Protocol

Tenecteplase in Wake-up Ischaemic Stroke Trial (TWIST)

A randomised-controlled trial of thrombolytic treatment with tenecteplase for acute ischaemic stroke upon awakening

EudraCT number: 2014-000096-80

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Signature page

Sponsor

_____

Director of Center for Quality Improvement and Development (signature and date)
University Hospital of North Norway

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Trial Co-coordinating Investigator and Head of Brain and Circulation Research Group (signature and date)
University Hospital of North Norway and University of Tromsø

Principal investigator

I have read this protocol and agree that it contains all the necessary details for carrying out the study. I will conduct the study as outlined herein and will complete the study within the time designated.

I will provide copies of the protocol and all pertinent information to all individuals responsible to me who assist in the conduct of the study. I will discuss this material with them to ensure that they are fully informed regarding the study intervention and the conduct of the study.

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Principal investigator (signature and date)

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Name of institution

1. Contents

2. Summary

Tenecteplase in Wake-up Ischaemic Stroke Trial (TWIST) is a randomised-controlled, open-label trial of thrombolytic treatment with tenecteplase for acute ischaemic stroke upon awakening.

The trial aims to answer the following questions:

- Can thrombolytic treatment with tenecteplase given within 4.5 hours of wake-up reduce the risk of poor functional outcome at 3 months?
- Can findings on plain CT and CT angiography (and CT perfusion at selected centres) identify patients who benefit from such treatment, compared to patients without such findings?

Patients are eligible if they have ischaemic stroke causing measurable neurologic deficits, and can be given tenecteplase within 4.5 hours after awakening. Patients will undergo brain CT and CT angiography (if possible) to exclude large infarction or other contraindications to thrombolytic treatment. Plain CT and CT angiography (if possible) will be repeated on day 2.

Patients will be allocated to tenecteplase 0.25 mg/kg as a bolus (maximum dose 25 mg) or to control. The primary effect variable is functional outcome at 3 months, as measured by the modified Rankin Scale (mRS).

The target is to include a total of 500 patients from centres in Norway, Sweden, Denmark, Finland, Estonia and Lithuania and the United Kingdom (UK). The sponsor for the trial is University Hospital of North Norway.

3. Background and rationale

3.1 Introduction

Thrombolytic treatment with recombinant tissue plasminogen activator (rt-PA) given within 4.5 hours of onset improves clinical outcome after ischaemic stroke, despite an increased risk of intracranial haemorrhage.[1,2] Patients who have new stroke symptoms when they wake up from sleep ("wake-up stroke") are excluded for such treatment, because the time of stroke onset is unknown. About one in five strokes occur during sleep,[3-5] and it is unknown whether patients with wake-up stroke will benefit from thrombolytic treatment.

There are reasons to expect that patients with wake-up stroke will benefit from treatment. Several studies have shown that the onset of stroke during sleep is close to awakening,[6,7] and that patients with wake-up stroke share many clinical and radiological findings with patients with stroke duration less than 4.5 hours.[8-13] Studies have also shown that thrombolytic treatment is safe in patients with wake-up stroke up to 4.5 hours from wakening,[14-19] and recently the WAKE-UP trial showed benefit of treatment with alteplase in patients selected based on DWI/FLAIR mismatch on MRI.[20] In a Cochrane systematic review[21] we have otherwise identified only one completed, small randomised-controlled trial of only nine patients,[22] but three trials are on-going (EXTEND,[23] THAWS,[24] and WASSABI,[25] see Appendix). All three trials use alteplase, and all use advanced imaging techniques for selection of patients. We will use a different strategy, and perform a large trial of tenecteplase, using a pragmatic study design, without exclusion of patients based on MRI findings. The trial will be complementary to the other trials, and is planned in advance to be included in our Cochrane meta-analysis of all trials of thrombolytic treatment for wake-up stroke, so that smaller, but still worthwhile effects can be detected that may be missed by the individual trials.

3.2 Potential benefits and harms of tenecteplase

Pharmacological properties

Tenecteplase is an alteplase molecule that is genetically engineered to have pharmacological advantages over alteplase: it has a 14-fold greater fibrin specificity and a very rapid onset of action compared to alteplase, has a longer half-life, and can be given as a single bolus, whereas alteplase must be given as a continuous infusion over one hour.[26,27] Tenecteplase also has a smaller effect on plasma fibrinogen levels,[28] which is beneficial for avoidance of intracranial haemorrhage,[29] and weaker pro-thrombotic effects.[30-32]

Data from pre-clinical studies

Animal models have shown that tenecteplase acts faster[33] and more potent[33-35] than alteplase, and that tenecteplase produces a more rapid and complete recanalisation of occluded arteries.[31,34,36] Models have also shown that experimentally induced infarcts were less frequently converted to haemorrhage with tenecteplase than with bio-equivalent doses of alteplase[36,37] and that the risk peripheral or major haemorrhage[31] and intracranial haemorrhage[36-41] was equal to or lower than the risk with alteplase.

