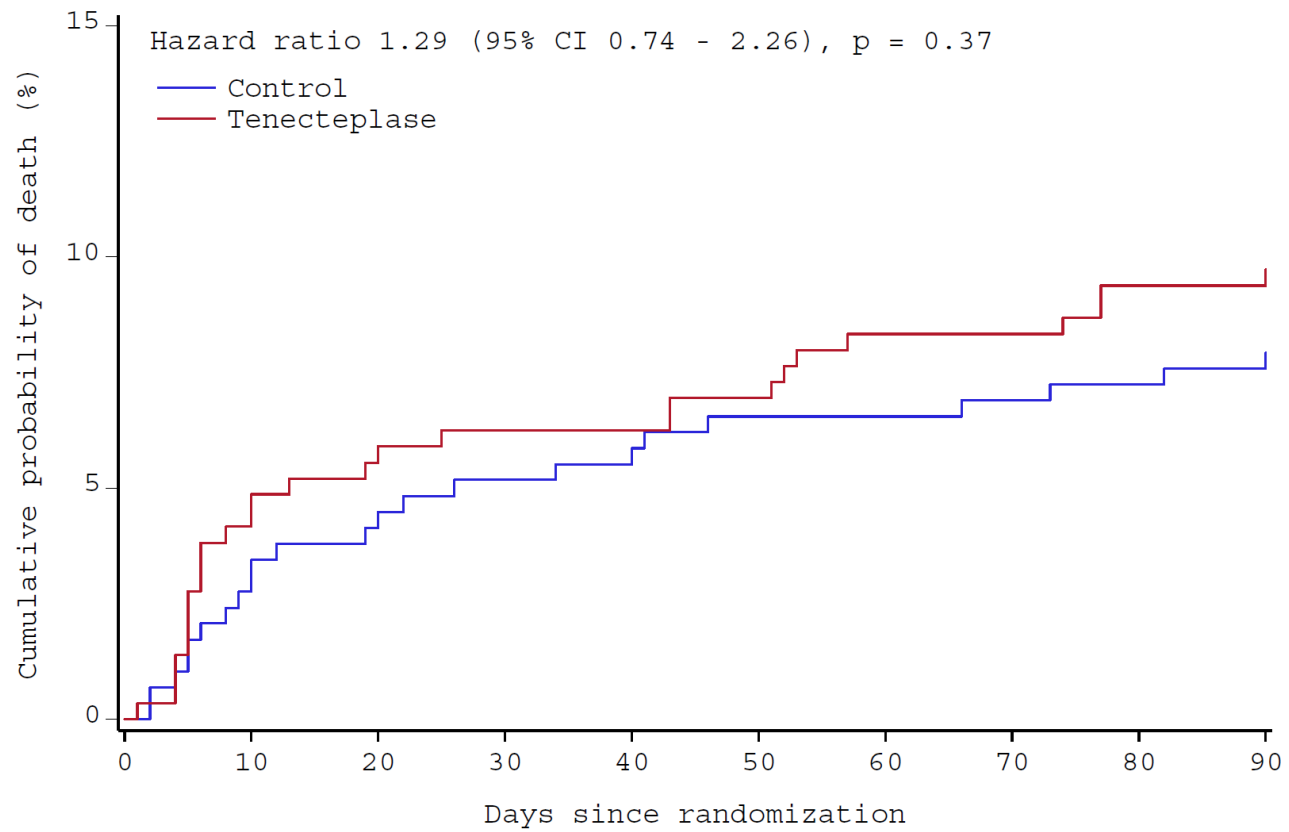


Figure 2.



No at risk

Tenecteplase	288	276	272	270	270	268	264	264	261	261
Control	290	282	278	275	274	271	271	270	269	268

Figure 3

Supplementary Material

Supplementary Appendix

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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. Rätty, 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

Christchurch Hospital (14), T. Wu (PI).

