Official Title: A Phase III, Prospective, Double-Blind, Randomized, Placebo-Controlled Trial of Thrombolysis in Imaging-Eligible, Late-Window Patients to Assess the Efficacy and Safety of Tenecteplase (Timeless)

NCT Number: NCT03785678

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STATISTICAL ANALYSIS PLAN

TITLE:	**A PHASE III, PROSPECTIVE, DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL OF THROMBOLYSIS IN IMAGING-ELIGIBLE, LATE-WINDOW PATIENTS TO ASSESS THE EFFICACY AND SAFETY OF TENECTEPLASE (TIMELESS)**
PROTOCOL NUMBER:	ML40787
STUDY DRUG:	Tenecteplase (RO5490263)
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SPONSOR:	Genentech, Inc.
PLAN PREPARED BY:	[redacted], Ph.D. Everest Clinical Research Corporation [redacted], Ph.D. Genentech, Inc.
DATE FINAL:	See electronic signature and date stamp on the final page of this document.

SAP AMENDMENT, VERSION 5:
SUMMARY OF CHANGES

A summary of changes from version 4 of the SAP is provided below:

- Added one additional model adjustment covariate, which indicates the protocol version under which a patient was randomized (i.e., Protocol V1-4 vs Protocol V5), to the analysis of the efficacy endpoints (primary, secondary, and exploratory).
- Added several additional subgroup analyses of the primary endpoint:
    - Baseline occlusion type: ICA vs M1 vs M2
    - Protocol version: Protocol V1-4 vs Protocol V5
    - Mechanical thrombectomy planned at baseline: Yes vs No
    - Mechanical thrombectomy performed (defined as successful clot manipulation): Yes vs No
    - Time (in minutes) between study drug administration and groin puncture: groups defined by tertiles of the time variable.

In addition, minor changes have been made to improve clarity and consistency. This amendment represents cumulative changes to the original SAP.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
ADL	activities of daily living
AE	adverse event
AESI	adverse event of special interest
AHA	American Heart Association
AIS	acute ischemic stroke
ALT	alanine aminotransferase
ASA	American Stroke Association
AST	aspartate aminotransferase
BI	Barthel Index
CBC	complete blood count
CI	confidence interval
CT	computed tomography
CTA	computed tomography angiography
CTCAE	Common Terminology Criteria for Adverse Events
CTP	computed tomography perfusion
CRF	case report form
DWI	diffusion-weighted imaging
eCRF	electronic Case Report Form
ECC	endovascular capable center
FDA	U. S. Food and Drug Administration
FLAIR	fluid-attenuated inversion recovery
GOS	Glasgow Outcome Scale
GRE	gradient recalled echo
ICA	internal carotid artery
ICH	intracranial hemorrhage
iDMC	independent Data Monitoring Committee
ITT	intent-to-treat (population)
IV	intravenous
IWRS	Interactive Web Response Systems
LOCF	last observation carried forward
LPI	last patient in (the study)
LPLV	last patient, last visit
MCA	middle cerebral artery
MAR	missing at random
MedDRA	Medical Dictionary for Regulatory Activities
MRA	magnetic resonance angiography
MRI	magnetic resonance imaging
MRP	magnetic resonance perfusion

Abbreviation	Definition
mRS	modified Rankin Scale
MT	mechanical thrombectomy
NCI	National Cancer Institute
nECC	non-endovascular capable center
Neuro-QoL	Quality of Life in Neurological Disorders
NIHSS	National Institutes of Health Stroke Scale
OR	odds ratio
PH1	parenchymal hematoma type 1
PH2	parenchymal hematoma type 2
PT	preferred term
SAE	serious adverse event
SAP	Statistical Analysis Plan
SI	International System of Units
sICH	symptomatic intracranial hemorrhage
SOC	system organ class
TEAE	treatment-emergent adverse event
TICI	Thrombolysis in Cerebral Infarction
T_{max}	time to maximum of the residue function
TNK	tenecteplase
tPA	tissue plasminogen activator
ULN	upper limit of normal
WHO	World Health Organization
WMW GenOR	Wilcoxon-Mann-Whitney Generalized Odds Ratio

1. BACKGROUND

Acute ischemic stroke (AIS) is the fifth leading cause of death in the U.S. Approximately 795,000 people suffer a stroke annually, with 23% due to recurrent stroke. Projections estimate that by the year 2030 an additional 3.4 million U.S. adults 18 years and older, representing 3.88% of the adult population, will experience a stroke, which is a 20.5% increase in prevalence from 2012 (Ovbiagele et al. 2013). Stroke is also the leading cause of serious, long-term disability. Approximately 2% of females and 3% of males are disabled from stroke (CDC 2017). From 2013 to 2014, the direct and indirect costs of stroke in the U.S. totaled $40.1 billion. It is projected that total direct medical costs will more than double, from $36.7 billion to $94.3 billion, between 2015 and 2035 (Benjamin et al. 2018).

Intravenous thrombolysis with alteplase remains the standard of care prior to thrombectomy for eligible patients within 4.5 hours of ischemic stroke onset (AHA/ASA 2018). However, alteplase succeeds in reperfusing large vessel arterial occlusion prior to thrombectomy in only a minority of patients (Campbell et al. 2018). Results of previous non-randomized studies have suggested that patients who have a mismatch between the volume of brain tissue that may be salvaged and the volume of infarcted tissue as seen on imaging could benefit from reperfusion of occluded proximal anterior circulation vessels, even when the reperfusion is performed more than 6 hours after the patient was last known to be well (Jovin T et al. 2011; Lansberg et al. 2015). Treatment benefit is strongly dependent on time-to-reperfusion and, as many patients have treatment delays due to inter-hospital transfers (or in some cases difficult vascular access), improved approaches to intravenous thrombolysis that decrease the need for transfer could substantially improve patient outcomes.

Tenecteplase (TNKase®) is a modified form of human tissue plasminogen activator (tPA) that binds to fibrin and converts plasminogen to plasmin. In vitro studies demonstrated that in the presence of fibrin, tenecteplase conversion of plasminogen to plasmin is increased relative to its conversion in the absence of fibrin. This fibrin specificity decreases systemic activation of plasminogen and the resulting degradation of circulating fibrinogen compared with a molecule lacking this property, which could potentially decrease the incidence of bleeding.

For further background information, see Section 1 of the Protocol.

In this trial, the goal is to determine if pre-treatment with an intravenous (IV) thrombolytic (tenecteplase) results in good clinical outcomes. The trial will evaluate whether treatment with IV tenecteplase, administered between 4.5 and 24 hours of a patient's last-known well time (DEFUSE 3 [Albers et al. 2018] and DAWN [Nogueira et al. 2017]), is superior to placebo in patients who have a vessel occlusion and evidence of salvageable tissue on their baseline computed tomography perfusion (CTP) or magnetic resonance imaging (MRI). Placebo is being used as the comparator since a thrombolytic is only U.S. Food and Drug Administration (FDA)-approved in the United States for use out to 3 hours, and the standard of care guidelines support use out to 4.5 hours.

This Statistical Analysis Plan (SAP) describes the statistical methods to be used for the clinical study report of study ML40787. This version of the SAP has been developed using version 4 of the study protocol dated 30 Jan 2020. The SAP is jointly authored by Genentech and Everest Clinical Research statisticians and will be finalized by Everest Clinical Research and approved by Genentech, Inc. before database lock and unblinding.

2. STUDY DESIGN

This is a Phase III, prospective, double-blind, randomized, placebo-controlled trial of tenecteplase in patients with AIS and evidence of salvageable tissue on their baseline CTP or MRI who present in the 4.5- to 24-hour time window with an internal carotid artery (ICA) or middle cerebral artery (MCA; M1 or M2) occlusion. Patients who meet inclusion criteria will be randomized 1:1 to tenecteplase (0.25 mg/kg, maximum 25 mg) or placebo, both administered as a single bolus injection over 5 seconds.

All patients will receive standard-of-care therapy according to American Heart Association (AHA)/ American Stoke Association (ASA) clinical guidelines (2018). For patients with ICA/M1 occlusions, this will include adjuvant endovascular therapy following study drug (tenecteplase/placebo) if criteria, as stipulated in the guideline, are met. Per guideline recommendations, patients with M2 occlusions generally will not undergo endovascular therapy following treatment with study drug (tenecteplase/placebo). Study drug administration is recommended to occur before the start of endovascular therapy (defined as groin puncture). It is recommended that patient should not be randomized if it appears that it will be challenging to administer study drug prior to the femoral groin puncture for planned thrombectomy. However, if the patient has already been randomized, and due to unforeseen circumstances the drug cannot be administered prior to groin puncture for the planned thrombectomy, the drug must be administered as soon as possible and prior to manipulation of the clot.

To determine eligibility for randomization, all patients will undergo multimodal computed tomography (CT) or MRI at baseline. Only patients with a vessel occlusion (ICA or MCA) and penumbral tissue will be randomized.

Randomization will be stratified by age (≤70 vs. >70 years), type of occlusion (ICA/M1 vs. M2), baseline National Institutes of Health Stroke Scale (NIHSS; ≤15 vs. >15), and randomization site (endovascular capable center [ECC] vs. non-endovascular capable center [nECC]). The primary goal of this trial is to compare the effect of treatment with tenecteplase vs. placebo and standard of care on the 90-day functional outcome (i.e., ordinal modified Rankin Scale [mRS] score) in patients who present in the 4.5- to 24-hour time window with an ICA or MCA (M1, M2) occlusion and evidence of target mismatch on multimodal CT or MRI of the brain. FDA approved and validated perfusion imaging software for determination of core volumes will be used.

Both patients transferred from an nECC as well as patients admitted directly to an ECC are eligible for the study.

Patients will undergo two follow-up MRI scans. Patients who cannot undergo an MRI will undergo a multimodal CT at the first follow-up and a non-contrast CT at the second follow-up. The first follow-up scan will be obtained at approximately 24 (±6 hours) after randomization and will include an assessment of recanalization and reperfusion, per standard of care. The second MRI will be obtained between 72 to 96 hours after randomization for assessment of final infarct volumes.

All patients will be evaluated using mRS at discharge/Day 5, Day 30, and Day 90 after randomization for clinical outcome evaluation.

2.1 PROTOCOL SYNOPSIS

The protocol synopsis is provided in Appendix 1 and the schedule of assessments is provided in Appendix 2. The study flow chart is provided in Figure 1 in Appendix 2.

2.2 ENDPOINTS

This study will evaluate the efficacy and safety of tenecteplase compared with placebo. Specific objectives and corresponding endpoints for the study are outlined below.

2.2.1 Primary Efficacy Endpoints

The primary endpoint is the ordinal mRS score at Day 90.