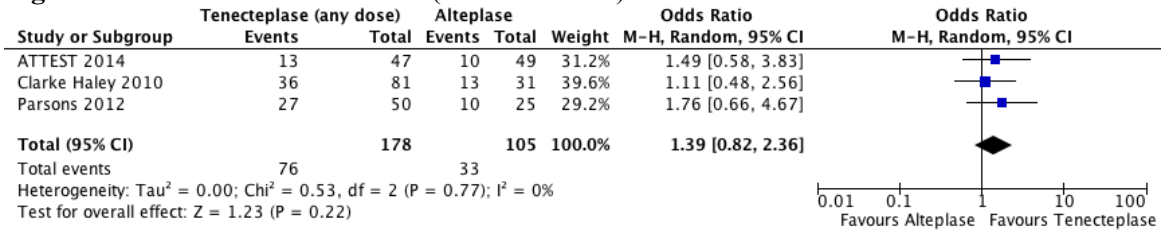
Data from clinical studies

Tenecteplase is preferred over alteplase for treatment of myocardial infarction, because it has lower risk of bleeding complications, and because of the pharmacological advantages listed above.[42]

A number of observational studies in patients with ischaemic stroke have shown that, compared to alteplase, tenecteplase is associated with similar or better recanalisation rates and clinical outcomes, and a similar or lower risk of intracranial haemorrhage.[43-47] These studies have also concluded that the optimal dose of tenecteplase for stroke is 0.25 mg/kg.[28,45,48,49]

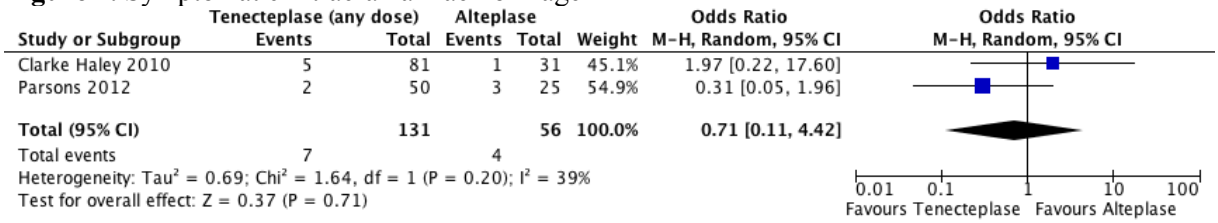
There has also been three small randomised-controlled trials of tenecteplase vs. alteplase in acute ischaemic stroke.[45,47,48] Meta-analysis of these trials with a total of 283 patients have shown that 76/178 patients (43%) patients given tenecteplase (doses ranging from 0.1 to 0.4 mg/kg) achieved good functional outcome (mRS score 0-1), compared to 33/105 patients (31%) patients given alteplase (odds ratio (OR) 1.39, 95% confidence interval (CI) 0.82-2.36, Figure 1).

Figure 1. Good functional outcome (mRS score 0-1) at three months



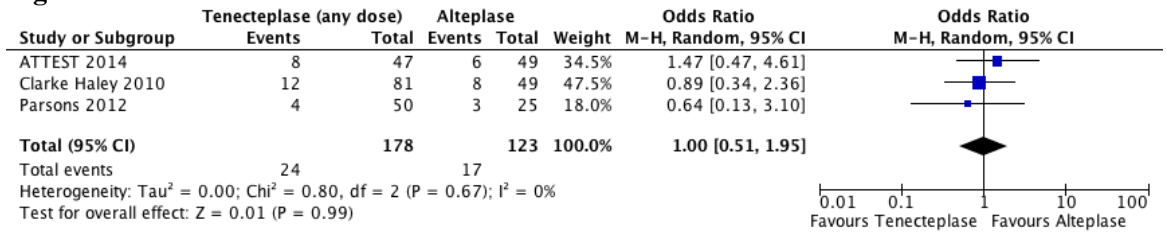
There was also a non-significant lower risk of symptomatic intracranial haemorrhage in patients given tenecteplase (Figure 2). The risks were 7/131 (5%) in patients given tenecteplase and 4/56 (7%) in patients given alteplase (OR 0.71, 95% CI 0.11-4.42). ATTEST 2014 did not provide data on symptomatic intracranial haemorrhage.

Figure 2. Symptomatic intracranial haemorrhage



There was no significant difference in the risk of death at three months (Figure 3). In total, 24 of the 178 patients (13%) given tenecteplase and 17 of the 123 patients (14%) given alteplase were dead at three months (OR 1.00, 95% CI 0.52-1.95).

Figure 3. Death of all causes at three months



Another meta-analysis comparing different doses of tenecteplase concluded that 0.25 mg/kg is the preferred dose over alteplase.[28,50] There are also observational studies that support this conclusion.[28,49] Furthermore, an analysis of pooled data for tenecteplase 0.25 mg/kg compared to alteplase 0.9 mg/kg showed trends in favour of tenecteplase.[50] Tenecteplase had higher odds than alteplase for neurological improvement at 24 hours (OR 3.3, 95% CI 1.5-7.2, p=0.09), excellent clinical outcomes at three months (OR 1.9, 95% CI 0.8-4.4, p=0.3), and good clinical outcomes at three months (OR 1.9, 95% CI 0.5-7.2, p=0.4); also, tenecteplase had a lower risk of brain haemorrhage than alteplase (OR 0.3, 95% CI 0.2-1.8, p=0.4). Recently, NOR-TEST showed no difference in functional outcome at three months between patients with ischaemic stroke treated with tenecteplase 0.4 mg/kg or alteplase 0.9 mg/kg within 4.5 hours[51] and the EXTEND-IA TNK trial showed that tenecteplase 0.25 mg/kg was superior to alteplase 0.9 mg/kg in improving recanalisation and functional outcome at three months when given within 4.5 hours and prior to intra-arterial intervention.[52] Other trials of tenecteplase in patients with acute ischaemic stroke are ongoing: TASTE[53], TEMPO-2[54] and ATTEST2.[55]

Conclusion: Potential benefits and harms of tenecteplase

The bolus administration and the very rapid onset of action makes tenecteplase an attractive option for patients with wake-up stroke, because the time to recanalisation of an occluded cerebral artery can be reduced by up to one hour, compared to alteplase.[17] Available data from pharmacological in-vitro studies, pre-clinical studies and clinical studies also indicate that tenecteplase 0.25 mg/kg is at least as safe and at least as effective as alteplase for the treatment of acute ischaemic stroke. The favourable profile of tenecteplase seen in these studies and in studies of acute myocardial infarction, in combination with the pharmacological characteristics and advantages of bolus administration, provides a good rationale for testing tenecteplase in patients with wake-up stroke.

