Statistical Analysis Plan for the Randomized Controlled Trial Tenecteplase in Wake-Up Ischaemic Stroke Trial (TWIST)

Agnethe eltoft (✉ agnethe.eltoft@unn.no)

Universitetssykehuset Nord-Norge <https://orcid.org/0000-0001-9808-879X>

Tom Wilsgaard

UiT Arctic University of Norway: UiT Norges arktiske universitet

Melinda B. Roaldsen

University Hospital of North Norway: Universitetssykehuset Nord-Norge

Mary-Helen Søyland

Universitetet i Tromsø Helsevitenskapelige fakultet Helsefak: UiT Norges arktiske universitet Det helsevitenskapelige fakultet

Erik Lundström

Uppsala Universitet

Jesper Petersson

Skånes universitetssjukhus Malmö Diagnostiskt centrum avdelning Malmö: Skanes universitetssjukhus Malmö Diagnostiskt centrum avdelning Malmö

Bent Indredavik

St Olavs Hospital University Hospital in Trondheim: St Olavs Hospital Universitetssykehuset i Trondheim

Jukka Putaala

Helsinki University Central Hospital: Helsingin seudun yliopistollinen keskussairaala

Hanne Christensen

Bispebjerg Hospital

Janika Kõrv

University of Tartu Faculty of Medicine: Tartu Ülikool Arstiteaduskond

Dalius Jatužis

Vilnius University: Vilniaus Universitetas

Stefan T. Engelter

University Hospital Basel: Universitätsspital Basel

Gian Marco De Marchis

University Hospital Basel: Universitätsspital Basel

David J. Werring

University College London Stroke Research Centre

Thompson Robinson

University of Leicester College of Life Sciences

Amstein Tveiten

Kristiansund sykehus

Ellisiv B. Mathiesen

UiT Arctic University of Norway: UiT Norges arktiske universitet

Research Article

Keywords: Ischemic stroke, Trombolysis, Wake-up stroke, Tenecteplase, TWIST, Acute stroke therapy

Posted Date: January 25th, 2022

DOI: <https://doi.org/10.21203/rs.3.rs-1064172/v1>

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Abstract

Background: Patients with wake-up ischemic stroke are frequently excluded from thrombolytic treatment due to unknown symptom onset time and limited availability of advanced imaging modalities. The Tenecteplase in Wake-up Ischaemic Stroke Trial (TWIST) is a randomized controlled trial of intravenous tenecteplase 0.25 mg/kg and standard care versus standard care alone in patients who wake up with acute ischemic stroke and can be treated within 4.5 hours upon awakening based on non-contrast CT findings.

Objective: To publish the detailed statistical analysis plan for TWIST prior to unblinding.

Methods: The TWIST statistical analysis plan is consistent with reporting standards for clinical trials and provides clear and open reporting.

Discussion: Publication of the statistical analysis plan serves to reduce potential trial reporting bias and outlines transparent pre-specified analyses.

Trial registration: ClinicalTrials.gov NCT03181360. EudraCT Number 2014-000096-80. WHO ICRT registry number ISRCTN10601890.

Introduction

Patients with wake-up ischemic stroke are traditionally ineligible for thrombolytic treatment due to unknown symptom onset. Recent randomized controlled trials have shown that thrombolytic treatment may be safe and effective in wake-up stroke patients selected by findings on advanced imaging such as MRI and CT perfusion.¹⁻³ However, these imaging modalities are not readily available at all hospitals 24/7. Tenecteplase in Wake-up Ischaemic Stroke Trial (TWIST) is an international, investigator-initiated, multi-center, prospective, randomized controlled, open-label, blinded end-point superiority trial of intravenous administered tenecteplase 0.25 mg/kg and standard care versus standard care alone in patients who wake up with acute ischemic stroke and can be treated within 4.5 hours (h) upon awakening. Patients are selected based on findings non-contrast computer tomography (NCCT) and randomized (1:1) to tenecteplase or best standard care. Both treatment arms will receive best standard care, including endovascular interventions for proximal cerebral artery occlusion on indication. The study is recruiting patients from 10 countries. Based on a revised sample size estimation (details in supplemental material) the aim was to include 600 patients (300 in each treatment arm).