2.2.2 Secondary Efficacy Endpoints

The following secondary efficacy endpoints are defined:

- Proportion of patients with functional independence, defined as an mRS of 0–2 at Day 90 from randomization.
- Proportion of patients with angiographic reperfusion (Thrombolysis in Cerebral Infarction [TICI] 2b or TICI 3) at completion of angiographic procedure (endovascular patients only).
- Median National Institutes of Health Stroke Scale (NIHSS) score at Day 90 from randomization.
- Proportion of patients with a Barthel Index (BI) score ≥95 at Day 90 from randomization.
- Proportion of patients with a good recovery based on the Glasgow Outcome Scale (GOS) at Day 90 from randomization.
- Proportion of patients with reperfusion at 24 hours post-randomization, defined as >90% reduction in T_{max}>6s lesion volume.
- Proportion of patients with recanalization at 24 hours post-randomization, defined as complete recanalization on computed tomography angiography (CTA)/magnetic resonance angiography (MRA).

Further detail is described in Section 4.6.2.

2.2.3 Exploratory Efficacy Endpoints

The following exploratory efficacy endpoints will be analyzed:

- Median final infarct volumes at 72-96 hours visit or discharge (if patient is being discharged prior to 72-96 hours visit).
- Median infarct growth at 72-96 hours visit or discharge (if patient is being discharged prior to 72-96 hours visit).
- Ordinal mRS score at Day 90 by occlusion type (M1/ICA or M2 occlusions).
- Ordinal mRS score at Day 30 and Day 90 by randomization site (ECC or nECC).
- Proportion of patients who are randomized at an nECC and transferred to an ECC for endovascular therapy for whom planned thrombectomy was not performed.
- Proportion of patients readmitted within 30 days from discharge.
- Proportion of patients requiring one pass with endovascular therapy device (endovascular patients only).
- Mean Quality of Life in Neurological Disorders (Neuro-QoL) scores by domain.
- Proportion of patients where planned thrombectomy was not performed.
- Overall survival at Day 90.

2.2.4 Pharmacokinetic Endpoints

No pharmacokinetic endpoints are planned for this study.

2.2.5 Safety Endpoints

The safety endpoints include:

- Incidence of symptomatic intracranial hemorrhage (sICH) within 36 hours.
- Incidence and severity of adverse events (AE).
- Mortality rate up to Day 30 and Day 90.
- Proportion of patients with parenchymal hematoma type 2 (PH2) at the 72-96 hours visit.

Further detail is described in Section 4.8.

2.3 DETERMINATION OF SAMPLE SIZE

Approximately 456 patients will be enrolled in this study. Assuming the distribution of mRS scores at Day 90 in the two treatment arms as shown in Table 1 and a 5% dropout rate in the study, approximately 228 patients in each treatment group will provide at least 90% power to detect the specified difference in the distribution of the mRS scores at the 2-sided 0.049 significance level (after adjustment for one efficacy interim analysis). This corresponds to being able to detect a common odds ratio (OR) of at least 1.76 (tenecteplase vs. placebo), with a total sample size of approximately 432 evaluable patients, assuming a 5% dropout rate.

Table 1 Assumed Distribution of 90-Day mRS in Use in Sample Size Determination

mRS	0	1	2	3	4	5	6
Tenecteplase (% of patients)	17	20	16	15	13	7	12
Placebo (% of patients)	13	13	12	14	18	12	18

mRS = modified Rankin Scale

The sample size estimation is highly dependent on the distribution assumption of 90-day ordinal mRS score for both treatment arms; thus, additional power calculation were performed with 456 total patients, assuming 5% drop-out rate, for different distribution scenarios on the mRS at Day 90, derived from previous stroke studies. These resulted in >88% and >99% power to detect the specified difference in the distribution of the mRS scores. For more details, see Section 6.1 of the Protocol.

2.4 ANALYSIS TIMING

This study has two planned safety interim analyses and one planned efficacy interim analysis. The safety interim analyses will occur when the first 25 and 50 patients have completed the 72-96 hours assessment post-randomization, respectively. More information on the safety interim analyses is available in Section 3.3.

The efficacy interim analysis will occur when 50% (i.e., 228) of the total patients have completed the 90-day assessment to monitor both the efficacy and safety of tenecteplase vs. placebo. For more details regarding the efficacy interim analysis, see Section 4.10.

For all of the aforementioned interim analyses, only the patients whose study schedules have reached the designated visits (i.e. 72-96 hours assessment for safety only interims and 90-day assessment for efficacy interim) or who have discontinued the study or died with status available to the Sponsor by the clinical cut off date will be included. Thus, patients who haven't reached the specific scheduled visits due to later study entrance will not be included.

The end of study is defined as the date when the last patient, last visit (LPLV) occurs, or the date at which the last data point required for safety follow-up is received from the last patient, whichever occurs later. This date is expected to occur 90±14 days after randomization of the last patient in (LPI) the study. The total length of the study is expected to be approximately 3 years. Analysis of data from all patients will be performed when all patients have completed or discontinued the study, all data from the study are in the database, and the database is locked.

3. STUDY CONDUCT

3.1 RANDOMIZATION

Once patients' eligibility requirements are confirmed, patients are randomized 1:1 to receive a single bolus injection of tenecteplase or placebo through the Interactive Web Response System (IWRS). The study treatment kit number is also assigned by IWRS at that time. Patients are treated as soon as possible after randomization. Patients are considered enrolled in the study once they have provided the informed consent and been randomized.

Randomization is stratified by age (≤70 vs. >70 years of age), type of occlusion (ICA/M1 vs. M2), baseline NIHSS (≤15 vs. >15), and randomization site (ECC vs. nECC). A permuted block randomization is used to achieve an approximately 1:1 ratio between tenecteplase and placebo arms within each randomization stratum.

3.2 INDEPENDENT REVIEW FACILITY

An independent neurologist is contracted with the Sponsor to conduct independent imaging reading for all study related images collected by the study sites. During the study, neurological images obtained at the study sites will be sent to the independent reading center following a predefined timeline. After receiving the images, the neurologist will conduct his reading independently to collect study related neurological variables. Imaging data read by the independent neurologist will be transferred to the Sponsor on a regular basis according to a schedule agreed by both parties. Both the schedules for data transfer from study sites to independent reader and from independent reader to the Sponsor should accommodate (i.e. expedite the turnaround time) any occurrence of symptomatic intracranial hemorrhage events. More details about the role and process of independent imaging reader are provided in the independent imaging review charter.

3.3 DATA MONITORING

An independent Data Monitoring Committee (iDMC) monitors patient safety, treatment efficacy, and study conduct on an ongoing basis. Members of the iDMC are external to the Sponsor and follow a charter that outlines the iDMC roles and responsibilities. Unblinded data summaries for iDMC review will be provided by an independent statistical entity external to the Sponsor. While the iDMC will review unblinded summaries, the Sponsor will remain blinded to treatment group until the time of formal study unblinding.

During the conduct of the study, regularly scheduled safety data reviews by the iDMC will occur. After the first patient is enrolled, safety review will occur after the first 25 patients have completed the 72-96 hours assessments post-randomization and again after the first 50 patients have completed the 72-96 hours assessments post-randomization. Thereafter, the iDMC will meet at a frequency determined by the iDMC and the Sponsor according to the emerging safety profile. The iDMC or Sponsor may

also request ad hoc reviews at any time to address potential safety concerns. Additional safety reviews have been requested by the iDMC when the first 100 and 200 patients have completed the 72-96 hours assessments post-randomization.

The iDMC will also review unblinded efficacy data at the planned efficacy interim analysis when 50% of the planned patients have completed 90-day follow-up post-randomization. At this review, the iDMC will evaluate the benefit-risk profile of tenecteplase treatment through reviewing unblinded safety and efficacy data.

The iDMC may recommend stopping the study early or amending the study for either significant safety concerns or outstanding efficacy results. The final decision of acting upon the iDMC's recommendations will rest with the Sponsor. Further details about the definition, role, and the responsibilities of the iDMC are provided in the iDMC Charter.

4. STATISTICAL METHODS

Descriptive statistics will be produced for all endpoints. Categorical variables will be summarized as the number and percentage of patients or occurrences in each response category. Continuous variables will be summarized using descriptive statistics (number of observations, mean, standard deviation, median, minimum, maximum). Any required confidence intervals (CI) will be constructed as two-sided 95% CIs. Data listings will be generated for specific endpoints as appropriate.

4.1 ANALYSIS POPULATIONS

4.1.1 Efficacy Analysis Population

All efficacy analyses will be based on the intent-to-treat (ITT) population, defined as all randomized patients who provided informed consent, and analyzed according to the treatment assignment at randomization.

4.1.2 Safety Analysis Population

The Safety population is defined as all randomized patients who provided informed consent and received any amount of study drug. For safety analyses, patients will be grouped according to the treatment actually received. Patients who did not receive treatment as randomized will be listed as described in Section 4.8.1.

4.1.3 Per Protocol Population

The Per Protocol (PP) population is defined as all patients from the ITT population, who did not have any key major protocol deviations. The list of key major protocol deviations will be finalized in a blinded fashion prior to database lock.

4.2 ANALYSIS OF STUDY CONDUCT

The number and percentage of patients who were randomized, received treatment, and completed the study will be summarized. For randomized patients, the number and percentage of patients who completed the study and discontinued the study, along with

the reasons for premature discontinuation, will be tabulated overall and by treatment group. A listing of patients who discontinued early from the study will be generated. Relatedness to COVID-19 will be determined by the Sponsor prior to the interim and final analysis in a blinded manner, and included in the listing and the summary.

A summary of the duration of study participation will be produced. Duration in days will be calculated as the last assessment date according to the schedule of study activities minus the randomization date + 1. Categorical summaries of number and percentage of patients who complete each scheduled visit will also be displayed, showing the overall completion as well as a breakdown by mode (in-person, phone or telemedicine). Summaries of missed visits will tabulate relatedness to COVID-19.

The number and percentage of patients included and excluded from the analysis populations (ITT, Safety and PP) will also be tabulated overall and for each treatment group. Reason(s) for exclusion from each population will be summarized. Patients excluded from analysis populations will be listed.

Major protocol deviations will be listed and summarized by treatment group. The summary of exclusions from the PP population will distinguish the major deviations related and unrelated to COVID-19. Relationship of major protocol deviations to COVID-19 will also be listed.

4.3 ANALYSIS OF TREATMENT GROUP COMPARABILITY

Demographic and baseline characteristics such as age, sex, race, ethnicity, baseline mRS, and baseline NIHSS will be summarized for the ITT population overall and by treatment group.

The number of non-missing observations, mean, standard deviation, median, minimum and maximum values of the continuous variables will be summarized by treatment group. Categorical variables will be summarized using numbers and percentages of patients in each category; unknown or missing will be included as a separate category.

Baseline for mRS, NIHSS, vital signs and lab parameters will be defined as the last available assessment prior to initiation of study medication. For patients who do not receive study medication, baseline values will be defined as the last available assessment prior to randomization. These will be included in listings.

4.4 MEDICAL HISTORY

Medical history is collected as general (as open ended response) as well as targeted conditions of myocardial infarction, hypertension, atrial fibrillation, hypercholesterolemia, diabetes, and prior stroke. Open ended responses will be coded using the latest version of the Medical Dictionary for Regulatory Activities (MedDRA) and summarized by system organ class and preferred term. Patients reporting more than one condition/diagnosis

within a body system will be counted only once for that body system. Targeted medical history will be summarized by the pre-specified term. All medical history data will be listed.