3.3 Multi-modal CT for the selection or patients to thrombolytic therapy

The WAKE-UP trial and the three on-going trials of thrombolytic treatment for wake-up stroke use advanced imaging techniques for selection of patients.[20,23-25] Two trials (WASSABI[25] and EXTEND[23]) use CT or MRI perfusion techniques to identify patients with ischaemic penumbra. The penumbra is an area of ischaemic, but still viable brain tissue surrounding the core of a cerebral infarction, which is assumed to be salvageable if blood flow can be promptly restored. The WAKE-UP trial[20] and the on-going THAWS[24] use DWI/FLAIR mismatch on MRI to select patients. Infarct changes on DWI are thought to represent alterations to water diffusion and can be visualised within minutes of onset. Infarct changes on FLAIR are thought to represent cytotoxic oedema and cannot be identified until a few hours after onset. Findings suggestive of ischaemic stroke on DWI, but absence of such findings on FLAIR (DWI/FLAIR mismatch), may therefore identify patients with a short time since onset of cerebral infarction.

Demonstration of penumbra or DWI/FLAIR mismatch identifies patients with a good prognosis, but it does not necessarily identify patients with a better response to thrombolytic treatment than other patients. Only one randomised-controlled trial (MR RESCUE, of intra-arterial interventions) has examined the value of advanced imaging for patient selection[56], but did not find a better effect of therapy in patients with penumbra, than in patients without penumbra.[56]

On the contrary, there is a risk that selection of patients based on such techniques will exclude patients from receiving an effective treatment. First, it is possible that patients without these specific radiological findings benefit equally well as those who are selected for participation in these trials, as indicated in a recent study.[19] Second, the techniques are far from perfect in characterising patients, for example, DWI/FLAIR mismatch can be absent in as many as 40% of patients with known stroke duration of less than three hours.[57] Third, these MRI techniques are time-consuming and are not available in the emergency setting in many hospitals, which means that time to thrombolytic treatment will be increased, and that some patients will be denied thrombolytic treatment altogether.

We will therefore base patient inclusion on wider and more pragmatic criteria, such as time since wake-up in combination with accepted CT exclusion criteria. Inclusion based on wider criteria will avoid excluding a large number of patients that might benefit from treatment. This will make the results applicable to patients that are encountered in everyday clinical practice, and will dramatically increase the proportion of stroke patients who can be treated. Patient can be included based on plain CT alone, but CT angiography (and CT perfusion at selected centres) can be routinely done in many hospitals, can be done without significant delay, and can give information that will help in the selection of patients with wake-up stroke for thrombolytic treatment.[58-62]

4. Trial design, questions, and hypotheses

4.1 Trial design

Randomised-controlled, open-label trial of tenecteplase in patients with acute ischaemic stroke upon awakening.

4.2 Study questions and objectives

The objectives of the trial is two answer the following questions, for patients who have an acute ischaemic stroke upon awakening:

- Can thrombolytic treatment with tenecteplase given within 4.5 hours of wake-up reduce the risk of poor functional outcome at 3 months?
- Can findings on plain CT and CT angiography (and CT perfusion at selected centres) identify patients who benefit from such treatment, compared to patients without such findings?

4.3 Hypotheses

- Thrombolytic treatment with tenecteplase will reduce the risk of poor functional outcome at 3 months. For details about size of effect, see Statistical calculations, below.
- CT angiography and CT perfusion identifies patients who benefits more from tenecteplase than other patients

5. Study population

Patients with acute ischaemic stroke upon awakening who can be given treatment within 4.5 hours after awakening.

5.1 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

5.2 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 plain CT or CT perfusion
  - Intracranial haemorrhage, structural brain lesions which can mimic stroke (e.g cerebral tumour)
- 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 andexanet) 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)
  - 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; haemostatic 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 mRS score ≥3), or life expectancy less than 12 months
- Patient unavailability for follow-up (e.g. no fixed address)

6. Patient screening

6.1 Patient screening

All patients who are admitted with new stroke symptoms on awakening should be screened for inclusion into the trial. Patients will be evaluated for vital signs, physical examination, neurological status (NIHSS), ECG and CT with CT angiography (section 6.2). Routine blood tests will include blood glucose and prothrombin time/INR*, aPTT*, TT and/or eucarin clotting time if it is suspected the patient is taking direct thrombin inhibitors or direct factor Xa inhibitors.

*Although it is desirable to know the results of these tests before giving intravenous tenecteplase, fibrinolytic therapy should not be delayed while awaiting the results unless (1) there is clinical suspicion of a bleeding abnormality or thrombocytopenia, (2) the patient has received heparin or warfarin within 48 hours, or (3) the patient has received other anticoagulants/direct thrombin inhibitors or direct factor Xa inhibitors.

A limited number of centres will be asked to make records of all patients considered for inclusion into the trial (screening logs), to account for patients that were screened, but not included into the trial.