Norway: National Coordinator E. B. Mathiesen

Akershus University Hospital (44), A. Reichenbach (PI), T.T. Moss, H.Y. Nilsen, R. Hammer-Berntzen, L.M. Nordby, T.A. Weiby, K. Nordengen. **Bærum Hospital** (2), H. Ihle-Hansen (PI). **Førde Central Hospital** (9), M. Stankiewicz (PI), O. Grotle, M. Nes, K. Thiemann, I.M. Særvold, M. Fraas. **Hammerfest Hospital** (2), S. Størdahl (PI). **Levanger Hospital** (5), J. W. Horn (PI), H. Hildrum, C. Myrstad. **Telemark Hospital Skien** (9), H. Tobro (PI), J-A. Tunvold, O. Jacobsen, N. Aamodt, H. Baisa, V.N. Malmberg. **St Olavs University Hospital** (27), G. Rohweder (PI), H. Ellekjær, F. Ildstad, E. Egstad, B.H. Helleberg, H.H. Berg, J. Jørgensen, E. Tronvik, M. Shirzadi. **Sørlandet Hospital Arendal** (3), R. Solhoff (PI), M-H. Søyland. **Sørlandet Hospital Flekkefjord** (5), R. Van Lessen. **Sørlandet Hospital Kristiansand** (11), A. Tveiten (PI), M-H. Søyland, A. Vatne, K. Forselv. **University Hospital of North Norway, Harstad Hospital** (7), H. Frøyshov (PI) M.S. Fjeldstad (PI), L. Tangen, S. Matapour, K. Kindberg, C. Johannessen, M. Rist, I. Mathisen, T. Nyrnes. **University Hospital of North Norway, Narvik Hospital** (1), A. Haavik (PI). **University Hospital of North Norway, Tromsø** (18), A. Eltoft (PI), G. Toverud, K. Aakvik, M. Larsson, K. Ytrehus, S. Ingebrigtsen, T. Stokmo, C. Helander, I.C. Larsen, T.O. Solberg. **Ålesund Hospital** (14), Y. M. Seljeseth (PI), S. Maini, I. Bersås.

Sweden: National Coordinators E. Lundström and J. Petersson

Capio St Görans Hospital (2), J. Mathé (PI). **Danderyd Hospital** (15), E. Rooth (PI), A-C. Laska, A-S. Rudberg. **Hässleholm Hospital** (3), M. Esbjörnsson (PI). **Karlstad Central Hospital** (8), F. Andler (PI), A. Ericsson, O. Wickberg. **Sahlgrenska University Hospital** (3), J-E. Karlsson (PI), P. Redfors, K. Jood.

Skåne University Hospital (11), F. Buchwald (PI), K. Mansson, O. Gråhamn, **Uppsala University Hospital** (6), K. Sjölin (PI), E. Lindvall, Å. Cidh, A. Tolf, O. Fasth. **Ängelholm Hospital** (1), B. Hedström (PI).

Switzerland: National Coordinator G. M. de Marchis

Groupement Hospitalier Ouest Lémanique (1), J. Niederhauser (PI). **University Hospital of Basel** (23), G. M. de Marchis (PI), J. Fladt, T. D. Dittrich, L. Kriemler.