4.5 PRIOR AND CONCOMITANT MEDICATIONS

Prior medications refer to those stopped prior to study day 1. Concomitant medications refer to those used by a patient from 7 days prior to initiation of study drug to the study completion/discontinuation visit. These will be coded using WHO (World Health Organization) Drug Dictionary and summarized by medication class and preferred term.

4.6 EFFICACY ANALYSIS

Efficacy analyses will be performed using the ITT population with patients grouped according to treatment assigned at randomization. Available data regardless of adherence to the protocol will be included in the efficacy analyses; this includes data from patients who discontinued prematurely from the study.

Deviation from the AHA/ASA treatment guidelines for standard of care (i.e. mechanical thrombectomy [MT] is planned for the patient but not performed due to various reasons) is considered an intercurrent event in this study. COVID-19 infections are considered another type of intercurrent event. COVID-19 infections during the follow-up period will be captured as AEs. In efficacy analyses, treatment policy strategy will be applied to both COVID-19 and non-COVID-19 intercurrent events. That is, all collected data will be used regardless of the occurrence of these intercurrent events.

Visit windows will be derived according to Table 2 and applied for all clinical efficacy assessments. If there are multiple assessments within the same window, the one closest to the target time point will be used. Assessments that are outside of the defined windows will not be used in analysis, except they may provide a value for the last observation carried forward (LOCF) imputation in the case of mRS (see Section 4.9.1.1).

Table 2 Definition of Visit Windows

Visit	Target Time	Window: mRS, GOS, BI, Neuro-QoL*	Window: NIHSS	Window: imaging
24 hours	24 hours	N/A	18 to 30 hours	18 to 30 hours
72-96 hours	84 hours	N/A	60 to 96 hours	60 to 120 hours
Day 5	5 days	4 to 6 days	5 to 6 days	N/A
Day 30	30 days	15 to 60 days	15 to 60 days	N/A
Day 90	90 days	61 to 120 days	61 to 120 days	N/A

BI = Barthel Index; GOS = Glasgow Outcome Scale; mRS = modified Rankin Scale; Neuro-QoL = Quality of Life in Neurological Disorders; NIHSS = National Institutes of Health Stroke Scale.

* Assessed only at Day 90 Visit

All analyses of efficacy endpoints (primary, secondary, and exploratory) will be evaluated for superiority. Analyses will be adjusted for all or a subset of the following variables:

- Age (≤70 vs. >70 years of age)
- Type of occlusion (ICA/M1 vs. M2)
- Baseline NIHSS (≤15 vs. >15)
- Randomization site (ECC vs. nECC)
- Mechanical thrombectomy planned (Yes vs. No)
- Protocol version (Protocol V1-4 vs Protocol V5).

In order to avoid small number of patients in the resulting strata, some variables may be removed from this list and not be adjusted for. The algorithm to perform this assessment is provided in Appendix 5.

For endpoints that are based on the imaging data where both local and central reader's evaluation are collected, the central reader's evaluation will be used for analysis. Post-baseline final infarct volume will only be recorded by the central reader. The local recording for the baseline image will be used to calculate change from baseline in final infarct growth at 72-96 hours (or discharge if patient is being discharged prior to 72-96 hours visit).

Analyses of NIHSS, BI and GOS will only utilize data collected in-person or by telemedicine. NeuroQoL will only utilize data collected in-person. If any of these assessments are conducted by a mode that is not allowed for the assessment, the data will be listed, but excluded from analysis and not replaced by imputations.

4.6.1 Primary Efficacy Endpoint

The primary endpoint is the ordinal mRS score at Day 90. mRS is an assessment of disability with values from 0 (no symptoms at all) to 5 (severe disability); death will be scored as a 6. During the study, mRS is collected at Baseline, Day 5 or hospital discharge, and 30 and 90 days after randomization. If death occurs prior to a scheduled assessment day, the mRS will be considered available and will be set to 6. The methodology for imputations related to the primary endpoint is described in Section 4.9.1.1.

The distribution of mRS at Day 90 will be summarized descriptively, and plotted using Grotta bars.

Analysis of the primary endpoint will test the hypothesis as follows:

$$H_0: OR_{\{TNK\ vs\ Placebo\}} = 1\ vs.\ H_1: OR_{\{TNK\ vs\ Placebo\}} \neq 1,$$

where $OR_{\{TNK\ vs\ Placebo\}}$ stands for the overall odds ratio of achieving a lower level vs. higher level mRS between the tenecteplase and the placebo arms. Superiority of tenecteplase over placebo will be declared if the p-value of the estimated treatment effect is ≤0.003 (two-sided) at the efficacy interim analysis or ≤0.049 (two-sided) at the final analysis, after adjustment for one efficacy interim analysis.

In case there are issues with fitting the proportional odds model (such as e.g. a quasi-complete separation), the stratified Wilcoxon-Mann-Whitney Generalized Odds Ratio statistic (WMW GenOR) as described below will be employed. Otherwise, the proportional odds assumption will be tested. For this purpose, a proportional odds model will be fitted for the Day 90 mRS outcome, which will include treatment and adjusting variables. If the p-value for the overall score test of the proportional odds hypothesis is >0.05, the proportional odds assumption will be deemed acceptable. The assessment of the proportional odds assumption will be independent of the results from the primary efficacy analysis from the proportional odds model.

If the proportional odds hypothesis is not rejected, the parametric proportional odds model described above will be used to analyze the primary efficacy endpoint. The estimated common odds ratio of a lower mRS score (better outcome) will be presented for treatment (TNK vs. placebo). The corresponding Wald p-value and 95% CI will also be obtained.

If the proportional odds hypothesis is rejected, the stratified WMW GenOR will be computed to compare the odds of having a lower mRS in the treatment group compared to placebo. This method does not require the proportional odds model assumption and is easily interpretable, computationally simple, and allows for confidence intervals to be constructed (Churilov et al. 2014). See details in Appendix 4.

An unadjusted analysis of mRS distributions by treatment group will also be performed using the Wilcoxon-Mann-Whitney test (PROC NPAR1WAY) as supportive analysis.

4.6.2 Secondary Efficacy Endpoints

Secondary endpoints will be compared between the two treatment groups, gated on the success of the primary efficacy comparison (2-sided p-value ≤0.003 at the interim analysis or ≤0.049 at the final analysis). Hypothesis testing of the key secondary endpoints will be performed in a sequential manner at the 2-sided significance level of 0.049 (or 0.003 for interim analysis), the amount available after testing the hypothesis for the primary endpoint. The order of testing is presented in the following table and testing will continue according to this order until the p-value of the hypothesis being tested is >0.049 (or >0.003 for interim analysis).

Table 3 Order of Hypothesis Testing for the Secondary Endpoints

Order	Endpoint
1	Proportion of patients with functional independence
2	Proportion of patients with recanalization at 24 hours post-randomization
3	Proportion of patients with reperfusion at 24 hours post-randomization
4	Proportion of patients with angiographic reperfusion (TICI 2b or TICI 3) at completion of angiographic procedure
5	Median NIHSS score at Day 90
6	Proportion of patients with a BI score of ≥95 at Day 90
7	Proportion of patients with good recovery based on the Glasgow Outcome Scale at Day 90

BI = Barthel Index; TICI = Thrombolysis in Cerebral Infarction.

4.6.2.1 Functional Independence

A patient with functional independence is defined as having an mRS of 0–2 at Day 90. The proportion of patients with functional independence will be analyzed using logistic regression. The model will be adjusted as specified in Section 4.6. Observed counts and percentages for tenecteplace and placebo arms, and model based odds ratio, corresponding Wald 95% CI and p-value will be produced. This analysis will be performed with the same imputations used in the primary analysis (see Section 4.9.1).

4.6.2.2 Recanalization at 24 Hours Post-Randomization

During the follow up CT or MRI, the degree of recanalization will be evaluated by the central reader. Possible outcome includes no recanalization, partial recanalization, or complete recanalization.

The proportion of patients with complete recanalization at 24 hours post-randomization will be analyzed using logistic regression. The model will be adjusted as specified in Section 4.6. Observed counts and percentages for tenecteplace and placebo arms, and model based odds ratio, corresponding Wald 95% CI and p-value will be produced.

4.6.2.3 Reperfusion at 24 Hours Post-Randomization

Time to maximum of the residue function (T_{max}) is one of the measurements used to estimate salvageable tissue after a stroke and is comparable in MRI and CT perfusions (Seker et al., 2017). For this study, reperfusion at 24 hours post-randomization is defined as >90% reduction in T_{max}>6s lesion volume, compared to the baseline scan.

The proportion of patients with reperfusion at 24 hours post-randomization will be analyzed using logistic regression adjusted as specified in Section 4.6. Observed counts and percentages for tenecteplace and placebo arms, and model based odds ratio, corresponding Wald 95% CI and p-value will be produced.

4.6.2.4 Angiographic Reperfusion

Angiography is a tool that can measure reperfusion, or restoring the flow of blood, after a stroke or heart attack. The Thrombolysis in Cerebral Infarction (TICI) scale is a grading system that is commonly used to measure the response of thrombolytic therapy in ischemic strokes, using angiographic reperfusion (Higashida RT et al, 2003). TICI scores range from grade of 0 (no reperfusion) to grade 3 (complete reperfusion). Grade 2 (partial reperfusion) is further subdivided into 2a and 2b.

In this study, a secondary efficacy endpoint is the proportion of patients with angiographic reperfusion, defined as achieving TICI 2b or TICI 3 at completion of the angiographic procedure. This analysis will be limited to endovascular patients only. Logistic regression will be used to determine the odds of having an angiographic reperfusion, adjusted as specified in Section 4.6. Observed counts and percentages for tenecteplace and placebo arms, and model based odds ratio, corresponding Wald 95% CI and p-value will be produced.

4.6.2.5 National Institutes of Health Stroke Scale

National Institutes of Health Stroke Scale is a questionnaire tool used to quantify the impairment caused by stroke. Scores are calculated by summing the responses on the survey, with higher values indicative of a more severe stroke. Missing responses will not be imputed and will result in a missing total NIHSS score. The range for the score is 0 to 42.

For this study, the NIHSS will be conducted at baseline, 24 hours, 72-96 hours, discharge or Day 5, 30 days, and 90 days. It will also be collected at the time of unscheduled imaging to support determination of sICH. All NIHSS scores will be allocated to analysis windows, and a single value per window will be selected for analysis according to the rules in Section 4.6. NIHSS score at Day 90 is a secondary endpoint.

NIHSS scores at each planned post-baseline time point will be summarized descriptively (mean, median, etc.). The van Elteren's test will be used to compare the distribution of tenecteplase and placebo arms, adjusting as specified in Section 4.6. The median treatment differences will be presented with 95% CI based on the Hodges-Lehmann approach for the location shift (median treatment difference).