6.2 Plain CT and CT angiography for patient screening before randomisation

All patients should be screened with plain CT, and CT angiography should be performed, if possible. Findings on plain CT shall only be used to exclude patients who are unlikely to benefit from treatment (See Exclusion criteria in Section 5). If other, advanced imaging examinations are used (see sections 6.3 and 6.4, below), findings on such examinations shall not influence the decision to include the patient, unless the results of such imaging show that the patient should or should not receive thrombolytic treatment, in the opinion of the investigator.

6.3 CT perfusion at selected centres before randomisation (substudy)

Some centres routinely use CT perfusion in patients with wake-up stroke, and these centres will be invited to participate in a substudy of CT perfusion. All CT scans taken in the trial will be sent to the Trial Co-ordinating Centre for central, blinded analysis.

6.4 Routine use of MRI before randomisation

Some centres routinely use MRI perfusion or MRI DWI/FLAIR in patients with wake-up stroke. These examinations are not part of the trial, but MRI examinations done of patients included into the trial shall be sent to the Trial Co-ordinating Centre for central, blinded analysis. Extra brain scanning (CT perfusion or MRI) must not delay treatment, and we will discourage extra scanning if randomisation is delayed more than 20 minutes.

7. Patient inclusion

7.1 Patient information and consent/assent

Written, informed consent will be sought from all eligible patients. If the patient can consent but cannot sign, non-written consent from the patient (witnessed by non-participating health care personnel) can be accepted. If the patient is unable to consent, his/her legal representative and/or next of kin can consent on the patient's behalf depending on national legislation. The regulations governing these procedures may vary from jurisdiction to jurisdiction. The procedures for obtaining consent will therefore have to be approved for each jurisdiction, and will be described in separate standard operating procedures. It is noted that legal restrictions in some EU Member States prevent performing any trial-specific interventions (e.g. treatment or trial-specific diagnostic procedures without obtaining prior informed consent).

If a patient was unable to give consent at trial entry, but regains capacity to receive information and give consent, he/she will be given information as soon as possible and asked whether he/she is willing to continue his/her participation in the trial. The patient and his/her legal representative will be informed of the right to withdraw from the trial and object to the use of his/her data.

7.2 Randomisation procedure and recording of baseline characteristics

Patients will be randomly allocated to tenecteplase or control in a 1:1 ratio. Randomisation will be performed by central computer over the Internet. The investigators will record patient details via a secure web interface before randomisation takes place. Investigators may also contact the Trial Coordinating Centre via the 24-hours help-line.

The randomisation procedure will include a standard minimisation algorithm which will ensure that the treatment groups are balanced for key prognostic factors, such as sex, age, NIHSS score, stroke type (OCSP subgroup), delay to thrombolytic treatment, and use of antiplatelet drugs within the past 24 hours. To avoid predictable alternation of treatment allocation, and thus potential loss of allocation concealment, patients will be allocated with a probability of 0.80 to the treatment group that would minimise the difference between the groups on the key prognostic factors.

8. Trial treatment

Patients will be randomly allocated to open-label tenecteplase plus best standard care or to best standard care alone.

8.1.1 Open-label tenecteplase plus best standard care

The total dose of tenecteplase is 0.25 mg per kg of body weight (maximum 25 mg). The dose shall be given as an intravenous bolus. Patients will be on bed rest in accordance with local routine following administration of tenecteplase.

After infusion, vital signs, neurological signs and blood pressure will be monitored every 15 minutes for two hours, then every 30 minutes for 6 hours, than 60 minutes until 24 hours from the start of treatment. Blood pressure should be maintained at or below 180/105 mmHg during the first 24 hours. Placement of intra-arterial catheters, indwelling bladder catheters, and nasogastric tubes shall be avoided for 24 hours if the patient can be safely managed without them. Venipunctures should be performed and monitored carefully.

8.1.2 Best standard care alone

Patients randomised to control shall not be given tenecteplase or any other thrombolytic agent.

8.1.3 Best standard care and other treatments

Both arms will receive best standard care, including intra-arterial interventions for proximal cerebral artery occlusion. If the patient is given tenecteplase, then aspirin or other antiplatelet or anticoagulant drugs shall not be given until 24 hours after termination of infusion and after the control CT brain scan. If the patient was allocated to control, he/she will receive aspirin 300 mg as a loading dose as soon as possible after randomisation. After the first 24 hours the recommended daily dose of aspirin is 75 mg once daily in both the tenecteplase group and the control group. Best standard care during the first week also includes treatments to maintain normal homeostasis (temperature, blood glucose, hydration, nutrition), as well as lipid lowering and blood pressure lowering drugs, in accordance with clinical guidelines. Clinical examinations, including additional CT scans will be performed as clinically indicated.

8.1.4 Management of serious adverse events related to study treatment

The management of serious adverse events that can be related to treatment with tenecteplase will be guided by specific, detailed standard operating procedures. All patients with neurological deterioration will undergo emergency head CT. In case of a major bleeding (e.g. intracranial

haemorrhage), antifibrinolytic agents or transfusion of cryoprecipitate, fresh frozen plasma or platelets may be indicated and should be considered. The investigator should institute any supplementary investigations of serious adverse events based on their clinical judgement of the likely causative factors. This may include seeking further opinion from a specialist in the field of the adverse event.

9. Visits and examinations from trial entry till end of follow-up

The duration of the follow-up period is 3 months. During this period, all patients should be treated according to standard clinical guidelines, at the discretion of the clinician. Table 2 gives an overview of all visits and examinations in the trial.