United Kingdom: National Coordinators David Werring and Thompson Robinson

Addenbrookes Hospital (15), N. Hannon (PI), E. Amis, S. Finlay, J. Mitchell-Douglas, J. Mcgee, **Arrowe Park Hospital** (2), R. Davies (PI), V. Johnson, **Calderdale Royal Infirmary** (2), A. Nair (PI), M. Robinson, J. Greig, **Charing Cross Hospital** (5), O. Halse (PI), P. Wilding, S. Mashate, **Countess of Chester Hospital** (11), K. Chatterjee (PI), M. Martin, S. Leason, J. Roberts, **Gloucestershire Royal Hospital** (2), D. Dutta (PI), D. Ward, **Hull University Teaching Hospital** (1), R. Rayessa (PI), E. Clarkson, **King's College Hospital** (3), J. Teo (PI), C. Ho, S. Conway, M. Aissa, **Leeds General Infirmary** (10), V. Papavasileiou, S. Fry, D. Waugh, J. Britton, A. Hassan, **Leicester Royal Infirmary** (7), L. Manning (PI), S. Khan, **Luton and Dunstable University Hospital** (8), A. Asaipillai (PI), C. Fornolles, M.L. Tate **Morrison Hospital** (1), S. Chenna (PI), T. Anjum, **Musgrove Park Hospital** (4), D. Karunatilake (PI), J. Foot, L. VanPelt, **Nottingham City Hospital** (12), A. Shetty (PI), G. Wilkes, A. Buck, B. Jackson, L. Fleming, **Pinderfields Hospital** (8), M. Carpenter (PI), L. Jackson, A. Needle, T. Zahoor, **Royal Cornwall Hospital** (2), T. Duraisami (PI), K. Northcott, **Royal Devon and Exeter** (9), J. Kubie (PI), A. Bowring, S. Keenan, D. Mackle, **Royal Derby Hospital** (17), T. England (PI), B. Rushton, A. Hedstrom, **Royal London Hospital** (2), S. Amlani (PI), R. Evans, **Royal Stoke University Hospital** (11), G. Muddegowda (PI), A. Remegoso, P. Ferdinand, R. Varquez, **Royal Victoria Infirmary** (16), M. Davis (PI), E. Elkin, R. Seal, M. Fawcett, C. Gradwell, C. Travers, B. Atkinson, S. Woodward, L. Giraldo, J. Byers, **Salford Royal Hospital** (1), B. Cheripelli (PI), S. Lee, **Southampton General Hospital** (1), R. Marigold (PI), S. Smith, **St George's Hospital** (3), L. Zhang (PI), R. Ghatala, C.H. Sim, **University Hospitals Coventry & Warwick** (4), U. Ghani (PI), K. Yates, **University College London** (2), D. Werring (PI), S. Obarey, **University Hospital of Birmingham** (2), M. Willmot (PI), K. Ahlquist, M. Bates, **Yeovil District Hospital** (4), K. Rashed (PI), S. Board.

Non-Enrolling Sites

Estonia

East Tallin Central Hospital, T. Toomsoo (PI). West Tallin Central Hospital, K. Gross-Paju (PI).

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

Karolinska University Hospital, E. Lundström (PI). Lund University Hospital, G. Andsberg (PI). Skaraborg Hospital Skövde, B. Cederin (PI).

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).

Trial Boards, Committees and Administrative staff

Trial Coordinating center: Ellisiv B. Mathiesen (Chief Investigator), Melinda B. Roaldsen, Agnethe Eltoft, David Perry, Mary-Helen Søyland, Tone Bratteng.

Trial Steering Committee: Bent Indredavik (Chair), Thompson G. Robinson, David Werring, Arnstein Tveiten, Jesper Petersson, Hanne Christensen, Helle Iversen, Jukka Putaala, Janika Kõrv, Dalius Jatuzis, Gian Marco De Marchis, Stefan Engelter, Erik Lundström, Tom Wilsgaard and Ellisiv B. Mathiesen.

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).

Image Analysis Centre: Andrew Bivard (Chair, Senior Reader), Mark Parsons (Senior Reader), Michael Valente, Amy Chen, Angelos Sharobeam, Leon Edwards, Christopher Blair.

Medical Monitors

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

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

Reference numbers from Research ethical committees:

Denmark: H-16031906, Estonia: 288/M-19, Finland: 145/13/03/00/16, Latvia: 1-47/616, Lithuania: P-16-57/2, New Zealand: 19/STH/87/AM02, Norway: 2014-000096-80, Sweden: 2016/359, Switzerland: 2017-00368, UK: 16/EM/0322 IRAS project ID 202096

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 haemorrhage, 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 haemorrhage 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 $100,000/\text{mm}^3$ (but patients on antiplatelet agents can be included)
 - Ischaemic stroke or myocardial infarction in previous 3 months, previous intracranial haemorrhage, 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)