4.6.2.6 Barthel Index Score

The Barthel Index is an ordinal scale used to measure performance in activities of daily living (ADL). This 10-item scale describes ADL and mobility (e.g., feeding, bathing, grooming, dressing, bowel control, bladder control, toileting, chair transfer, ambulation, and stair climbing). Each performance item is rated on this scale with a given number of points assigned to each level or ranking. BI scoring ranges from 0 to 100, and lower scores representing greater dependency.

The proportion of patients with a BI score $\geq$95 at Day 90 will be analyzed using logistic regression adjusted as specified in Section 4.6. Observed counts and percentages for

tenecteplace and placebo arms, and model based odds ratio, corresponding Wald 95% CI and p-value will be produced.

4.6.2.7 Glasgow Outcome Scale

The GOS is a scale used to assess recovery of patients with brain damage. The scale has 5 categories, with scale ranging from 1 (death) to 5 (good recovery). For the purpose of analysis, the scale will be reversed so that 1=good recovery and 5=death, to align with the common practice in clinical trials.

If death occurs prior to Day 90, GOS will be considered available and will be set to 5.

The proportion of patients with good recovery, i.e., rescaled GOS score = 1, at Day 90 will be analyzed using logistic regression adjusted as specified in Section 4.6. Observed counts and percentages for tenecteplace and placebo arms, and model based odds ratio, corresponding Wald 95% CI and p-value will be produced.

4.6.3 Exploratory Efficacy Endpoints

4.6.3.1 Subgroup Analyses for the Primary Endpoint

The primary efficacy endpoint, mRS score at Day 90, will be analyzed by the following subgroups: (1) Occlusion type (ICA/M1 or M2 occlusions) and (2) Randomization site (ECC or nECC). These subgroup analyses will be conducted using the same imputations and analysis methodology as the primary endpoint (see Section 4.6.1), however there will be no adjustment by the variable defining the subgroup. Proportionality of odds will not be re-evaluated for each subgroup. mRS score at Day 30 will be analyzed in a similar manner for the randomization site subgroups (ECC or nECC). Additional subgroup analyses for the primary efficacy endpoint are described in Section 4.6.5.

4.6.3.2 Final Infarct Volumes and Infarct Growth

Two follow-up imaging scans will be obtained after randomization, one at 24±6 hours and one at 72-96 hours (or discharge if patient is being discharged prior to 72-96 hours visit) after study drug administration. The scan at 72-96 hours visit (or discharge if patient is being discharged prior to 72-96 hours visit) will be used to evaluate the final infarct volume (captured as infarct volume on CT scan page and diffusion-weighted imaging (DWI) lesion volume on MRI scan page). Infarct growth will be defined as change from baseline at 72-96 hours visit (or discharge if patient is being discharged prior to 72-96 hours visit) in infarct volume. Patient who died before 72-96 hours visit and were not discharged prior to death will be excluded from the analysis.

Final infarct volumes and infarct growth at 72-96 hours visit assessment or discharge will be summarized descriptively (mean, median, etc.) and analyzed with nonparametric methods. The van Elteren's test will be used to produce p-values for the comparison of the distribution of tenecteplase versus placebo arms, adjusting as specified in Section 4.6. The median treatment differences will be presented with 95% CI based on the Hodges-Lehmann approach for the location shift (median treatment difference).

4.6.3.3 Planned Thrombectomy Not Performed for nECC to ECC Transfers

Some patients who were randomized at an nECC may be transferred to an ECC for endovascular therapy. Occasionally, a planned thrombectomy may not need to be performed on such patient, for example, if symptom improvement was observed prior to the planned thrombectomy. This improvement could occur before, during, or after the transfer of the patient.

The proportion of patients where a planned thrombectomy was not performed for patients randomized at an nECC and transferred to an ECC for endovascular therapy will be summarized by treatment group and analyzed using logistic regression. The model will be adjusted as specified in Section 4.6 but excluding the planned thrombectomy variable. Observed counts and percentages for tenecteplace and placebo arms, and model based odds ratio, corresponding Wald 95% CI and p-value will be produced.

4.6.3.4 Readmission Within 30 Days of Discharge

The proportion of patients readmitted to the hospital within 30 days of discharge will be summarized by treatment group and analyzed using logistic regression. The model will be adjusted as specified in Section 4.6. Observed counts and percentages for tenecteplace and placebo arms, and model based odds ratio, corresponding Wald 95% CI and p-value will be produced.

4.6.3.5 Patients Requiring One Pass with Endovascular Therapy Device

This endpoint will be defined for endovascular patients for whom the mechanical thrombectomy was performed (i.e. the clot was able to be reached) and a final TICI of 2b or 3 was achieved at the completion of the procedure. The number of passes to reach final TICI 2b/3 will be categorized as 1 vs >1, and analyzed using logistic regression, adjusting as specified in Section 4.6 but excluding the planned thrombectomy variable. If multiple mechanical thrombectomies were reported for a subject, only the initial procedure will be used to define this endpoint. Observed counts and percentages for tenecteplace and placebo arms, and model based odds ratio, corresponding Wald 95% CI and p-value will be produced.

4.6.3.6 Quality of Life in Neurological Disorders

Quality of Life in Neurological Disorders (Neuro-QoL) is a set of self-reported measures that assesses the physical, mental, and social effects experienced by adults living with neurological conditions. In this study, the short forms (domains) collected are the adult versions of: Lower Extremity Function (Mobility), Depression, Ability to Participate in Social Roles and Activities, and Cognitive Function. Patients will be assessed using the Neuro-QoL at Day 90. Raw Neuro-QoL scores are normalized using data from the US general population with a T-score mean of 50 and standard deviation of 10. Tables for converting the raw scores are available in the Neuro-QoL user's manual for each short form (Neuro-QoL Scoring Manual, v2.0, 2015).

Missing values within a Neuro-QoL short form will be handled according to the rules described in the manual. First, confirm that 4 or 50% of items, whichever is greater, have been answered. If there a sufficient number of items is not missing, sum the response scores from the items that were answered. Multiply this sum by the total number of items in the short form. Finally, divide by the number of items that were answered. If fewer than 4 questions or 50% of the total number of questions are answered, then scores will be set to missing.

For each domain, Neuro-QoL scores will be summarized descriptively (mean, median, etc.). Linear models will be used to compare the mean score of the treatment group versus placebo, adjusting as specified in Section 4.6.

4.6.3.7 Planned Thrombectomy Not Performed

A planned thrombectomy may not be performed in some cases. This includes patients with partial or complete recanalization of the qualifying vessel occlusion who did not go to angiography suite because non-invasive imaging demonstrated recanalization, or patients who have undergone catheter angiography and are found to no longer to have an occlusion of the qualifying vessel.

The proportion of patients where a planned thrombectomy was not performed due to partial or complete recanalization will be summarized by treatment group and analyzed using logistic regression adjusted as specified in Section 4.6 but excluding the planned thrombectomy variable. Observed counts and percentages for tenecteplace and placebo arms, and model based odds ratio, corresponding Wald 95% CI and p-value will be produced.

4.6.3.8 Overall Survival at Day 90

Cumulative incidence of all deaths will be presented by means of Kaplan-Meier plots if there are sufficient events (at least 5 in each treatment group). Time to death since randomization will be analyzed with a stratified log-rank test using variables as specified in Section 4.6. Death occurred up to Day 90 visit (or within 120 days since randomization, which is the study day cut-off for efficacy outcome evaluation) will be included in the analysis. Patients who did not die up to Day 90 visit will be censored at the last study assessment date. In addition, death occurred after Day 90 visit and the relatedness of death is determined by having a related fatal AE will also be included in the analysis.

4.6.4 Sensitivity Analyses

4.6.4.1 Analysis Using Hypothetical Strategy for COVID-19 Intercurrent Events

The analysis of the primary efficacy endpoint will be repeated in the ITT population, applying the hypothetical strategy for COVID-19 intercurrent event. This will exclude any mRS data collected after being infected with COVID-19. Missing Day 90 mRS scores, including those being excluded due to COVID-19 intercurrent event, will be imputed according to the methodology described in Section 4.9.1.1. In case of non-existence of post-baseline mRS data after applying the hypothetical strategy, subject(s) will be

excluded from the analysis. In this analysis, treatment policy strategy will still be used for non-COVID-19 intercurrent event.

4.6.4.2 Per Protocol Analysis

The analysis of the primary efficacy endpoint will be conducted in the PP population. This analysis will be based on the mRS data at Day 90 with the LOCF imputation.

4.6.4.3 Tipping Point Analysis

To assess robustness of the primary efficacy analysis result, a tipping point analysis will be conducted. In this analysis, multiple imputation (see Section 4.9.1.2 for details) will be employed to impute the missing mRS scores at Day 90.

In these imputations, the log-odds for a favorable outcome (mRS ≤ k) will be penalized in the TNK treatment group via an additional term Δ (see Tang 2018). Thus, exp(−Δ) is the additional term in the odds ratio of a favorable outcome between the TNK and Placebo groups under various assumptions of missing data mechanism, where Δ=0 corresponds to missing at random (MAR) and Δ>0 denotes missing not at random (MNAR). The value of Δ will be gradually increased until the p-value crosses the pre-specified nominal alpha level (0.003 at interim, 0.049 at final analysis).

Rubin's standard error will be used in the normal approximation formula to construct confidence intervals and p-values (Little et al. 2002). If the p-value does not reach the nominal alpha level within a reasonable range of data, then it will be concluded that the finding about the treatment difference is sufficiently robust to violations of the MAR assumption. If the p-value exceeds the nominal alpha level for some value of Δ, then a plot of p-values versus Δ will be provided. A monotone smoother will be added to the plot to help locate the tipping point.

4.6.5 Subgroup Analyses

In addition to the exploratory subgroup analyses specified in Section 4.6.3.1, the primary efficacy endpoint will also be analyzed on the following subgroups:

- Age: <80 vs ≥80 years
- Baseline NIHSS: ≤15 vs >15
- Baseline infarct volume: <20 mL, ≥20 mL and <50 mL, ≥50 mL
- Time (in hours) between stroke onset to randomization: <16 hours vs ≥16 hours
- Baseline occlusion type: ICA vs M1 vs M2
- Protocol version: Protocol V1-4 vs Protocol V5
- Mechanical thrombectomy planned at baseline: Yes vs No
- Mechanical thrombectomy performed (defined as successful clot manipulation): Yes vs No

- Time (in minutes) between study drug administration and groin puncture: groups defined by tertiles of the time variable. The analysis will include all patients who had groin puncture performed regardless of final performance of mechanical thrombectomy.

These subgroup analyses will be conducted using the same imputations and analysis methodology as the primary efficacy analysis (see Section 4.6.1), but proportionality of odds will not be re-evaluated for each subgroup.

4.7 PHARMACOKINETIC AND PHARMACODYNAMIC ANALYSES

No pharmacokinetic or pharmacodynamics analyses are planned for this study.

4.8 SAFETY ANALYSES

Safety analyses will be performed using the Safety population, with patients grouped according to the treatment actually received. Hypothesis testing will not be performed for any safety analysis.