Table 2. Scheme for visits and examinations

	Days			Months
	1	2	7	3
Clinical visit	x		x	
Routine blood analysis	x		x	
CT + CT angiography (if possible) before randomisation	x			
CT perfusion, at selected centres	(x)			
CT + CT angiography (if possible) 24±6 hrs after randomisation		x		
Centralised follow-up				x

Day 1 is the day of entry into the trial. *Day 7 or day of discharge, whichever occurs first.

9.1.1 Visits and examinations during stay in hospital

Clinical visits: These will be performed on day 1 (at baseline) and day 7 (or on the day of discharge, whichever occurs first). NIHSS will be performed at both times. ECG will be performed on day 1. Clinical events will be recorded on day 7 (or on the day of discharge). Outcome assessors will be blinded to the assigned treatment group.

Routine blood analysis will be performed on day 1 (at baseline) and day 7 (or on the day of discharge, whichever occurs first). Tests on day 1 will include: haemoglobin, creatinine, blood glucose, platelets, prothrombin time/INR, aPTT, TT and/or eucarin clotting time if it is suspected that the patient is taking direct thrombin inhibitors or direct factor Xa inhibitors.

Plain CT and CT angiography (if possible): All patients will undergo plain CT and CT angiography (if possible) before randomisation, and again at 24±6 hours after randomisation. CT angiography at 24±6 hours will only be performed in patients with a positive finding on CT angiography before randomisation.

CT perfusion will be performed at selected centres.

9.1.2 Centralised follow-up via telephone or mail at 3 months (90 ±7 days)

At 90±7 days, the Trial Coordinating Centre will contact patients (or their carers) via telephone (alternatively, via postal mail), blinded to the treatment that the patients received. We may also need to contact the patients' general practitioners and local hospitals to get information from the patients' medical records. We will record information about clinical events, functional status (mRS), activities of daily living (BI), health-related quality of life (EuroQol), and cognitive status (MMSE, TICS-M).

9.1.3 Long-term follow-up by record linkage with central registries

We will collect data up to three years from trial entry, by using record linkage with data from central registries such as national patient registries and the cause-of-death registries in the participating countries.

10. Effect variables

10.1 Primary effect variable

Functional outcome (defined by the mRS) at 3 months

10.2 Secondary effect variables

Clinical events:

- Intracranial haemorrhage during follow-up
- Stroke progression during follow-up
- Recurrent ischaemic stroke during follow-up
- Death from all cause during follow-up

Clinical events are defined in the Appendix.

Clinical outcomes:

NIHSS score, Barthel Index score, EuroQol score, and MMSE scores at 3 months

Radiological outcomes

Radiological outcomes will be defined in a separate imaging protocol.

Health-economic variables:

Costs related to:

- Length of hospital stay
- Nursing home care after discharge
- Re-hospitalisations during first 3 months

11. Analysis and statistical considerations

11.1 Estimation of sample size

Based on the results of a Cochrane systematic review of the effect of rt-PA within 4.5 hours of stroke onset, we assume a 9% absolute risk reduction in patients treated with tenecteplase (as expressed by the common odds ratio from an ordinal regression analysis).[63]

We anticipate that 30% of the patients in the control group will have a good functional outcome at 3 months, defined as mRS score 0 to 2. Using a conventional sample size calculation (dichotomising mRS using logistic regression) we would need 433 patients in each group, or 866 patients in total (assuming a 5% two-sided level of significance and 80% statistical power). Ordinal regression analysis (shift analysis) will increase statistical power substantially, equivalent to a reduction of the order of 30% in the sample size without loss of statistical power. Adjusting the analyses for prespecified covariates will reduce heterogeneity and further increase statistical power.[64, 65] We therefore plan a study of 500 patients (250 patients in each group).

There are about 50,000 patients with first-ever strokes annually in the Nordic countries, and about 15% of these patients have wake-up strokes (7,500 patients/year). If 3.5% of these patients can be recruited (260/year), recruitment can be completed within two years.

11.2 Statistical analyses

A detailed Statistical Analysis Plan will be drawn up before breaking the randomisation code. We will analyse the data according to the intention-to-treat principle. Functional outcome will be primarily compared between the study groups by means of ordinal logistic regression, after adjustment for covariates. In a secondary analysis, functional outcome will be dichotomised (mRS score 0-1 vs. 2-6) and analysed by means of logistic regression.

For clinical events we will estimate odds ratios and 95% confidence intervals using logistic regression and estimate hazard ratios and 95% confidence intervals using the Cox proportional hazards model. Interactions will be tested using likelihood ratio tests. The risks of clinical events will be compared using Kaplan-Meyer survival analyses and log-rank tests, after adjustment for confounding variables. All analyses will use 5% two-sided level of significance.

We will perform prespecified subgroup analyses of patients with findings suggestive of penumbra (on CT perfusion), of patients included based on presence or absence of DWI/FLAIR mismatch on MRI, of patients with proximal cerebral artery occlusion, and of patients with different time intervals since awakening, controlling for imbalances in baseline characteristics. Subgroup analyses will be prespecified in the Statistical Analysis Plan. Patients treated with intra-arterial intervention for proximal cerebral artery occlusion will also be analysed separately, and the primary effect variable for this analysis will be cerebral arterial patency before intra-arterial intervention.

Any missing components of the baseline NIHSS will be imputed using a regression-based technique, using age and all other components of the NIHSS. A worst-case approach will be used when handling incomplete dates and times for adverse events. For example, events will be assumed to be treatment emergent unless it is clear even from the partial date/time that this is not the case.