A description of the TWIST trial protocol has been published previously.⁴ The purpose of this Statistical Analysis Plan (SAP) is to outline in detail the pre-determined plan for the statistical analyses associated with the primary report of the trial results prior to unblinding and analysis of the trial primary outcome. The SAP has been finalized before completion of the data collection and will be adhered to in analyzing data. The plan for secondary analyses and content of all subsequent publications cannot be specified in detail, but where appropriate, we set out the general analytical approach.

Aims And Hypothesis

The primary aim of TWIST is to test the hypothesis that treatment with intravenous (IV) tenecteplase within 4.5 h of awakening will improve functional outcome at 3 months in patients with wake-up ischemic stroke selected by NCCT brain imaging compared to standard care (no IV thrombolysis).

Patient population

Patients aged 18 years and older with new stroke symptoms acquired during sleep and a clinical diagnosis of stroke with limb weakness and National Institute of Health Stroke Scale (NIHSS) score ≥ 3 , or dysphasia, are eligible for inclusion. Detailed inclusion and exclusion criteria have been published previously⁴ and are listed in the supplementary material. In short, the main exclusion criteria are NIHSS score >25 or NIHSS consciousness score >2 , infarct size larger than $>1/3$ of the middle cerebral artery territory or intracranial hemorrhage on NCCT.

Randomization

A remote, web-based, computer-generated randomization procedure is used. All online submissions are secured by the use of password site entry and data encryption procedures via the TWIST online webpage. The investigators record patient details via a secure web interface before randomization takes place. The randomization procedure includes a standard minimization algorithm which ensures that the treatment groups are balanced across all centers in all countries for the key prognostic factors; age, NIHSS score and time since wake-up. To avoid predictable alternation of treatment allocation, and thus potential loss of allocation concealment, patients are allocated with a probability of 0.80 to the treatment group that would minimize the difference between the groups on the key prognostic factors. Patients will be randomly allocated to open-label IV tenecteplase plus best standard care or to best standard care alone (i.e. no IV thrombolysis).

Study procedures

Details on the background and study design are documented in the trial protocol and published protocol.⁴ Both patients and investigators are unblinded to treatment allocation. Primary outcome assessments are performed by telephone interview at 3 months (90 ± 7 days) by authorized study personnel, blinded to treatment allocation. Results of 3-month follow-up interviews are securely stored and separated from the investigators.

Only the Data Monitoring Committee (DMC) has access to interim data and results. An independent statistician, who otherwise is not involved in the trial, prepares semiannually unblinded reports of interim data and results to the DMC. If DMC judges 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.

Trial Objectives and Outcomes

The primary outcome is functional outcome at 3 months on an ordinal scale (0-6), defined by the modified Rankin Scale (mRS) score. The use of a categorical shift in mRS at 3 months after stroke has been suggested to provide a more comprehensive index of the clinical impact of acute stroke treatment.⁵ This accounts for the fact that thrombolytic treatment is often not completely curative, but has potential to improve patient outcome over the whole range of functional measurements. The table below outlines all relevant outcomes for the primary report.

Table 1. Objectives and outcomes of the Tenecteplase in Wake-up Ischaemic Stroke Trial

