AcT trial: Statistical Analysis Plan (SAP)

1. Introduction

This Statistical Analysis Plan (SAP) is for the Alteplase Compared to Tenecteplase in patients with Acute Ischemic Stroke (AcT) Trial. Intravenous thrombolysis with alteplase is widely used in patients with acute ischemic stroke presenting early after symptom onset. Recent phase II trials have suggested that intravenous tenecteplase may be safer and potentially achieves higher early reperfusion rates than alteplase. This study investigates whether intravenous tenecteplase is non-inferior to intravenous alteplase with respect to clinical outcomes.

2. Trial Objectives

The Alteplase compared to Tenecteplase (AcT) trial will therefore seek to demonstrate the non-inferiority of intravenous tenecteplase compared to intravenous alteplase in terms of 90-day functional outcome assessed using the modified Rankin Score. The secondary objectives of this study are to compare intravenous tenecteplase to alteplase in terms of safety and relevant secondary outcomes.

3. Study Design

The AcT trial is a pragmatic, registry linked, prospective, randomized (1:1) controlled, open-label parallel group clinical trial with blinded endpoint assessment of 1600 patients to test if intravenous tenecteplase (0.25 mg/kg body weight, max dose 25 mg) is non-inferior to intravenous alteplase (0.9 mg/kg body weight, max dose 90 mg) in patients with acute ischemic stroke otherwise eligible for intravenous thrombolysis as per standard care. The trial will recruit patients from the emergency departments of participating primary or comprehensive stroke centers across Canada. Study outcomes will be collected through the trial and/or through linkage to ongoing registries and national administrative databases.

4. Randomization

Randomization will be centralized, secure and concealed using a real-time web-based server, to prevent confounding due to allocation bias. Investigators can access the randomizer either through the internet, secure text or through a local telephone. The trial will have allocation concealment and blinded endpoint assessment. Given the pragmatic design of the trial and the time sensitive nature of acute stroke, blinding the enrolling health personnel to treatment allocation is not practical. A 1:1 randomization will be used to allocate patients to intravenous tenecteplase (0.25 mg/kg body weight, max dose 25 mg) or intravenous alteplase (0.9 mg/kg body weight, max dose 90 mg). Randomization will use a validated minimal sufficient balance (MSB) algorithm to assure balance by site.

5. Sample size

The primary outcome will be 90-day mRS score which will be determined by the Rankin Focused Assessment (RFA-A) method using centralized telephone interview by trained study personnel blinded to treatment allocation.

A total of 1600 subjects will be randomly assigned to receive either intravenous tenecteplase or alteplase in a 1:1 ratio, assuming a missingness of primary outcome data/loss to follow-up rate <5%. Based on prior literature, the incidence of primary outcome (mRS 0-1) 90 days after randomization is assumed to be 38% and 35% respectively for tenecteplase vs. alteplase. Assuming a one-sided non-inferiority margin of 5%, a one-sided significance Type I error of 2.5% and 90% power to show that tenecteplase is non-inferior to alteplase, 759 subjects are needed in each arm of the trial. The choice of 5% as a non-inferiority margin represents 50% of the estimate of effect size (10%) for intravenous alteplase administered within 3 hours of stroke symptom onset vs. control for the outcome mRS 0-1 measured at 90 days obtained from the largest patient level pooled meta-analysis of such data. The choice of 5% as the non-inferiority margin in this trial means that at least half of the point estimate of effect for intravenous alteplase vs. control will be preserved. Hence the non-inferiority margin is guaranteed to be less than the lowest reasonable estimate of alteplase vs. control (placebo) effect size.

6. Interim Monitoring

Schedule for interim analyses (at every 1/3rd of total patients enrolled) will be finalized in consultation with the Data Safety Monitoring Committee (DSMC). The overall principle of interim analyses is to determine early if tenecteplase causes more mortality or is significantly inferior to alteplase at interim. Early stopping of the trial for efficacy is generally to be avoided. The guidance on stopping for safety pertains to a substantial mortality difference favoring alteplase at interim. This may be met if the observed p-value for mortality comparing the two randomized groups is below a threshold defined using a power family approach to alpha-spending using ϕ=1, and if the numeric rate of mortality favors alteplase (e.g., if it is found that tenecteplase is substantially and significantly inferior to alteplase in terms of mortality at interim). For inferiority of tenecteplase, the stopping may be defined in terms of absolute difference between the tenecteplase and alteplase rates of mRS 0-1 at 90-120 days. As an example, if at interim, the difference Δ for mRS 0-1 at 90-120 days in the tenecteplase vs. alteplase group is lower (worse) than an indicated value (see table in DSMC Charter for some suggested thresholds), the trial may be stopped for significant inferiority of tenecteplase. Details are provided in the AcT trial DSMC charter.

7. Definition of the target populations

7.1. Intention to Treat population

All patients enrolled in the trial randomized on an intent-to-treat basis.

7.2. Per-protocol population

All patients enrolled in the trial who received any dose of study drug and met all the inclusion and exclusion criteria per current Canadian Stroke Best Practices Recommendations. Since the trial has pragmatic eligibility criteria, patients who may have been inadvertently enrolled and received thrombolysis beyond 4.5 hours from stroke onset and any treatment crossovers are defined as protocol deviations for analysis.

8. Blinding

Treatment assignment is open label. Blinding of the outcome assessment at 90-120 days will be ensured by having central personnel trained on administration of the Rankin Focused Assessment, blinded to treatment allocation, and not involved in the acute treatment period conduct the assessment via telephone.