As subgroup analyses are of an exploratory nature, no adjustment for multiple comparisons will be made.

12. Trial conduct and practices/procedures

12.1 Compliance with regulations and guidelines

The trial will conform to the EU Clinical Trials Directive (2001/20/EC) and national applicable regulatory requirements. EudraCT number: 2014-000096-80. ISRCTN number: 10601890. ClinicalTrials.gov number: NCT03181360.

12.2 Data protection

Personal identifiers will not be stored together with clinical information about the patient, but will be stored on a separate, password-protected computer with access only for persons in the Trial Coordinating Centre who are responsible for central follow-up. The code linking personal identifiers with clinical data will be destroyed 15 years after the publication of the primary report, of the trial. The procedures for protection of personal information will be approved by the Research Ethics Committee, and data protection officials at the University Hospital of North Norway.

12.3 Ethical conduct

The trial will be 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 (CETS No.: 164), the ICH Harmonized Tripartite Guideline for Good Clinical Practice (CPMP/ICH/135/95) and the Declaration of Helsinki (Edinburgh, October 2000).

We will seek informed consent from all patients, and approval from the ethics committees, according to local or national regulations. All patients will be monitored carefully during treatment and follow-up, and procedures for management of adverse drug reactions, unmasking of the trial treatment, and reporting of serious adverse events are given in the protocol. All events will be evaluated, and the trial will be monitored, audited, and inspected according to applicable regulatory requirements.

12.4 Liability

Patients are indemnified by insurance specific for patients participating in trials of an Investigational Medicinal Product (IMP). Patients are also covered for non-negligent liability by the product indemnity provided by the supplier.

12.5 Recording and reporting of serious adverse events

Serious adverse events will be recorded in the case report forms. In case of unexpected serious adverse events, the Trial Coordinating Centre should be notified immediately and within 24 hours at the latest, in accordance with the EC guidance document 2011/C 172/01 ("CT-3"). The report of the SAE must include an assessment of whether there is a reasonable possibility that the IMP caused the event.

Reports of suspected unexpected serious adverse reactions (SUSARs), with all relevant information, will be reported in an expedited manner by the Sponsor, to the competent authority, the ethics committee, and the Data Monitoring Committee, according to the EU Clinical Trials Directive (2001/20/EC) and applicable regulatory requirements. Copies of such reports will be sent to the principal investigators and the Sponsor. (Definitions of serious adverse events and SUSARs: See Appendix).

Serious adverse events that are expected will not be reported in an expedited manner by the Sponsor, for example recurrent ischaemic stroke, intracranial haemorrhage, myocardial infarction, or death. These are events that are expected, and that will be reported as end-points in the case report forms. Expected adverse reactions listed in Section 4.8 of the Summary of Product Characteristics will also not be reported in an expedited manner.

12.6 Event adjudication

A central Event Adjudication Committee will evaluate all events, blinded to treatment allocation.

12.7 Monitoring of data quality

The central computer randomisation system will check eligibility for all patients entered in the trial. Data entered over the Internet (at trial entry) and data in case report forms will be checked for validity and internal consistency, to ensure high data quality and completeness, and coherence with the protocol. Centres with poor standards will be contacted and appropriate measures will be taken. During the course of the study the Sponsor will visit all centres at start-up and least once for monitoring purposes, with review of the CRF, comparison with source documents, and observation of the conduct of the trial and adherence to the Protocol. It will be particularly important to check that each patient exists, that a valid consent form is present in the hospital notes, to confirm patient information and adherence to the protocol. There will also be frequent telephone contacts, with the purpose of facilitating the work and fulfilling the objectives of the study.

12.8 Handling of protocol violations and protocol amendments

The nature and reasons for the protocol violation shall be recorded in the CRF, in the source documents and in the monitoring visit report. All of this serious non-compliance will be followed up and reported to RA and IEC as per local regulations. In parallel, corrective and/or preventive actions will be undertaken and documented, including any retraining of the investigator and site staff. All patients who have been included in the trial will be followed up, irrespective of whether treatment was discontinued prematurely, or whether the protocol was violated. If treatment discontinuations or protocol violations become frequent the Data Monitoring Committee will consider whether there is a need to increase the number of patients to be included in the trial.

All important changes to the trial will be specified in protocol amendments. Amendments must be approved by the Steering Committee, and the Sponsor has the responsibility to seek approval from the competent authorities the ethics committees. Completed and signed protocol amendments will be circulated to all those who were on the circulation list for the original protocol. Amendments will be listed in chapter 17.

12.9 Monitoring of effectiveness and safety

During the course of the trial the Data Monitoring Committee (DMC) will every 6 months perform an unblinded review of SAEs, in all patients, and in the prespecified subgroups. The DMC will also perform an unblinded interim analysis of the primary efficacy and safety variables (functional outcome and intracranial haemorrhage) when half of the patients have been included. 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. Unless this happens, the Steering Committee will remain ignorant of the interim results.

The DMC will also be responsible for monitoring the overall conduct of the trial, and may formulate recommendations relating to the selection, recruitment, or retention of participants, or their management, or to improving their adherence to protocol-specified regimens, and the procedures for data management and quality control.

The DMC will convene before the start of the trial to agree on the exact terms of reference for the committee, and will meet annually during the course of the trial.
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12.10 Audit and inspection

All source data and all trial data and material will be made directly available for audit and inspection. Source data is all information in original records and certified copies of original records that is necessary for the reconstruction and evaluation of the trial.