Objectives	Outcome
Primary	
Improve functional outcome in participants with wake-up ischemic stroke	Functional outcome defined as shift across the ordinal modified Rankin Scale (mRS) (0-6) at 3 months follow-up
Secondary	
Increase the proportion of patients with excellent functional outcome	Proportion of participants free from disability defined as functional outcome mRS score of 0-1 at 3 months follow-up
Increase the proportion of patients with good functional outcome	Proportion of participants functionally independent defined as mRS score 0-2 at 3 months follow-up
Increase the proportion of patients with response to treatment stratified by baseline stroke severity	Proportion of patients with response to treatment; mRS 0 for patients with mild deficits at study entry (NIHSS ≤ 7), mRS 0-1 for patients with moderate deficits (NIHSS 8-14), and mRS 0-2 for patients with severe deficits (NIHSS >14)
Reduce mortality rate	Proportion of participant mortality over the 3 months study period
Determine safety based on rate of symptomatic intracranial hemorrhage (SICH)	Proportion of patients with SICH as defined by SITS-MOST criteria ⁶ Proportion of patients with SICH as defined by IST-3 criteria ⁷
Determine safety based on rate of parenchymal hemorrhage type 2 (PH-2) ^{8*}	Proportion of patients with parenchymal hemorrhage type 2 on follow-up imaging at 24 ($\pm$ 6) hours
Determine safety based on rate of any intracranial hemorrhage	Proportion of patients with intracranial hemorrhage detected on follow-up imaging at 24 ($\pm$ 6) hours
Reduce poor functional outcome or death	Proportion of patients with mRS score of 4-6 at 3 months

*Hematoma occupying 30% or more of the infarcted tissue, with obvious mass effect⁸

Additional clinical, radiological and health care system related outcomes are included in the supplemental material. Details about definition and adjudication of clinical events and expert imaging readings are also listed in the supplementary material and Appendix 2.

Statistical analysis plan

Analysis principles and general considerations

All analyses will be conducted on data from all randomly assigned patients according to the intention to treat (ITT) principle, i.e. patients will be analyzed in the group they were randomized to, no matter what treatment they received, and regardless of whether they deviated from the protocol in any way. All outcomes and analyses are prospectively categorized as primary, secondary, or exploratory.

Ordinal logistic regression is pre-specified as the method for the analysis of the primary outcome using common odds ratio (OR) as the corresponding effect size measure, restricting adjustment to age, symptom severity (baseline NIHSS score) and time since wake-up. Differences in all outcomes between the two treatment groups will be tested independently at the two-tailed 0.05 level of significance. All estimates of treatment effects will be presented with 95% confidence intervals (CIs). No formal adjustments will be undertaken to constrain the overall type I error associated with the secondary and exploratory analyses including subgroup analyses. Their purpose is to supplement evidence from the primary analysis to better characterize the treatment effect. Results from the secondary and exploratory analyses will be interpreted in this context. Pre-specified subgroup analyses (as outlined in the supplementary material) will be carried out irrespective of whether there is a significant treatment effect on the primary outcome.

Sensitivity analyses for the primary outcome will be performed to test for the robustness of the primary analysis with regards to protocol violations, baseline imbalance, clustering effects and missing data (details in supplementary material).

Analyses will be conducted primarily using Stata statistical software. Proposed tables and figures for the main publication are presented in Appendix 2.

Trial profile

Flow of patients through the study will be displayed in a standard Consolidated Standards of Reporting Trials diagram. The report will include the number of patients included, withdrawn, lost to follow-up, the number who received the allocated treatment and the number of patients analyzed.

Patient characteristics and baseline comparisons

To assess balance, description of collected baseline characteristics will be presented for the tenecteplase and control groups. Discrete variables will be summarized as frequencies and percentages. Unless otherwise indicated, percentages will be calculated according to the number of patients for whom data are available. If there are more than 5% missing values, the denominator will be added as a footnote in the corresponding summary table. Continuous variables will be summarized using either mean and standard deviation, or median and interquartile range (IQR). Time intervals will be summarized by medians and IQRs. Some of these variables were used in the minimization algorithm determining

randomization allocation, and good balance would thus demonstrate successful operation of the algorithm.