4.8.1 Exposure of Study Medication

A summary of exposure to study drug, including any reasons not administered (if applicable), dose administered, and total volume administered, will be produced for the safety population.

4.8.2 Adverse Events

All verbatim adverse event terms will be coded using the latest version of MedDRA available at the time of reporting. Adverse event severity will be graded according to National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE, v5.0).

A treatment-emergent adverse event (TEAE) is defined as any new adverse event reported or any worsening of an existing condition on or after study drug administration. Adverse events with missing onset date will be considered to be treatment emergent. Adverse events with partially missing onset date will also be considered as treatment emergent when the partial date is identified to be on or later than the treatment administration date. An AE will also be considered treatment emergent if the event occurred before the start of treatment and the end date of the AE is either missing or on or after the start of treatment and AE increased in severity or either the initial or most extreme severity grade is missing. TEAEs will be tabulated by preferred terms (PT) within each system organ class (SOC) and will be presented in descending frequency.

Summaries will be provided for each of the following categories:

- All AEs
- Non-serious AEs reported in at least 5% of patient in a treatment group
- All AEs by NCI CTCAE severity

- Serious adverse events (SAEs)
- AEs related to study treatment as assessed by the investigator
- AEs leading to study discontinuation
- AEs resulting in death
- Adverse events of special interest (AESIs) as defined in the protocol (see Section 4.8.2.2)

Listings of AEs, SAEs, AESIs will also be provided.

4.8.2.1 Symptomatic Intracranial Hemorrhage

Intracranial hemorrhage events (ICH) are identified by neuroimaging and are recorded as AEs. Preferred terms for ICH will be based on the latest version of the MedDRA Standardized Medical Query (SMQ) “haemorrhagic central nervous system vascular conditions” (narrow).

For this study, Symptomatic ICH (sICH) is defined as ≥4 points of clinical worsening on the NIHSS compared with the most proximal NIHSS reported, attributed to a bleed on CT scan (preferred) or MRI performed within 36 hours after study drug administration.

To ensure accurate ICH and sICH reporting, central reader data will be compared with the case report forms (CRFs). Sites will be queried if there is a bleeding identified by the central reader without a matching ICH entry in the AE CRF. Counts of ICH and sICH events will be based on the AE reporting. The percentage of patients with sICH within 36 hours will be presented by treatment group and the ICH (symptomatic and asymptomatic) adverse events will be listed. Central reader’s evaluation of ICH, including subtypes, will be presented by treatment group.

4.8.2.2 Adverse Events of Special Interest

AESIs for this study include the following non-serious AEs:

- sICH events.
- The non-drug specific AESIs:
  - Cases of potential drug-induced liver injury that include an elevated alanine aminotransferase (ALT) or aspartate aminotransferase (AST) in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's Law. This includes
    - Treatment-emergent ALT or AST $>3 \times$ upper limit of normal (ULN) in combination with total bilirubin $>2 \times$ ULN
    - Treatment-emergent ALT or AST $>3 \times$ ULN in combination with clinical jaundice
  - Suspected transmission of an infectious agent by the study drug, as defined below:
    - Any organism, virus, or infectious particle (e.g., prion protein transmitting transmissible spongiform encephalopathy), pathogenic or non-pathogenic,

is considered an *infectious agent*. A transmission of an infectious agent may be suspected from clinical symptoms or laboratory findings that indicate an infection in a patient exposed to a medicinal product. This term applies only when a contamination of the study drug is suspected.

AESIs will be summarized by treatment group using the safety population and listed.

4.8.2.3 Mortality

Mortality rate up to study day 30 and day 90 will be summarized. These will be tabulated by primary cause of death. Deaths that occurred after the Day 90 visit date (up to 120 days since randomization) but captured in the database (e.g., as an outcome of an ongoing SAE) will not be included in the safety summaries unless the event is believed to be related with study treatment. All known deaths will be listed.

Details of any deaths will be presented in the form of individual patient listings.

4.8.2.4 Parenchymal Hematoma Type 2

The proportion of patients with PH2 intracranial hemorrhage within 72-96 hours follow-up visit will be summarized by treatment group.

4.8.3 Laboratory Data

Baseline lab results will be converted to International System of Units (SI) and summarized by treatment group.

4.8.4 Vital Signs

Baseline vital signs, including pulse rate, respiratory rate, weight, temperature, and systolic/diastolic blood pressure, will be converted to SI units and summarized by treatment group.

4.9 MISSING DATA

Missing data will be maintained as missing in the analysis datasets, unless specified otherwise. For variables where missing data are imputed, the analysis dataset will contain a new record with the imputed value and the original variable value will be maintained as missing.

4.9.1 mRS

For patients with no post-baseline mRS assessments, missing post-baseline mRS scores will not be imputed and these patients will be excluded from all mRS related analyses.

4.9.1.1 Last Observation Carried Forward

For patients who died prior to completing the Day 90 study visit, the missing mRS score at Day 90 will be imputed as 6. Otherwise, missing mRS data at Day 90 will be imputed using a LOCF technique, where the missing mRS score at Day 90 will be imputed by the

last observed mRS score, Day 30 or Day 5 / hospital discharge mRS value but not the baseline value.

4.9.1.2 Multiple Imputation

Missing mRS data will be imputed using a sequential regression method. Particularly, mRS will be sampled from a multinomial distribution. This distribution will be estimated by ordinal logistic regressions, using baseline covariates and the previous mRS value.

The ordinal logistic regressions estimate the cumulative odds for achieving an mRS outcome of at most k:

$$\text{logit}[\Pr(mRS_t \leq k)] = \alpha_{kt} + X\beta_t + mRS_{t-1}\delta_t$$

where t is the time point index (Day 5, Day 30, Day 90), k = 0 to 5, α_{kt} are intercepts, X is the design matrix for the baseline covariates and β_t are the corresponding regression coefficient vectors, and mRS_{t-1} is the previous mRS result (baseline, Day 5 or hospital discharge, or Day 30) with its coefficient δ_t. The list of covariates will include age, sex, race (white vs. non-white), baseline NIHSS, occlusion type and discharge destination (home vs. non-home).

Separate regressions will be fitted for each treatment group to avoid any assumptions about the treatment effect. In order to limit the number of parameters and prevent estimation issues, it will be assumed that the covariate effects β_t and δ_t do not depend on k (the proportional odds assumption). The model may be revised if estimation issues are encountered (e.g. quasi-complete separation). Logit is the usual link transformation, associated with a logistic regression.

For each fixed value of Δ, imputation is carried out in the following steps. These steps are repeated to impute missing mRS first at Day 30, then at Day 90 (using imputed Day 30 results):

- Fit the ordinal logistic regressions for mRS at time t, separately in the TNK and placebo groups.
- For patients with missing mRS at time point t, obtain the predicted cumulative probabilities. In the placebo group, use the probabilities predicted by the model. In the TNK group, recalculate the probabilities using the following equation.

$$logit[\Pr(mRS_t \leq k)] = \widehat{\alpha_{kt}} + X\widehat{\beta_t} + mRS_{t-1}\widehat{\delta_t} - \Delta$$

  where $\widehat{\alpha_{kt}}$, $\widehat{\beta_t}$ and $\widehat{\delta_t}$ are the estimated coefficients from the TNK model. The penalty term Δ reduces the odds for a favorable outcome. This penalty will only be applied the first time mRS is missing for the patient; its effect will be propagated to subsequent time points by the mRS_{t-1} term in the regression.

- Draw mRS at time t from the above distribution, with these additional considerations:

  (a) Intermittently missing mRS cannot be imputed with mRS=6. Where mRS=6 is generated for imputation, mRS=5 will be imputed instead.

  (b) Once mRS=6 is imputed, it will be propagated to all subsequent missing time points.

Note that by this algorithm, all ITT patients with baseline covariates will have Day 90 either observed or imputed. The process will be repeated to obtain 100 independent copies of the imputed dataset.

To analyze the multiply imputed data, apply the primary analysis model to each of the imputed dataset to arrive at 100 estimated treatment effects, $\gamma^{(m)}$ and their standard errors $S^{(m)}$, m = 1 to 100. The multiple imputation estimate and its standard error are then provided by the multiple imputation inference (SAS PROC MIANALYZE). The confidence interval and p-value can also be obtained from these estimates.

4.9.2 Adverse Events

Missing AE dates will be imputed and treatment emergent AEs will be identified according to the Sponsor's standard rules, provided in the ADaM dataset specification document. AE data listings will display the AE start and stop dates as collected, rather than the imputed dates.

4.9.3 Laboratory Values

Laboratory values of '>=x' or '<=x' will be taken as the value of x in the analyses. If a laboratory value is prefixed with '>': the available original value +0.001 will be used for table summaries; if a laboratory value is prefixed with '<', then the original value –0.001 will be used in table summaries.

4.10 INTERIM ANALYSES

This study has two planned safety interim analyses and one planned efficacy interim analysis. The interim safety analyses will occur when the first 25 and 50 patients have completed the 72-96 hours assessment post-randomization, respectively. Moreover, two additional safety interim analyses were requested by the iDMC to be conducted when the first 100 and 200 patients completed the 72-96 hours assessment post-randomization. Bleeding is a potential safety concern of the study drug and of special interest is symptomatic intracranial hemorrhage (sICH). Non-binding sequential boundaries will be used to monitor the sICH rate at the planned safety reviews (Ivanova 2005). The study may be halted if excessive number of sICH cases are observed, that is, if the number of patients with sICH is equal to or exceeds the threshold as shown in Table 4. This is a Pocock-type stopping boundary that yields a probability of crossing the boundary at ≤0.05 if the true sICH rate is equal to 10%.

Table 4 Non-binding Stopping Boundaries at Interim Safety Evaluations

Interim Evaluation	Stopping Boundary
Number of Patients Evaluated	***Number of Patients with sICH***
25	8
50	12
100	19
200	32
228	36

More information on the safety interim analyses is available in Section 3.3.

One efficacy interim analysis is planned for this study, when 50% (i.e., 228) of the total patients have completed the 90-day assessment to monitor both the efficacy and safety of tenecteplase vs. placebo. At the efficacy interim analysis, a type I error rate of 0.003 will be allocated for the interim efficacy evaluation based on the Lan-DeMets theory for α-spending function that approximates the O'Brien-Fleming boundary (DeMets and Lan 1994). In case of the positive efficacy result, the iDMC may recommend to stop the trial based on the benefit-risk profile of the study drug (see Section 3.3). In the event that the trial is stopped due to overwhelming efficacy results from the primary endpoint at the interim analysis, final analysis of the secondary endpoints will be performed based on the hierarchical testing procedure as described in the Section 4.6.2 at the 0.003 level to control the studywise type 1 error. If the study continues beyond the efficacy interim analysis, the critical value at the final primary efficacy analysis would be adjusted accordingly to maintain the protocol-specified overall type I error rate, i.e., the final efficacy analysis will be performed at the 0.049 significance level to maintain an overall family-wise type 1 error rate of 0.05.