Data monitoring of safety and efficacy

The Data Monitoring Committee reviewed unblinded safety and efficacy data based on closed reports from an independent statistician, distributed to the committee members in strictest confidence. If, in their view, there is credible evidence of harm, or overwhelming evidence of efficacy, the committee will advise the chairman of the Steering Committee. The DMC worked on the principle that a difference of at least 3 standard errors in an interim analysis of a major outcome event may be needed to justify halting, or modifying, a study before the planned completed recruitment (Haybittle-Peto rule). This criterion has the practical advantage that the exact number of interim analyses would be of little importance, and so no fixed schedule is proposed. A less-strict rule might be used if there was evidence that tenecteplase was unsafe. The DMC were to advise the chairman of the Trial Steering Committee if, in their view, sufficient evidence existed for (i) a clear conclusion to be made that, for all patients (or some), the treatment is clearly indicated or clearly contra-indicated or (ii) evidence that might reasonably be expected to materially influence future patient management. Unless this happened, the Trial Steering Committee, collaborators and central administrative staff were to remain unaware of any interim results. The Trial steering committee received no such information from the DMC and was therefore ignorant about any results throughout the course of the trial.

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 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. This decision was not based on interim analysis of trial data; the Trial Steering Committee remained blinded to the study results throughout the course of the trial. This decision was not based on interim analysis of trial data; the Trial Steering Committee remained blinded to the study results throughout the course of the trial. 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

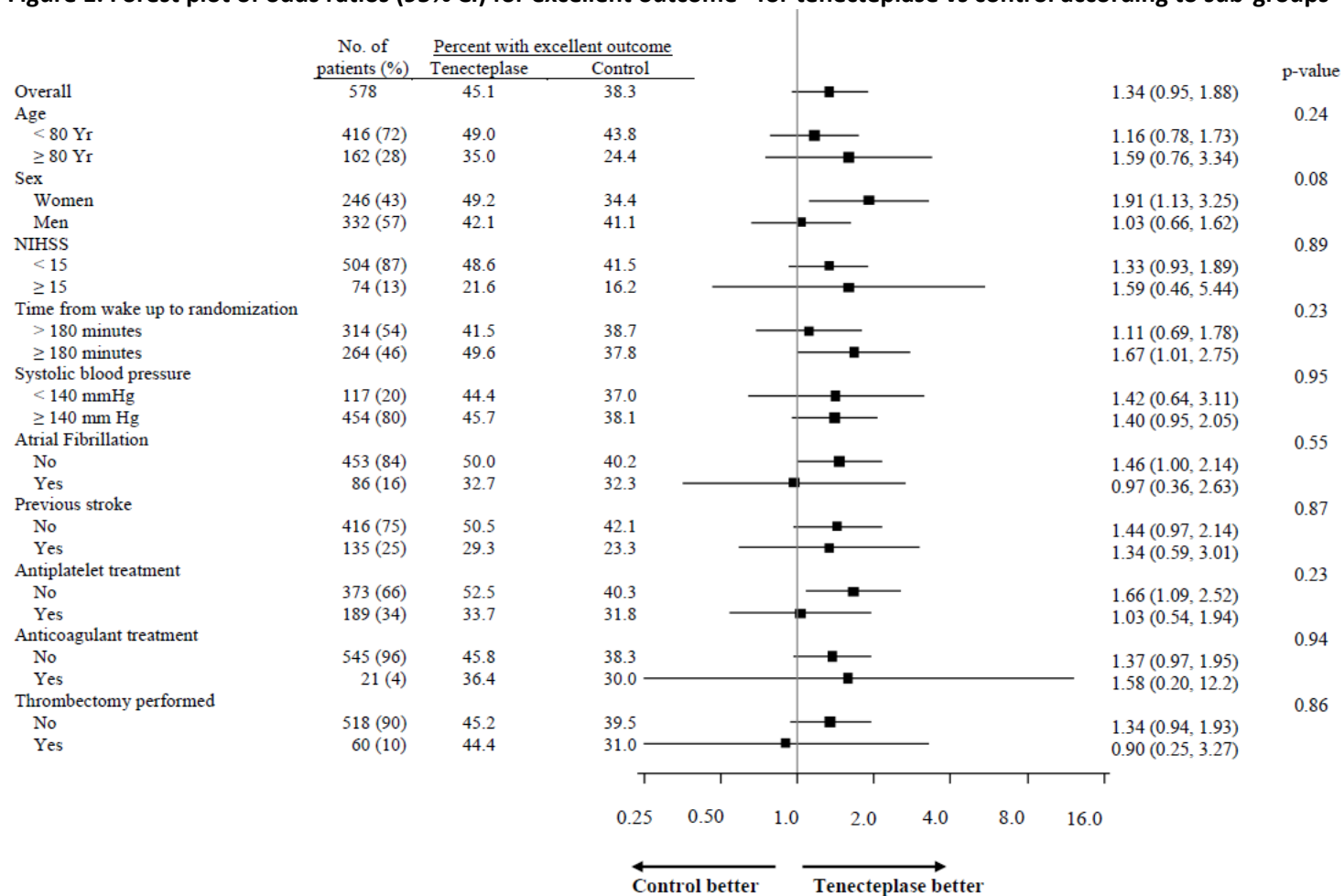
studies on patients with unknown stroke onset time,³ where favourable outcome was defined as mRS 0-2. The MRI-based inclusion criteria in WAKE-UP compared to the CT-based inclusion in TWIST could lead to a smaller treatment effect in TWIST. We assumed a treatment effect of 10% absolute difference in 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 randomisation	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 randomisation	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 randomisation, 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. Secondary efficacy outcomes (intention-to-treat population)