Primary outcome: Differences in 3 months functional outcome across the full mRS scale between treatment groups

Outcome: This outcome measure will occur at 3 months follow-up. The outcome is measured using the mRS ordinal scale (range 0-6)

Main analysis: Common OR from an ordinal logistic regression model adjusted for age, stroke severity (baseline NIHSS score) and time since wake-up will be used if the proportional odds assumptions are satisfied (approximate likelihood-ratio test of proportionality of odds are not significant). However, if the proportional odds assumptions are not satisfied, the assumption free Wilcoxon–Mann–Whitney Generalized Odds Ratios (WMW GenOR) will be used.⁹

Statistical hypotheses: The null hypothesis which is to be refuted in the ordinal logistic regression is that the common OR is equal to 1, i.e. there is no difference in treatment effect between the intervention and control groups. The null hypothesis to be refuted in WMW GenOR test is equality of ranks when ties are split evenly. The null hypothesis for the WMW GenOR test states that the probability that the treatment observation is better than the control observation is the same as the probability that the treatment observation is worse than the control observation (splitting the ties equally), i.e. the WMW GenOR is equal to 1.

Analysis of the primary outcome: If the analyses of the baseline characteristics of the patients in the trial show clear differences in key prognostic factors (age, stroke severity and time since wake-up) between treatment groups, this may complicate the estimation of the effect of treatment. The primary analysis of the effect of treatment on the primary outcome will therefore be adjusted for the following covariates: age, symptom severity (baseline NIHSS score) and time since wake-up. An unadjusted analysis will also be presented. A separate set of analyses will be performed stratified for patients who received endovascular treatment and patients who did not.

Secondary efficacy and safety outcomes: Differences in proportions of patients with clinical outcomes between treatment groups

Outcomes:

- *Excellent functional outcome (mRS 0-1) at 3 months poststroke:*

The outcome is measured using mRS dichotomized as excellent functional outcome (mRS 0-1 or return to baseline if premorbid $mRS \geq 2$) versus unfavorable (mRS 2-6).

- *Good functional outcome (mRS 0-2) at 3 months poststroke:*

The outcome is measured using the mRS dichotomized as good (mRS 0-2) versus unfavorable (mRS 3-6).

- *The proportion of patients with response to treatment on day 7 (or discharge)*

Response to treatment is defined as mRS 0 for patients with mild deficits at study entry (NIHSS ≤ 7), mRS 0-1 for patients with moderate deficits (NIHSS 8-14), and mRS 0-2 for patients with severe deficits (NIHSS >14).

- *Symptomatic intracranial hemorrhage as defined by SITS-MOST⁶*

- *Symptomatic intracranial hemorrhage as defined by IST-3⁷*

- *Parenchymal hemorrhage type 2⁸ on follow-up imaging at 24 ± 6 h*

- *Any intracranial hemorrhage detected on follow-up imaging at 24 ± 6 h*

- *Poor outcome defined as mRS score dichotomized as poor outcome (mRS 4-6) versus mRS*

0-3 at 3 months

These outcomes are each measured on a binary scale and are defined as positive in the presence of event and as negative otherwise.

Statistical hypotheses: For each separate outcome the set of statistical hypotheses are $p(\text{IV tenecteplase}) = p(\text{control})$ versus $p(\text{IV tenecteplase}) \neq p(\text{control})$, where $p(\text{IV tenecteplase})$ is the proportion of subjects with the specific clinical outcome in the IV tenecteplase group and $p(\text{control})$ is the proportion with the specific clinical outcome in the control group. If $OR=1$, the treatment effect is equal in both groups.

Analysis method: An unconditional logistic regression model will be fitted for each outcome separately to estimate the OR associated with treatment effect, restricting adjustment to age, baseline NIHSS score and time since wake-up. Corresponding 95% CIs will be provided. For the outcome *proportion of patients with response to treatment on day 7*, no adjustment for baseline NIHSS will be performed. An unadjusted analysis will also be presented. A separate set of analyses will be performed stratified for patients who received endovascular treatment and those who did not.

Differences in 3 months mortality between treatment groups

Outcome: This secondary safety outcome is measured as time-to-event data. Event is defined as death of any cause within 3 months post-stroke.