An independent statistical entity will be responsible for conducting the interim safety and efficacy analyses and preparing the reports for iDMC review. The Sponsor will remain blinded during the course of the study.

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Appendix 1
Protocol Synopsis

PROTOCOL SYNOPSIS

TITLE:	**A PHASE III, PROSPECTIVE, DOUBLE-BLIND, RANDOMIZED, PLACEBO-CONTROLLED TRIAL OF THROMBOLYSIS IN IMAGING-ELIGIBLE, LATE-WINDOW PATIENTS TO ASSESS THE EFFICACY AND SAFETY OF TENECTEPLASE (TIMELESS)**
PROTOCOL NUMBER:	ML40787
VERSION NUMBER:	5
EUDRACT NUMBER:	Not applicable
IND NUMBER:	139,995
TEST PRODUCT:	Tenecteplase (RO5490263)
PHASE:	Phase III
INDICATION:	Acute ischemic stroke
SPONSOR:	Genentech, Inc.

Objectives and Endpoints

This study will evaluate the efficacy and safety of tenecteplase compared with placebo in patients with acute ischemic stroke (AIS) and evidence of salvageable tissue on their baseline computed tomography perfusion (CTP) scan or magnetic resonance imaging (MRI) who present in the 4.5- to 24-hour time window with an internal carotid artery (ICA) or middle cerebral artery (MCA; M1 or M2) occlusion. Specific objectives and corresponding endpoints for the study are outlined below.

Primary Efficacy Objective	Corresponding Endpoint
• To compare the efficacy of tenecteplase with placebo	• Ordinal modified Rankin scale (mRS) score at Day 90
Secondary Efficacy Objective	**Corresponding Endpoints**
• To evaluate the efficacy of tenecteplase compared with placebo	• Proportion of patients with functional independence, defined as an mRS of 0–2, at Day 90 • Proportion of patients with angiographic reperfusion (TICI 2b or TICI 3) at completion of angiographic procedure (endovascular patients only) • Median National Institutes of Health Stroke Score (NIHSS) score at Day 90 (Appendix 3) • Proportion of patients with a Barthel Index (BI) score of ≥95 at Day 90 (Appendix 4) • Proportion of patients with good recovery based on the Glasgow Outcome Scale at Day 90 (Appendix 5)

	• Proportion of patients with reperfusion at 24 hours post-randomization, defined as >90% reduction in T_{max}>6s lesion volume • Proportion of patients with recanalization at 24 hours post-randomization, defined as complete recanalization on CT angiography (CTA)/magnetic resonance angiography (MRA)
Exploratory Efficacy Objective	**Corresponding Endpoints**
• To evaluate the efficacy of tenecteplase compared with placebo	• Median final infarct volumes at 72-96 hours visit or discharge (if patient is being discharged prior to 72-96 hours visit) • Median infarct growth at 72-96 hours visit or discharge (if patient is being discharged prior to 72-96 hours visit) • Ordinal mRS score at Day 90 by occlusion type (M1/ICA or M2 occlusions) • Ordinal mRS score at Day 30 and Day 90 by randomization site (non-endovascular capable center [nECC] or endovascular capable center [ECC]) • Proportion of patients where planned thrombectomy was not performed • Proportion of patients who are randomized at an nECC and transferred to an ECC for endovascular therapy for whom planned thrombectomy was not performed • Proportion of patients readmitted within 30 days from discharge • Proportion of patients requiring one pass with endovascular therapy device (endovascular patients only) • Mean Neuro-QoL scores by domain (Appendix 6) • Overall Survival at Day 90
Safety Objective	**Corresponding Endpoints**
• To evaluate the safety of tenecteplase compared with placebo	• Incidence of symptomatic intracranial hemorrhage (sICH [a]) within 36 hours • Incidence and severity of adverse events • Mortality rate up to Day 30 and Day 90 • Proportion of patients with parenchymal hematoma type 2 (PH2) at the 72-96 hours visit

BI=Barthel Index; CT = computed tomography; CTA=computed tomography angiography; ECC=endovascular capable center; MRA=magnetic resonance angiography; mRS=modified Rankin scale; nECC=non-endovascular capable center; NIHSS = National Institutes of Health Stroke Scale; sICH = symptomatic intracranial hemorrhage; PH1 = parenchymal hematoma type 1; PH2 = parenchymal hematoma type 2; TICI=thrombolysis in cerebral infarction (scale); T_{max}=time to maximum of the residue function.

[a] sICH is defined as ≥ 4 points of clinical worsening on the NIHSS compared with the most proximal NIHSS reported, attributed to a bleed on CT scan (preferred) or MRI performed within 36 hours after study drug administration.

Study Design

Description of Study

This is a Phase III, prospective, double-blind, randomized, placebo-controlled trial of tenecteplase in patients with AIS who meet predefined imaging criteria. Patients who meet inclusion criteria will be randomized to tenecteplase (0.25 mg/kg, maximum 25 mg) or placebo, both administered as a single bolus injection over 5 seconds.

All patients will receive standard-of-care therapy according to American Heart Association / American Stroke Association (AHA/ASA) clinical guidelines (2018). For patients with ICA/M1 occlusions, this will include adjuvant endovascular therapy following study drug (tenecteplase/placebo) if criteria, as stipulated in the guideline, are met. Per guideline recommendations, patients with M2 occlusions generally will not undergo endovascular therapy following treatment with study drug (tenecteplase/placebo).

Study drug administration is recommended to occur before the start of endovascular therapy (defined as groin puncture):

- If it appears that it will be challenging to administer study drug prior to the femoral groin puncture for a planned thrombectomy, it is recommended that the patient not be randomzied
- However, if the patient has already been randomized, and due to unforeseen circumstances the drug cannot be administered prior to groin puncture for the planned thrombectomy, the drug **must** be administered as soon as possible and prior to manipulation of the clot

To determine eligibility for randomization, all patients will undergo multimodal CT or MRI at baseline. Only patients with a vessel occlusion (ICA or MCA) and penumbral tissue will be randomized.

Randomization will be stratified by age (≤70 vs. >70 years), type of occlusion (ICA/M1 vs. M2), baseline NIHSS (≤15 vs. >15), and randomization site (endovascular capable center [ECC] vs. non-endovascular capable center [nECC]). The primary goal of this trial is to compare the effect of treatment with tenecteplase vs. placebo and standard of care on 90-day functional outcomes (i.e., ordinal mRS scores) in patients who present in the 4.5- to 24-hour time window with an ICA or MCA (M1, M2) occlusion and evidence of target mismatch on multimodal CT or MRI of the brain. U.S. Food and Drug Administration (FDA)-approved and validated perfusion imaging software for determination of core volumes will be used.

Both patients transferred from an nECC as well as patients admitted directly to an ECC are eligible for the study.

Patients will undergo two follow-up MRI scans. Patients who cannot undergo an MRI will undergo a multimodal CT at the first follow-up and a non-contrast CT at the second follow-up. The first follow-up scan will be obtained at approximately 24 hours (±6 hours) after randomization and will include an assessment of recanalization and reperfusion, per standard of care. The second MRI will be obtained between 72 to 96 hours after randomization for assessment of final infarct volumes.

All patients will be evaluated using mRS at discharge/Day 5, Day 30 and Day 90 after randomization for clinical outcome evaluation.

The primary analysis is to compare the efficacy of tenecteplase versus placebo in all patients at Day 90, as assessed by the mRS score with an ordinal analysis. As a key prespecified

subgroup analysis, the trial will also assess the effect of tenecteplase separately in patients with ICA/M1 and in patients with M2 occlusions.

Number of Patients

Approximately 456 patients with AIS will be enrolled in this study at approximately 90 sites (consisting of both ECCs and nECCs) primarily in the U.S. and Canada.

Target Population

Inclusion Criteria

Patients must meet the following criteria for study entry:

1. Patient/legally authorized representative has signed the Informed Consent Form
2. Age ≥18 years
3. AIS symptom onset within 4.5 to 24 hours
   Stroke onset is defined as the time the patient was last known to be at their neurologic baseline. (Wake-up strokes are eligible if they present within the 4.5- to 24-hour time limits of last known well.)
   a. Note: All study-related treatment needs to be initiated within 24 hours.
4. Signs and symptoms consistent with the diagnosis of an acute anterior circulation ischemic stroke involving occlusion of the ICA, M1, or M2 vessels
5. Functionally independent (mRS 0–2) prior to stroke onset
6. Baseline NIHSS ≥5 and that remains ≥5 immediately prior to randomization
7. **Neuroimaging:** ICA or M1, M2 occlusion (carotid occlusions can be cervical or intracranial, with or without tandem MCA lesions) by magnetic resonance angiography (MRA) or computed tomography angiography (CTA) **AND** target mismatch profile on CT perfusion or MR perfusion (ischemic core volume <70 mL, mismatch ratio is ≥ 1.8 and mismatch volume is ≥ 15 mL)

   – The mismatch volume is determined by FDA-approved imaging software in real time based on the difference between the ischemic core lesion volume and the time to maximum of the residue function (T_{max})>6s lesion volume. If both a CT perfusion and a multimodal MRI scan are performed prior to enrollment, the later of the 2 scans is assessed to determine eligibility. For patients screened with MRA, only an intracranial MRA is required (cervical MRA is not required). Cervical and intracranial CTA are typically obtained simultaneously in patients screened with CTA, but only the intracranial CTA is required for enrollment.
   – Enrollment of patients with an ICA (including proximal and tandem ICA occlusions) will be capped at no more than 15% of the target study population.

   Alternative neuroimaging:

   – If CTA (or MRA) is technically inadequate: T_{max}>6s perfusion deficit consistent with an ICA or M1, M2 occlusion **AND** target mismatch profile (ischemic core volume <70 mL, mismatch ratio ≥ 1.8 and mismatch volume ≥ 15 mL as determined by RAPID software)
   – If MR perfusion (MRP) is technically inadequate: ICA or M1, M2 occlusion by MRA **AND** diffusion-weighted imaging (DWI) lesion volume ≤ 25 mL for an M1 or ICA occlusion and ≤ 15 mL for an M2 occlusion. If MRA is technically inadequate, a CTA can be used if performed within 60 minutes prior to the MRI. Carotid occlusions can be cervical or intracranial; with or without tandem MCA lesions.
   – If CTP is technically inadequate: patient can be screened with MRI and randomized if neuroimaging criteria are met.
8. Ability to comply with the study protocol, in the investigator's judgement

Exclusion Criteria

Patients who meet any of the following criteria will be excluded from study entry:

General

1. Current participation in another investigational drug or device study
2. Known hypersensitivity or allergy to any ingredients of tenecteplase
3. Active internal bleeding
4. Known bleeding diathesis
5. Known hereditary or acquired hemorrhagic diathesis, coagulation factor deficiency; recent oral anticoagulant therapy with INR >1.7
6. Use of one of the new oral anticoagulants within the last 48 hours (dabigatran, rivaroxaban, apixaban, edoxaban)
7. Treatment with a thrombolytic within the last 3 months prior to randomization
8. Intracranial neoplasm (except small meningioma), arteriovenous malformation, or aneurysm

    Any patient with an aneurysm located anywhere where a catheter or wire may be used in the thrombectomy procedure should be excluded. Additionally, patients with proximally thrombosed aneurysms suspected to be the mechanism of thromboembolism of the affected vascular territory should not be considered for enrollment due to the need for additional surgical or endovascular treatment that may require additional antithrombotic therapy.