12.11 Handling of patient data

All patients will be assigned a unique code number. The patient data will be linked to this number, and the patients' names or other personal identifiers will not be included in the database. The patient database will be kept on a separate, secure computer. The code will be stored on another, secure computer, and will be deleted 15 years after the results of the trial have been published. The trial's procedures for data protection will conform to the Norwegian applicable regulatory requirements, and to the conditions set by the Norwegian Data Inspectorate.

12.12 Handling of the list of treatment codes

The lists of random treatment codes will be produced by the Statistical Centre at the University of Tromsø. Copies of the list will be kept by the person setting up the central randomisation system, and the Trial Statistical Centre. The list will be kept secret for all other people involved in the trial until the closure of the patient database.

12.13 Screening logs

Selected centres will be asked to make records of all patients considered for inclusion in the trial (screening logs).

12.14 Financial conduct

Contracts will be agreed between the Sponsor and each of the investigators/institutions. The Coordinating Investigator or other people centrally involved in the trial will not have any financial or other conflicts of interest in connection to the trial. Patients participating in the trial will be reimbursed for their travel expenses.

12.15 Publication and data sharing policy

The trial will be published in accordance with the CONSORT guidelines and will be presented by a writing committee on behalf of the investigators. All participating centres and collaborators will be acknowledged in the main publication. The primary results and results of any substudies will be presented at international meetings and in public media.

12.16 User involvement

A Patient Advisory Board will included representatives from the three major stroke patient organisations in Norway ("Norsk forening for slagrammede", "LHL Hjerneslag", and "Landsforeningen for slagrammede"). The board will have a representative in the Steering Committee, and will be consulted in all phases of the study.

13. Central trial organisation

13.1 Sponsors and funding bodies

The University Hospital of North Norway will be the Sponsor of the trial. Address: Sykehusveien 38, NO-9019 Tromsø, Norway. Telephone number: +47 91507766. The Director of Research will act as the Sponsor's legal representative.

The trial has received basic funding from the Norwegian Ministry of Health and Care Services (Clinical Therapy Research in the Specialist Health Services research programme (KLINBEFORSK)) and the National Association for Public Health. The trial has also received funding from the British Heart Foundation and from the Swiss Heart Foundation. Boehringer-Ingelheim GmbH will reimburse the Sponsor for the costs of tenecteplase.

13.2 Trial Coordinating Centre

The Trial Coordinating Centre will be based at the Brain and Circulation Research Group at the University Hospital of North Norway and the University of Tromsø.

The Trial Coordinating Centre consists of the following persons: Trial Co-coordinating Investigator and Head of Brain and Circulation Research Group (Ellisiv B. Mathiesen), Trial Co-coordinating Investigator (Eivind Berge), Trial Manager (Melinda B. Roaldsen), Assistant Trial Managers (Mirza Jusufovic, Mary-Helen Søyland, Linn Hofsøy Steffensen), Trial IT Manager (David Perry), Trial Secretary (to be appointed), Research Nurse (Tone Bratteng).

13.3 Trial Statistical Centre

Tom Wilsgaard (chair), others.

13.4 Executive Committee

Melinda Roaldsen, Mirza Jusufovic, Ellisiv B. Mathiesen, Eivind Berge.

13.5 Imaging Scientific Committee

Anders Christensen, Arnstein Tveiten, representatives from other centres/countries.

13.6 Trial Steering Committee

Bent Indredavik (Chair), Ellisiv B. Mathiesen, Tom G. Robinson, David Werring, Arnstein Tveiten, Ole Morten Rønning, Jesper Petersson, Hanne Krarup Christensen, Jukka Putaala, Janika Kõrv, Dalius Jatuzis, Gian Marco De Marchis, other national co-ordinating investigators, statistician (to be appointed), representative from the user organisations (to be appointed), Eivind Berge.

13.7 Data Monitoring Committee

Terje R. Pedersen (Chair), Peter Sandercock, Hans Wedel (statistician).

13.8 Event Adjudication Committee

To be appointed.

14. Time table and end of trial

First patient included: July 2017
Last patient included: July 2019
End of follow-up: December 2019
Presentation of main results: Spring 2020
Follow-up by record linkage with central registries: December 2022

The end of the trial is defined as the last visit of the last patient included.

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16. Appendix

16.1 Completed and on-going studies of thrombolytic treatment for wake-up stroke

Trial (clinicaltrials.gov ID)	Planned enrolment	Participants	Interventions	Primary outcome	Recruitment period
WAKE-UP (NCT01525290)	800 (503 enrolled)	Ischaemic stroke; <4.5 hours after awakening; MRI DWI/FLAIR mismatch	1. IV alteplase 2. Placebo	mRS score at 90 days	Nov 2012 – June 207
WASSABI (NCT01455935)	90	Ischaemic stroke on wake-up; <24 h of onset; NIHSS 8-22; 18-80 years; Penumbra on CT perfusion	1. Best medical care 2. IV alteplase 3. IA treatment	mRS score at 90 days	Nov 2011 -
EXTEND (NCT01580839)* *Not a study of wake-up stroke per se, but a small proportion of the patients are likely to be patients with wake-up stroke.	400	NIHSS 4-26; Penumbra mismatch on MRI or CT	1. IV alteplase 2. Placebo	mRS score at 90 days	June 2010 -
THAWS (NCT02002325)	300	Initial NIHSS ≥5 and ≤25; DWI/FLAIR mismatch on MRI	1. IV alteplase 2. Control	mRS at 90 days	April 2014 -

16.2 Definitions of clinical events

16.2.1 Serious adverse events

A serious adverse event is any untoward medical occurrence that, at any dose:

- results in death
- is life threatening
- requires hospitalisation or prolongation of an existing hospitalization
- results in disability/incapacity
- is a congenital abnormality / birth defect.