Statistical hypothesis: The set of statistical hypotheses is hazard (IV tenecteplase) = hazard (control) versus hazard (IV tenecteplase) $\neq$ hazard (control). If HR=1, the hazard of death is equal in both groups.

Analysis method: The effect of treatment allocation on survival will be assessed using time-to-event analysis by Cox proportional hazards models adjusted for age, baseline NIHSS score and time since wake-up. The risk related to treatment will be presented as HRs with the corresponding 95% CI and corresponding survival plots will be presented. Unadjusted Kaplan-Meier survival curves with the log-rank test will also be presented. A separate set of analyses will be performed stratified for patients who received endovascular treatment and those who did not.

Exploratory analyses for additional outcomes, planned subgroup and meta-analyses are included in the supplementary material

Trial status

Recruitment started in June 2017 and ended with a total of 578 included patients on September 30th, 2021. Collection of primary endpoints are ongoing until January 2022.

Summary And Conclusions

TWIST will provide evidence on whether wake-up ischemic stroke patients can be treated safe and effectively with tenecteplase 0.25 mg/kg within 4.5 h of awakening based on findings on non-contrast CT.

Abbreviations

TWIST; Tenecteplase in Wake-up Ischaemic Stroke Trial

h; hours

NCCT; non-contrast computer tomography

SAP; Statistical Analysis Plan

IV; intravenous

NIHSS; National Institute of Health Stroke Scale

DMC; Data Monitoring Committee

mRS; modified Rankin Scale

SICH; symptomatic intracranial hemorrhage

PH-2; parenchymal hemorrhage type 2

ITT; intention to treat

OR; odds ratio

CI; confidence intervals

IQR; interquartile range

WMW GenOR; Wilcoxon–Mann–Whitney Generalized Odds Ratios

Declarations

Ethics approval and consent to participate

TWIST has received approval from medical research ethical committees and medical agencies in all 10 participating countries. Written, informed consent is obtained from all eligible patients according to approved national regulations.

Acknowledgement

We are forever thankful and indebted to Eivind Berge for his pivotal contribution to the trial as Co-coordinating Investigator until his death in Feb. 2020.

Consent for publication

Not applicable

Availability of data and material

Once completed, TWIST will contribute data to a planned future individual patient data meta-analysis. Data may be made available upon request.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: DW reports grant from British Heart Foundation and personal fees from Bayer, Portola and Alnylam. TR reports grant from British Heart Foundation. EBM reports grants from the Norwegian Clinical Therapy Research in the Specialist Health Services Research Programme and from the Northern Norway Regional Health Authority. JK reports personal fees and non-financial support from Boehringer Ingelheim, Sevier and Pfizer. JPu reports personal fees from Boehringer Ingelheim, Portola, Herantis Pharma and Terve Media, personal fees, speakers honorary, advisory board, and research grant from BMS-Pfizer, Bayer and Abbott/St. Jude Medical, research collaboration, and stock ownership from Vital Signum, and grants from Business Finland and Amgen. SE reports advisory board compensation from Boehringer Ingelheim and Bayer, and grants from Daiichi-Sankyo.

Declaration of competing interests

The author(s) declared the following potential conflicts of interest with respect to the research, authorship, and/or publication of this article: The cost of tenecteplase is covered by an unconditional grant from Boehringer Ingelheim Norway KS.

Authors' contributions

EBM is the Chief investigator, obtained the funding for the trial and supervised its completion. AE is grant holder as postdoc in the trial, is involved in data collection and drafted the statistical analysis plan. TW is a senior statistician, a member of the trial steering committee and commented on drafts of the statistical analysis plan. All authors are involved in the conduct of the trial as Steering committee members (EL, JPe, BI, JPu, HC, JK, DJ, SE, GDM, DJW, TR, AT) or PhD students (MBR and MS) and have read, commented and approved the final manuscript.

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Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [09.11.21SAPsupplementals.docx](#)
- [Appendix1Listofimagingvariables.docx](#)
- [Appendix2Proposedtablesandfigures.docx](#)