    Aneurysms located elsewhere in the intracranial vasculature do not constitute an exclusion criterion *per se*, subject to the standards of care and the discretion of the treating physician. Additionally, previously treated intracranial aneurysms are not a criterion for exclusion, although, patients who underwent intracranial surgery within 2 months of screening are excluded.
9. Seizures at stroke onset if it precludes obtaining an accurate baseline NIHSS
10. Pre-existing medical, neurological, or psychiatric disease that would confound the neurological or functional evaluation

    COVID-19 positive and/or suspected (i.e., symptomatic) patients are not eligible unless previously tested positive for COVID-19 AND have been asymptomatic at a minimum 10 days from time of screening.
11. Severe, uncontrolled hypertension (systolic blood pressure >180 mmHg or diastolic blood pressure >110 mmHg)
12. For patients with suspected coagulopathy, platelet count must be checked prior to randomization and patient is excluded if baseline platelet count <100,000/μL
13. Baseline blood glucose >400 mg/dL (22.20 mmol/L)
14. Baseline blood glucose <50 mg/dL needs to be normalized prior to randomization
15. Clot retrieval attempted using a neurothrombectomy device prior to randomization
16. Intracranial or intraspinal surgery or trauma within 2 months

17. Other serious, advanced, or terminal illness with life expectancy is less than 6 months (investigator judgement)
18. History of acute ischemic stroke in the last 90 days
19. History of hemorrhagic stroke
20. Presumed septic embolus; suspicion of bacterial endocarditis
21. Any other condition that, in the opinion of the investigator, precludes an endovascular procedure or poses a significant hazard to the patient if an endovascular procedure was to be performed
22. Pregnant

Imaging

23. Unable to undergo a contrast brain perfusion scan with either MRI or CT
24. Extensive early ischemic change (hypodensity) on non-contrast CT estimated to be >1/3 MCA territory, or significant hypodensity outside the T_{max}>6s perfusion lesion that invalidates mismatch criteria (if patient is enrolled based on CT perfusion criteria)
25. Significant mass effect
26. Acute symptomatic arterial occlusions in more than one vascular territory confirmed on CTA/MRA (e.g., bilateral MCA occlusions, or an MCA and a basilar artery occlusion)
27. Evidence of intracranial tumor (except small meningioma) acute intracranial hemorrhage, neoplasm, or arteriovenous malformation

End of Study

The end of this study is defined as the date when the last patient, last visit (LPLV) occurs or the date at which the last data point required for safety follow-up is received from the last patient, whichever occurs later. The end of the study is expected to occur 90 days (±14 days) after randomization of the last patient in (LPI) the study.

Length of Study

The total length of the study, from screening of the first patient to LPLV, is expected to be approximately 3 years.

Investigational Medicinal Products

Test Product (Investigational Drug)

The investigational medicinal product (IMP) for this study is tenecteplase. The recommended total dose for this study is weight-based with 0.25 mg of tenecteplase per kg, not exceeding a maximum dose of 25 mg. A single bolus dose should be administered over 5 seconds based on patient weight.

Comparator

Placebo is being used as the comparator since a thrombolytic is only FDA-approved in the United States for use out to 3 hours, and the standard of care guidelines support use out to 4.5 hours.

Statistical Methods

Primary Analysis

The primary endpoint is the ordinal mRS score at Day 90. The distribution of the mRS scores will be compared between the treatment groups by a proportional odds model controlling for the randomization strata and baseline mechanical thrombectomy planned status. If the proportional-

odds assumptions are not met, alternative assumption-free ordinal analysis on the mRS score will be used (Agresti 1980; Howard et al. 2012; Churilov et al. 2014). Further details about the assumption-free method will be described in the Statistical Analysis Plan. Superiority of tenecteplase over placebo will be declared if the p-value of the estimated treatment effect from the proportional odds model is ≤0.049 (two-sided). An unadjusted analysis will also be performed using the Wilcoxon-Mann-Whitney test.

Determination of Sample Size

Approximately 456 patients will be enrolled in this study. Assuming the distribution of mRS scores at Day 90 in the two treatment arms and a 5% dropout rate in the study, approximately 228 patients in each treatment group will provide at least 90% power to detect the specified difference in the distribution of the mRS scores at the 2-sided overall 0.05 significance level based on the Wilcoxon-Mann-Whitney test. The final efficacy analysis will be performed at the 2-sided 0.049 significance level after adjustment for one interim efficacy analysis. This corresponds to being able to detect a common odds ratio of at least 1.76 (tenecteplase vs. placebo), with a total sample size of approximately 432 evaluable patients, assuming a 5% dropout rate.

Interim Analysis

Two safety only interim analyses are planned when the first 25 and 50 patients have completed the 72-96 hours visit assessment post-randomization, respectively. One efficacy interim analysis is planned when 50% (i.e., 228) of the total patients have completed the 90-day assessment to monitor both the efficacy and safety of tenecteplase vs. placebo. The safety data to be summarized in the safety interims include deaths, SAEs, non-serious AEs, treatment-related AEs, and AEs of special interest. The study may be halted if an excessive number of sICH events are observed and the details of the suggested stopping boundaries for interim safety evaluations will be provided in the the study SAP. At the efficacy interim analysis, where the primary efficacy analysis will be performed on 50% of patients, a type I error of 0.003 will be allocated for the interim efficacy evaluation based on the Lan-DeMets theory for α-spending function that approximates the O'Brien-Fleming boundary (DeMets and Lan 1994). Additional criteria for recommending that the study be stopped for positive efficacy may be added to the iDMC charter. If the study continues beyond the efficacy interim analysis, the critical value at the final primary efficacy analysis would be adjusted accordingly to maintain the protocol-specified overall type I error rate, i.e., the final efficacy analysis will be performed at the 0.049 significance level to maintain an overall family-wise type 1 error rate of 0.05.

Appendix 2
Schedule of Activities

	Screening and Treatment Visit	Visit 24 (±6) Hours After Randomization	Visit 72–96 Hours After Randomization [p]	Discharge or Day 5 (whichever occurs earlier)	Follow-Up [a]	
					30 (± 7) Days	90 (± 14) Days
Informed consent	x					
Medical history and baseline conditions [b]	x				x	x
Demographic data [b]	x					
Vital signs [c]	x					
Actual Weight[d]	x					
Glucose [d]	x					
Coagulation [d]	x					
CBC [e]	x					
Chemistry Panel[e]	x					
Pregnancy test [f]	x					
mRS [g]	x			x	x	x
NIHSS [h]	x	x	x	x	x	x
Brain imaging [i, m]	x	x	x			
Barthel Index				x	x	x
GOS	x			x	x	x
Neuro-QoL						x
Study drug administration [m]	x					
Concomitant medications [n]	x	x	x	x	x	x
Adverse events [h, o]	x	x	x	x	x	x

AIS= acute ischemic stroke; CBC = complete blood count; CT = computed tomography; DWI = diffusion-weighted imaging; eCRF = electronic Case Report Form; FLAIR = fluid-attenuated inversion recovery; GOS = Glasgow Outcome Scale; GRE = gradient recalled echo; IV=

intravenous; MRA = magnetic resonance angiography; MRI = magnetic resonance imaging; MRP = magnetic resonance perfusion; mRS = modified Rankin Scale; NIHSS = National Institutes of Health Stroke Scale; sICH=symptomatic intracranial hemorrhage; SOC=standard of care.

[a] Patients should return to the clinic for follow-up 30 and 90 days after treatment. At Day 90, patients should have the study completion visit. If an in-person visit is infeasible, only the mRS should be performed by telephone. All questionnaires can be collected by telemedicine except the Neuro-QoL.

[b] Medical history, including clinically significant diseases, surgeries, cancer history (including prior cancer therapies and procedures), reproductive status, smoking history, and use of alcohol and drugs of abuse, will be recorded at baseline. Demographic data will include age, sex, self-reported race/ethnicity, and veteran status.

[c] Vital signs will include measurements of respiratory rate, pulse rate, and systolic and diastolic blood pressure, and temperature. Temperature will be required prior to randomization (to rule out infection) and will be performed with subsequent frequency per SOC. The frequency of respiratory rate, pulse rate, systolic and diastolic blood pressure measurements should reflect post-IV thrombolytic monitoring in AIS per SOC.

[d] Patients should be weighed before randomization. Glucose (finger stick or blood draw) and coagulation tests (INR, aPTT, PT [only if the patient is taking an anticoagulant]) should be done. Results are required prior to randomization.

[e] Platelet count results must be checked prior to randomization in patients with suspected coagulopathy. Electrolytes will also be collected. All other CBC and electrolyte results are NOT required prior to randomization.

[f] All women of childbearing potential will have a urine/serum pregnancy test at screening. If a urine pregnancy test is positive, it must be confirmed by a serum pregnancy test.

[g] A pre-stroke mRS will be estimated a baseline. Thirty and 90 days after treatment, patients should be evaluated using mRS preferably in the clinic; if an in-person visit is not feasible, the patient can be evaluated by telephone (Appendix 2).

[h] A baseline NIHSS will be used to determine stroke severity and study eligibility. This assessment will be performed per institutional practice during hospitalization, upon hospital discharge and at the listed follow-up visits. In the event of an ICH event that occurs within 36 hours of drug administration, a NIHSS assessment is to be completed. See Appendix 3 for NIHSS. Please refer to Appendix 7 of the Protocol for brain imaging requirements.

[i] Patients will undergo two follow-up MRI scans (MRI/MRA/MRP at the first follow up and MRI at the second follow up). Patients who cannot undergo an MRI will undergo a multimodal CT/CTA/CTP at the first follow-up and a non-contrast CT at the second follow-up. The first follow-up scan will be obtained at approximately 24 (±6) hours after randomization. The second follow-up scan will be obtained at 72–96 hours after randomization. See Appendix 7 of the Protocol for brain imaging requirements.

[j] See Appendix 4 for the Barthel Index.

[k] See Appendix 5 for GOS.

[l] See Appendix 6 for Neuro-QoL.