	Tenecteplase (n=288)	Control (n=290)
Barthel index*		
>= 61, no. (%)	223 (94)	217 (91)
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)	221 (76)
Moderate (8–14)	40 (14)	43 (15)
Severe (≥15)	29 (10)	26 (9)
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 (85)	233 (80)
Moderate (8–14)	30 (10)	40 (14)
Severe (≥15)	14 (5)	17 (6)
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.

Number of missing values: Barthel index: n=102, MMSE: n=188, EQ5-D-VAS: n=141, NIHSS at 24 hours: n=9, NIHSS at 7 days: n=30.

Table S3. 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 (14)	30 (11)				
1	90 (32)	78 (28)				
2	44 (16)	62 (22)				
3	57 (20)	57 (20)				
4	19 (7)	27 (10)				
5	6 (2)	8 (3)				
6	28 (10)	22 (8)				
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 (46)	108 (38)	1.37 (0.98, 1.91)	0.069	1.40 (0.99, 1.97)	
Good functional outcome [¶]	173 (61)	170 (60)	1.05 (0.75, 1.48)	0.76	1.07 (0.74, 1.54)	
Response to treatment according to baseline neurological deficit ^{**}		53 (19)	1.41 (0.94, 2.10)	0.098	1.41 (0.94, 2.12)	
Safety outcomes						
Deaths within 90 days after intervention	28 (10)	22 (8)	1.35 (0.77, 2.38)	0.30	1.33 (0.75, 2.34)	0.33
Symptomatic intracranial haemorrhage						
As defined by SITS- MOST ^{††}	6 (2)	3 (1)	2.03 (0.50, 8.19)	0.32	2.17 (0.53, 8.88)	0.28
As defined by IST-3 ^{††}	12 (4)	7 (3)	1.75 (0.68, 4.52)	0.25	1.74 (0.67, 4.50)	0.25
Parenchymal haemorrhage type 2 ^{§§}	7 (3)	4 (1)	1.78 (0.51, 6.13)	0.36	1.85 (0.53, 6.46)	0.33
Any intracranial haemorrhage	33 (12)	28 (10)	1.21 (0.71, 2.06)	0.49	1.22 (0.71, 2.10)	0.48
Poor functional outcome or death ^{¶¶}	53 (19)	57 (20)	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 haemorrhage 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 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 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 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 (14)	32 (11)				
1	88 (31)	77 (27)				
2	46 (16)	60 (21)				
3	56 (20)	58 (21)				
4	17 (6)	25 (9)				
5	6 (2)	7 (3)				
6	28 (10)	23 (8)				
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)	109 (39)	1.32 (0.94, 1.84)	0.11	1.32 (0.94, 1.87)	
Good functional outcome [¶]	173 (62)	169 (60)	1.08 (0.77, 1.52)	0.65	1.08 (0.75, 1.56)	
Response to treatment according to baseline neurological deficit ^{**}	69 (25)	56 (20)	1.32 (0.89, 1.97)	0.17	1.33 (0.89, 1.99)	
Safety outcomes						
Deaths within 90 days after intervention	28 (10)	23 (8)	1.29 (0.74, 2.26)	0.37	1.29 (0.74, 2.25)	0.38
Symptomatic intracranial haemorrhage						
As defined by SITS- MOST ^{††}	6 (2)	3 (1)	2.04 (0.50, 8.22)	0.32	2.15 (0.53, 8.77)	0.29
As defined by IST-3 ^{††}	12 (4)	8 (3)	1.53 (0.62, 3.81)	0.36	1.54 (0.62, 3.82)	0.36
Parenchymal haemorrhage type 2 ^{§§}	7 (3)	5 (2)	1.42 (0.45, 4.53)	0.55	1.46 (0.46, 4.69)	0.52
Any intracranial haemorrhage	33 (12)	29 (10)	1.17 (0.69, 1.98)	0.57	1.18 (0.69, 2.03)	0.54
Poor functional outcome or death ^{¶¶}	51 (18)	55 (20)	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 haemorrhage 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 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 modified Rankin Scale score of 4 (moderately severe disability), 5 (severe disability) or 6 (death) at 90 days of follow-up.