Medical and scientific judgement should be exercised in deciding whether reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalisation, but may jeopardise the subject or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These should also be considered serious. Examples of such events are invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalisation, or development of drug dependency or abuse.

16.2.2 Asymptomatic intracranial haemorrhage

Intracranial haemorrhage on brain MRI or CT without: neurological deterioration, new headache, new acute hypertension, new nausea or vomiting or sudden decrease in conscious level.

16.2.3 Death

Death will be classified according to cause:

1. Initial stroke

2. Recurrent stroke
3. Myocardial infarction
4. Pneumonia
5. Other

16.2.4 Recurrent stroke

Neurological deterioration (increase of ≥2 on NIHSS, after exclusion of other causes for neurological deterioration) occurring after 72 hours will be considered as a recurrent stroke. Recurrent stroke will be classified as ischaemic, haemorrhagic or unknown (if not documented on imaging).

16.2.5 Symptomatic intracranial haemorrhage

The IST-3[1] definition of symptomatic intracranial haemorrhage:

1. Symptoms (neurological deterioration, new headache, new acute hypertension, new nausea or vomiting, or sudden decrease in conscious level).
2. Intracranial haemorrhage on brain MRI or CT

16.3 Abbreviations

aPTT	Activated partial thromboplastin time
ATTEST	Alteplase versus tenecteplase for thrombolysis after ischaemic stroke
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case report form
CT	Computed tomography
DWI	Diffusion-weighted imaging
ECG	Electrocardiography
ECT	Ecarin clotting time
EXTEND	Extending the Time for Thrombolysis in Emergency Neurological Deficits Trial
FLAIR	Fluid-attenuated inversion recovery
IEC	Independent ethics committee
IMP	Investigational medicinal product
INR	International normalized ratio
MMSE	Mini-mental state examination
MRI	Magnetic resonance imaging
MR-RESCUE	Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy
mRS	Modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
NOR-TEST	The Norwegian tenecteplase stroke trial
SSS	Scandinavian Stroke Scale
TASTE	Tenecteplase versus Alteplase for Stroke Thrombolysis Evaluation
THAWS	THrombolysis for Acute Wake-up and Unclear-onset Strokes With Alteplase at 0.6 mg/kg Trial
TT	Thrombin time
WAKE-UP	Efficacy and Safety of MRI-based thrombolysis in Wake Up Stroke
WASSABI	Wake Up Symptomatic Stroke in Acute Brain Ischemia

17. Summary of changes

17.1 Protocol version/date 160323

- Addition of signature page
- Addition of section 3.2: Potential benefits and harms of tenecteplase
- Change to section 4.2 (Study questions and objectives)
- Change to section 5.2 (Exclusion criteria):

  - New exclusion criterion: "NIHSS score >25 or NIHSS consciousness score >2, or seizures during stroke onset"
  - Change to wording of exclusion criterion "High risk of bleeding"
  - New exclusion criterion: "Contraindications to tenecteplase"
  - Change to wording of exclusion criterion "Childbearing potential, pregnancy, positive pregnancy test, breastfeeding"
- Change to section 6.1 (Patient screening)
- Change to section 7.1 (Patient information and consent/assent)
- Change to section 7.2 (Randomisation procedure and recording of baseline characteristics)
- Change to section 8 (Trial treatment)
- Change to section 9 (Visits and examinations from trial entry till end of follow-up)
- Change to section 11.2 (Statistical analyses)
- Change to section 12.2 (Data protection)
- Change to section 12.5 (Recording and reporting of serious adverse events)
- Change to section 12.7 (Monitoring of data quality)
- Change to section 12.8 (Handling of protocol violations)
- Change to section 12.9 (Monitoring of effectiveness and safety)
- Change to section 12.10 (Audit and inspection)
- Change to section 16.2 (Definitions of clinical events)
- Change to section 17 (Summary of changes)

17.2 Protocol version/date 160421

- Change to section 7.1 (Patient information and consent/assent)

17.3 Protocol version/date 170412

- Change to front page, signature page, sections 13.1 (Sponsor) and 13.2 (The Trial Coordinating Centre): Change of sponsor
- Change to section 12.2 (Data protection) and 12.11 (Handling of data): Change from "Norwegian Data Inspectorate" to "Data Protection Officials"
- Change to section 12.16 (User involvement): Change in text
- Change to section 13.7 (Data Monitoring Committee): New names

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- Change to section 5.2 (Exclusion criteria): Reversal of effect of anticoagulant drugs
- Change to section 10.2 (Secondary effect variables): New timing
- Change to 11.2 (Statistical analyses): Specification of secondary analysis
- Change to section 13.1 (Sponsor and Funders), 13.2 (Trial Coordinating Centre), 13.6 (Trial Steering Committee): Update
- Change to section 14 (Time table): Update

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- Section 3: Update on results of recently published relevant studies
- Section 5.1: Change to inclusion criterion: "NIHSS score ≥3"
- Sections 5.2, 6.1 and 8.1.3: Removal of exclusion criterion: "Patient will be treated with intra-arterial interventions for proximal cerebral artery occlusion"
- Section 10.2: Change to secondary effect variables
- Section 11.2: Pre-specification of secondary analyses
- Section 13.6: Change to Trial Steering Committee