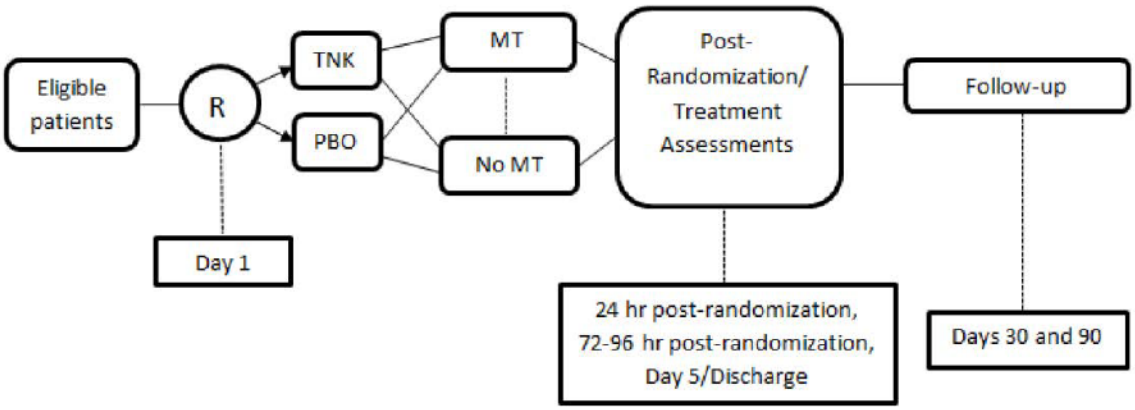
[m] Study drug administration needs to occur within 90 minutes of qualifying brain imaging. If 90 minutes is exceeded, CTP/MRP imaging must be repeated to validate that the patient is still eligible for the study. Study drug administration is recommended to occur before the start of endovascular therapy (defined as groin puncture). If it appears that it will be challenging to administer study drug prior to femoral groin puncture for a planned thrombectomy, it is recommended that the patient not be randomized. However, if the patient has already been randomized, and due to unforeseen circumstances the drug cannot be administered prior to groin puncture for the planned thrombectomy, the drug must be administered as soon as possible and prior to manipulation of the clot.

[n] In addition, all medications (e.g., prescription drugs, over-the-counter drugs, vaccines, herbal or homeopathic remedies, nutritional supplements) used by the patient within 7 days prior to initiation of study treatment will be recorded.

[o] New or worsened clinically significant abnormalities should be recorded as adverse events on the Adverse Event eCRF.

[p] Activities may be performed at discharge, if discharge is prior to the 72-96 hours visit. If the patient is discharged within 24 hours of the 24-hour visit, no 72-96 hours visit image collection is required.

Figure 1 Study Flow Chart



hr=hour; MT=mechanical thrombectomy; PBO=placebo; R=randomized; TNK=tenecteplase.
Note: The primary endpoint will be assessed at the Day 90 visit.

Appendix 3
SAS Code for Statistical Analysis

Analysis	Sample Code

Ordinal mRS parametric analysis and the test for proportional odds	`proc logistic;`
	`  class trt x1-x4;`
	`  model Y = trt x1-x4;`
	`run;`
	
	where Y is the ordinal mRS score, trt is treatment, and x1-x4 are randomization stratification factors. The score test for proportional odds is generated by default.

Multiple imputations for the tipping point analysis, ordinal mRS	I. Obtain prediction probabilities for mRS at time point t.
	`proc logistic;`
	`  by trt;`
	`  ...`
	`  model Yt = ... Yp;`
	`  output out=fit predicted=p;`
	`run;`
	
	where Yt is the ordinal mRS score at time t, trt is treatment group, Yp is the value of mRS at the previous visit.
	
	II. Apply the penalty to the predicted probabilities.
	`data fit;`
	`  set fit;`
	`  if not missing(p) then do;`
	`  oddsr=p/(1-p);`
	`  newoddsr=oddsr/delta;`
	`  newp=newoddsr/(1+newoddsr);`
	`  end;`
	`run;`
	
	III. Replace missing values of mRS at time t with random draws from the multinomial distribution defined by the vector of cumulative probabilities p or newp. newp is used for the values in the TNK group to impute for the first missing time point; other imputations will use p.
	
	`data outmi;`
	`  ...`

	x=rand('TABLE',p0,p1-p0,p2-p1,p3-p2,p4-p3,p5-p4)-1; ... run; The code will be executed first to impute Day 5 mRS, then Day 30 mRS, and then again to impute Day 90 mRS. Each time only the records with non-missing covariates and previous mRS value will be supplied.

Rubin's rules for the tipping point analysis, ordinal mRS (assuming proportional odds)	proc logistic data=outmi;
	model Y = trt x1-x4;
	by _Imputation_;
	ods output parameterestimates=PE;
	run;
	proc mianalyze parms=PE;
	modeleffects trt;
	run;
	
	where Y is the ordinal mRS score, trt is treatment, x1-x4 are randomization stratification factors, outmi is the dataset with multiple imputations, and _imputation_ is the index of the imputed dataset.

Wilcoxon-Mann-Whitney Generalized odds ratio	Derived through programming the formulas shown in Appendix 4 in data steps.

Appendix 4
Wilcoxon-Mann-Whitney Generalized odds Ratio

The Wilcoxon-Mann-Whitney generalized odds ratio (WMW GenOR) statistic provides a convenient way to compare two distributions of an ordinal outcome that does not require the proportional odds assumption. It is defined as follows:

Let Y_t be a randomly selected observation of mRS from the treatment group and let Y_p be a randomly selected observation from the placebo group. WMW GenOR is the ratio of the probability that Y_t is better than Y_p, to the probability that Y_p is better than Y_t, with an adjustment for ties:

$$\alpha = \frac{P(Y_t < Y_p) + 0.5 * P(Y_t = Y_p)}{P(Y_t > Y_p) + 0.5 * P(Y_t = Y_p)}$$

An earlier work by Agresti (1980) considered a similar statistic, but did not account for the ties. This measure was discussed in Churilov et al (2014), and was shown to have superior properties in the presence of ties than Agresti's statistic. Churilov et al (2014) argued that the asymptotic properties developed by Agresti extend to the WMW GenOR statistic, but did not present the formulas for the standard error. O'Brien and Castelloe offered a way to eliminate the ties and apply Agresti's formulas directly. Details of the derivation are presented below.

1. Eliminate the ties by splitting the counts observed for mRS=k in the placebo ($i.e. N_{2k}, k = 0, 1, 2, .., 6$) group equally between k-0.1 and k+0.1 (i.e. $N_{2k}/2, k = 0, 1, 2, \dots, 6$). Thus, observed mRS will be represented by the following table, where $N_{1k}, k = 0, 1, 2, \dots, 6$, stands for the counts observed for mRS=k in the treatment group:

mRS	Treatment	Placebo
-0.1	0	N_{20} / 2
0	N_{10}	0
0.1	0	N_{20} / 2
0.9	0	N_{21} / 2
1	N_{11}	0
1.1	0	N_{21} / 2
1.9	0	N_{22} / 2
2	N_{12}	0
2.1	0	N_{22} / 2
2.9	0	N_{23} / 2
3	N_{13}	0

3.1	0	N_{23} / 2
3.9	0	N_{24} / 2
4	N_{14}	0
4.1	0	N_{24} / 2
4.9	0	N_{25} / 2
5	N_{15}	0
5.1	0	N_{25} / 2
5.9	0	N_{26} / 2
6	N_{16}	0
6.1	0	N_{26} / 2

2. Let $P_{1i}=N_{1i}/\sum_i N_{1i}$ and $P_{2i}=N_{2i}/\sum_i N_{2i}$ be the sample proportions of patients with observed mRS=i in the treatment and placebo groups, respectively, where the index i is for the mRS value from the above table, from -0.1 to 6.1. Then the WMW GenOR statistic and its variance can be computed using Agresti's (Agresti A 1980) formulas (4.1) and (4.3), respectively

$$\hat{\alpha} = \sum_{j>i} P_{1i}P_{2j} \Big/ \sum_{j<i} P_{1i}P_{2j}$$

$$\hat{\sigma}^2 = \left\{ \frac{1}{N_1}\sum_j P_{1j}\left(\hat{\alpha}\sum_{i<j} P_{2i} - \sum_{i>j} P_{2i}\right)^2 + \frac{1}{N_2}\sum_j P_{2j}\left(\hat{\alpha}\sum_{i>j} P_{1i} - \sum_{i<j} P_{1i}\right)^2 \right\} \Big/ \left(\sum_{i>j} P_{1i}P_{2j}\right)^2$$

The more accurate confidence interval is derived for $\log(\alpha)$ and then exponentiated:

$$\exp\left(\log(\hat{\alpha}) \pm \frac{Z_{0.025}\hat{\sigma}}{\hat{\alpha}}\right)$$

3. For the stratified analysis, derive these statistics separately for each stratum (m=1,…, M) to arrive at $\hat{\alpha}_m$ and $\hat{\sigma}_m$. The following formulas can be used to estimate $\log(\alpha)$ and its variance:

$$\log(\hat{\alpha}) = \sum_m \hat{\alpha}_m^2 \log \hat{\alpha}_m / \hat{\sigma}_m^2 \Big/ \sum_m \hat{\alpha}_m^2/\hat{\sigma}_m^2$$

$$\widehat{Var}(\log(\hat{\alpha})) = \left(\sum_m \hat{\alpha}_m^2/\hat{\sigma}_m^2\right)^{-1}$$

Appendix 5
Selection of Adjusting Variables

This appendix presents the algorithm for selecting the variables to adjust efficacy analyses, starting from the initial list identified in section 4.6, excluding the protocol version variable.

- V1: Age (≤70 vs. >70 years of age)
- V2: Type of occlusion (ICA/M1 vs. M2)
- V3: Baseline NIHSS (≤15 vs. >15)
- V4: Randomization site (ECC vs. nECC)
- V5: Mechanical thrombectomy planned (Yes vs. No)

By default, the protocol version variable will be included in the model. Only if it is impossible to meet the minimum cell size requirement, the protocol version variable will be added to the above list as V6, and the variable selection algorithm will be repeated for all six variables.

A combination of variables C_i is any subset of these variables, i runs from 1 to 2^5. For example, (V1, V2, V5) selects Age, Type of Occlusion and MT Planned.

The combination's length L_i is the number of variables it includes. For the example of (V1, V2, V5), $L_i = 3$.

The combination's smallest cell size S_i is the smallest number of observations among 2^{L_i+1} cells formed by crossing all of the combination's variables and treatment. For the example of (V1, V2, V5), there are 16 cells to evaluate. If there are empty cells, $S_i = 0$.

The algorithm is applied to the dataset with non-missing outcome and adjusting variables, and proceeds as follows:

(1) Calculate L_i and S_i for i=1 to 2^5.

(2) Discard all C_i where $S_i \leq 1\%$ of subjects in the full ITT set (i.e., the same cell size threshold is used for all ITT analyses and subgroup analyses).

(3) Among the remaining C_i, prioritize the highest L_i and discard the rest. For example, if both (V1, V2, V5) and (V1, V3, V4, V5) have all cells >1%, (V1, V2, V5) will not be considered because it does not have the highest number of variables possible.

(4) Among the remaining C_i choose the one with the highest S_i.

(5) Among the remaining C_i (which have ties of both L_i and S_i), prioritize the C_i that contains V1. then V2, etc, until only one choice remains.

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System identifier: RIM-CLIN-474627

Approval Task	[redacted] Company Signatory 29-Mar-2023 22:03:03 GMT+0000