Table S5. 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 (14)	30 (11)				
1	90 (32)	78 (28)				
2	44 (16)	62 (22)				
3	57 (20)	57 (20)				
4	19 (7)	27 (10)				
5	6 (2)	8 (3)				
6	29 (10)	22 (8)				
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)	108 (38)	1.36 (0.97, 1.90)	0.074	1.39 (0.98, 1.95)	
Good functional outcome [¶]	173 (61)	170 (60)	1.05 (0.75, 1.46)	0.80	1.06 (0.74, 1.52)	
Response to treatment according to baseline neurological deficit ^{**}	69 (24)	53 (19)	1.40 (0.93, 2.09)	0.10	1.40 (0.94, 2.11)	
Safety outcomes						
Deaths within 90 days after intervention	29 (10)	22 (8)	1.40 (0.80, 2.45)	0.24	1.37 (0.78, 2.41)	0.27
Symptomatic intracranial haemorrhage						
As defined by SITS- MOST ^{††}	6 (2)	3 (1)	2.02 (0.50, 8.16)	0.32	2.15 (0.53, 8.81)	0.29
As defined by IST-3 ^{‡‡}	13 (5)	7 (3)	1.90 (0.75, 4.83)	0.18	1.89 (0.74, 4.82)	0.18
Parenchymal haemorrhage type 2 ^{§§}	7 (3)	4 (1)	1.77 (0.51, 6.11)	0.37	1.84 (0.53, 6.41)	0.34
Any intracranial haemorrhage	34 (12)	28 (10)	1.24 (0.73, 2.11)	0.42	1.26 (0.73, 2.16)	0.41
Poor functional outcome or death ^{¶¶}	54 (19)	57 (20)	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 haemorrhage 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 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 modified Rankin Scale score of 4 (moderately severe disability), 5 (severe disability) or 6 (death) at 90 days of follow-up.