9. Statistical Analysis

Primary analysis of the trial data to establish non-inferiority will be conducted using risk difference analysis. First, non-inferiority will be established if the lower boundary of the 95% confidence interval of the percentage difference in subjects achieving excellent outcome (mRS 0-1) in the tenecteplase versus the alteplase arm is greater than – 5% (the non-inferiority margin). If non-inferiority is demonstrated, then a test of superiority of tenecteplase vs. alteplase will be performed as part of secondary analysis. In addition, logistic regression will be used to provide an adjusted estimate of the effectiveness of tenecteplase over alteplase for the primary outcome. The risk ratio of good 90-day outcome (mRS01) associated with the treatment groups will be estimated using a mixed-effects logistic regression model that adjusts for age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.

Secondary analyses will evaluate key safety (mortality and symptomatic intracerebral hemorrhage as defined in the AcT trial MOP) and secondary outcomes using relevant tests of association. Frequency tables will be used to summarize categorical variables by treatment group. Descriptive statistics will be used to summarize continuous data variables by treatment group.

The secondary outcomes and the corresponding analyses are described as follows. Both unadjusted (not described in Table below) and adjusted (described in table below) will be reported. Unadjusted analysis will be tests of difference in proportions, means or medians or regression analysis as appropriate. All analyses will be conducted secondary analyses are conducted at α = 0.05.

Outcome	Analysis
mRS 0-1 i.e., excellent functional outcome (blinded)	Efficacy secondary analysis of the mRS 0-1. Logistic mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
mRS 0-2 i.e., good functional outcome (blinded)	Risk difference and the corresponding 95%CI to assess non-inferiority. Risk ratio and the 95%CI to evaluate efficacy

	Adjusted logistic mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Ordinal mRS (blinded)	Ordinal logistic mixed-effects regression with treatment (Tenecteplase vs Alteplase) as exposure; for age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Return to pre-stroke status (pragmatic outcome) (blinded)	Risk difference and the corresponding 95%CI to assess non-inferiority. Risk ratio and the 95%CI to evaluate efficacy Adjusted logistic regression analysis with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Euroqol 5-D Visual Analogue Scale (EQ5D-VAS) (blinded)	A linear mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
EQ5D-5L (blinded)	Adjusted and unadjusted ordinal logistic regression analyses will be conducted for each EQ5D item (mobility, self-care, usual activities, anxiety, and depression) as the outcome variable. Health utility index derived from the EQ5D-5L items. Linear regression analysis with robust standard errors with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as explanatory variables.

Home time*	A generalized linear mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects. In addition to home time, similar exploratory analysis will be conducted for other registry and administrative data outcomes such as length of hospital stay until discharge and discharge destination (home, home with home care, home with early supportive discharge, rehabilitation hospital, long term care and hospice) that will use appropriate regression analysis.
Mortality	Risk difference and the corresponding 95%CI A Kaplan-Meier survival distribution. Patients alive after 90/120 days were censored. Logistic mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Symptomatic intracerebral hemorrhage	Risk difference and the corresponding 95%CI Logistic mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Intracranial Hemorrhage on follow-up Imaging (Blinded) • Parenchymal hemorrhage (hemorrhagic	Difference in proportion of ICH categories and the corresponding 95%CI

infarction type 1, hemorrhagic infarction type 2, parenchymal hematoma type 1, parenchymal hematoma type 2, remote hemorrhagic infarction type 1, remote hemorrhagic infarction type 2, remote parenchymal hematoma type 1, remote parenchymal hematoma type 2) • Subdural hemorrhage • Subarachnoid hemorrhage • Intraventricular hemorrhage	
Peripheral Bleeding requiring Blood Transfusion	Risk difference and the corresponding 95%CI
Angioedema	Risk difference and the corresponding 95%CI
Proportion of patients receiving EVT	Risk difference and the corresponding 95%CI. Logistic mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Other SAEs and SUSARs	Risk difference and the corresponding 95%CI

10. Subgroup Analyses

Heterogeneity in treatment effects will be explored via subgroup analyses of pre-specified prognostic variables in the ITT population primarily and in the per-protocol population secondarily. These will include analysis of primary outcome (mRS01) and key safety outcomes by

a. age (continuous and as < 80 years vs. >= 80 years),

b. sex (male vs. female),
c. baseline stroke severity as measured by the National Institute of Health Stroke Scale (NIHSS; continuous and < 8, 8-15 vs > 15),
d. presence of large vessel occlusion on baseline CTA
e. stroke onset-to-needle time (continuous and as <=180 minutes vs. > 180 minutes)
f. registry (QuiCR vs. OPTIMISE),
g. type of enrolling hospital (PSCs vs. CSCs),

Evidence of a treatment-by-sub-group variable interaction will be tested by including a multiplicative interaction term (treatment*subgroup variable) in the model. Subgroup analyses will help to determine if there is efficacy or futility in any pre-specified subgroup. Statistical significance for each subgroup analysis will be exploratory and conducted at α = 0.05

11. Missing data

Since the trial enrolls participants using a deferred consent approach, participants with missing mRS because of refusal of consent will be excluded completely from the analysis. Under the ITT principle, all remaining patients who are randomized are included in the analysis. Thus, every effort will be made to keep all missing data to a minimum i.e., < 5%. If, despite best efforts, there are missing data, then for the primary outcome analysis, data will be assumed to be missing completely at random (MCAR). Sensitivity analyses will be conducted to examine the impact of MCAR assumption on study conclusions using available case analysis and multiple imputation methods. Similarly, assumptions and missing data methods will be adopted for analysis of secondary outcomes.