Table S6. 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	38	
Age, years					0.38
< 80	416 (72)	1.09 (0.77, 1.54)	49	44	
≥ 80	162 (28)	0.98 (0.71, 1.37)	42	39	
Sex					0.07
Women	246 (43)	1.61 (1.02, 2.52)	49	34	
Men	332 (57)	0.93 (0.63, 1.36)	42	41	
NIHSS					0.74
< 15	504 (87)	1.05 (0.60, 1.83)	49	42	
≥ 15	74 (13)	0.98 (0.43, 2.24)	22	16	
Time to wake-up to randomization, minutes					0.12
< 180	314 (54)	0.94 (0.64, 1.40)	42	39	
> 180	264 (46)	1.58 (1.02, 2.44)	50	38	
Country					0.68
Norway	157 (27)	0.93 (0.53, 1.64)	37	42	
Sweden	48 (8)	1.47 (0.51, 4.22)	64	46	
Denmark	33 (6)	1.21 (0.33, 4.46)	56	40	
Finland	32 (6)	2.00 (0.50, 8.04)	56	21	
Estonia	20 (3)	2.95 (0.45, 19.3)	38	8	
Latvia	11 (2)	1.10 (0.08, 14.4)	33	40	
Lithuania	74 (13)	2.04 (0.86, 4.85)	22	17	
United Kingdom	165 (29)	1.22 (0.70, 2.12)	54	46	
Switzerland	24 (4)	6.75 (0.80, 57.4)	50	38	
New Zealand	14 (2)	1.32 (0.16, 11.3)	83	50	
Systolic blood pressure, mmHg					
< 140	117 (20)	1.12 (0.58, 2.14)	44	38	
≥ 140	454 (80)	1.24 (0.89, 1.73)	46	38	
Atrial fibrillation					0.75
No	453 (84)	1.23 (0.88, 1.71)	50	40	
Yes	86 (16)	1.19 (0.53, 2.66)	33	32	
Previous stroke					0.03
No	416 (75)	1.46 (1.03, 2.06)	51	42	
Yes	135 (25)	0.70 (0.38, 1.28)	29	23	
Antiplatelet treatment					0.05
No	373 (66)	1.53 (1.06, 2.21)	53	40	
Yes	189 (34)	0.79 (0.47, 1.31)	34	32	
Anticoagulant treatment					0.19
No	545 (96)	1.15 (0.86, 1.56)	46	38	
Yes	21 (4)	4.16 (0.75, 23.1)	36	30	
Thrombectomy performed					0.48
No	518 (90)	1.23 (0.90, 1.67)	45	40	
Yes	60 (10)	0.79 (0.27, 2.32)	44	31	

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

Table S7. 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)	28 (11)			2 (11)	4 (10)		
1	84 (31)	70 (28)			6 (33)	9 (21)		
2	45 (17)	56 (23)			2 (11)	6 (14)		
3	55 (20)	52 (21)			3 (17)	7 (17)		
4	16 (6)	21 (9)			3 (17)	6 (14)		
5	6 (2)	4 (2)			0 (0)	4 (10)		
6	26 (10)	17 (7)			2 (11)	6 (14)		
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)	98 (40)	1.34 (0.94, 1.93)		8 (44)	13 (31)	0.90 (0.25, 3.27)	
Good functional outcome¶	167 (62)	154 (62)	1.10 (0.75, 1.61)		10 (56)	19 (45)	0.82 (0.23, 2.92)	
Response to treatment**	63 (23)	45 (18)	1.33 (0.86, 2.05)		7 (39)	11 (26)	1.32 (0.34, 5.04)	
Safety outcomes								
Deaths within 90 days after intervention	26 (10)	17 (7)	1.25 (0.66, 2.35)	0.49	2 (11)	6 (14)	0.95 (0.17, 5.39)	0.95
Symptomatic intracranial haemorrhage								
As defined by SITS-MOST††	5 (2)	1 (0.4)	5.87 (0.66, 52.4)	0.11	1 (6)	2 (5)	1.35 (0.09, 21.3)	0.83
As defined by IST-3‡‡	10 (4)	2 (0.8)	4.71 (1.02, 21.8)	0.047	2 (11)	6 (14)	0.92 (0.14, 6.00)	0.93
Parenchymal haemorrhage type 2§§	5 (2)	1 (0.4)	5.87 (0.66, 52.4)	0.11	2 (11)	4 (10)	2.25 (0.27, 19.0)	0.46
Any intracranial haemorrhage	27 (10)	13 (5)	1.95 (0.97, 3.89)	0.059	6 (33)	17 (41)	1.08 (0.28, 4.14)	0.91
Poor functional outcome or death¶¶	48 (18)	42 (17)	0.87 (0.52, 1.46)	0.61	5 (28)	16 (38)	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 haemorrhage 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 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 modified Rankin Scale score of 4 (moderately severe disability), 5 (severe disability) or 6 (death) at 90 days of follow-up.

Table S8. Details of all deaths during follow-up

Age	Sex	Baseline NIHSS	Treatment group	Intra-arterial intervention	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 ischaemic stroke†
76	F	17	Tenecteplase	N	Recurrent ischaemic 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 ischaemic 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 ischaemic 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)