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STATISTICAL ANALYSIS PLAN

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VERSION:	1.0

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1. BACKGROUND

Stroke is the fourth leading cause of death and a leading cause of disability in the United States (U.S.). More than half of all ischemic stroke patients have mild strokes at initial presentation in population-based studies (3; 10). Alteplase is the only approved therapeutic agent for the treatment of AIS (acute ischemic stroke) and is widely accepted as the standard of care for eligible patients (4; 6). The currently approved Activase® (alteplase) U.S. Package Insert (USPI) contains wording that recommends against use of alteplase in patients with "minor neurological deficit or with rapidly improving stroke symptoms" due to the fact this patient population has not been evaluated. Furthermore, the current American Heart Association/American Stroke Association Council Guidelines include "minor and rapidly improving stroke symptoms (clearing spontaneously)" as a relative exclusion criterion for alteplase (6). This is largely due to the aforementioned label wording not recommending treatment of patients with minor neurological deficit or with rapidly improving stroke symptoms (RISS), the lack of a consistent and consensus definition of who constitutes this patient population, and the absence of definitive evidence of alteplase efficacy in this setting (TREAT Task Force 2013). Therefore, this trial is designed to evaluate the efficacy and safety of alteplase in confirmed AIS patients with minor neurological deficit or with RISS, which will be referred to collectively as "mild stroke".

For further background information see Section 1 of the protocol.

The original plan was for approximately 948 patients with mild stroke to be enrolled and randomized in a 1:1 ratio to two treatment groups. However the study enrollment was prematurely terminated due to recruitment below target in December 2016 (at approximately 1/3 of original planned enrollment of 948) and the analysis will be performed using available data from patients randomized prior to study termination.

This Statistical Analysis Plan (SAP) describes the statistical methods to be used for the clinical study report of study ML29093. This version of the SAP has been developed using version 3 of the study protocol dated 08 Jan 2015. The SAP will be finalized by [redacted] and approved by Genentech, Inc. before database lock and unblinding.

2. STUDY DESIGN

This is a double-blind, multicenter, randomized, Phase IIIb study to evaluate the efficacy and safety of IV alteplase in AIS patients with mild strokes that do not appear to be clearly disabling. The trial will consist of a screening assessment and randomization, treatment followed by a 30-day phone call and a 90-day follow-up visit. Treatment with the IV study drug should start within 3 hours from last known well time (stroke symptom onset).

All subjects randomized before the study was terminated will be followed to complete all assessments as originally planned.

2.1 SYNOPSIS

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

2.2 OUTCOME MEASURES

2.2.1 Primary Efficacy Outcome Measures

The primary efficacy outcome measure for this study is:

- A favorable functional outcome, defined by a modified Rankin Scale (mRS) of 0 or 1 at Day 90 post randomization

All mRS assessments will be performed by investigators blinded to treatment assignment that are trained and certified in mRS administration.

2.2.2 Secondary Efficacy Outcome Measures

The secondary efficacy outcomes are measured at Day 90 and include:

- Ordinal mRS
- Global favorable recovery (Global Outcome Measure derived from mRS 0-1, National Institutes of Health Stroke Scale [NIHSS] 0-1, Barthel Index [BI] ≥ 95, and Glasgow Outcome Scale [GOS] 1)

2.2.3 Exploratory Efficacy Outcome Measures

The following exploratory outcome measures for this study are measured at day 90 and included in the analyses described in this SAP:

- Total score of NIHSS at day 90
- Instrumental activities of daily living, as measured by the Total score of the BI at day 90
- Raw score of the GOS at day 90
- Cognition and behavior (modified 30-minute battery):
  - Controlled Oral Word Association test;
  - Hopkins Verbal Learning Test-Revised [HVLT-R] trials 1, 2, and 3;
  - digit symbol coding from the Wechsler Adult Intelligence Scale III [WAIS III]; Forward and Backward Digit Span test;
  - Benton Judgment of Line Orientation test, form V;
  - HVLT-R trial 4 and recognition;
  - semantic fluency [Animal Naming test];
  - Boston Naming Test [BNT; 15-item short form]
- Ambulatory performance (as measured by walking speed)
- Depression (CES-D [Center for Epidemiologic Studies, Depression]) score
- Quality of Life (EQ-5D [European Quality of Life] questionnaire)
- Stroke Impact Scale-16 (SIS-16)

2.2.4 Safety Outcome Measures

The safety outcome measures for this study are as follows:

- Symptomatic Intracranial Hemorrhage (sICH) within 36 hours of receiving IV study drug (primary safety assessment)

  sICH is defined as any neurological decline attributed to new ICH seen on imaging by the investigator (based on AE reporting). New ICH seen on imaging will be later confirmed on independent reading by blinded central study radiologists
- Any ICH (based on central neuroimaging reading) within 36 hours of receiving IV study drug
- Mortality within 90 days

- Stroke-related and neurological deaths within 90 days
- Incidence, severity, and spectrum of all adverse events (AEs) and serious AEs (SAEs).

The exploratory safety measure for this study is:

- Stroke recurrence (based on AE monitoring) within 90 days

2.3 DETERMINATION OF SAMPLE SIZE

The sample size was originally determined as follows.

A sample size of 856 is required in order to achieve 80% power in the primary analysis to detect effect size of 9% absolute difference in the proportion of patients with favorable outcomes [mRS of 0 or 1 at Day 90] between the alteplase and control arms. The above power consideration assumes a control proportion of 65% and Type I error of 0.025 (one-sided), and uses a group sequential design with one interim analysis for futility (non-binding), based on O'Brien and Fleming boundary, conducted after 50% of the anticipated sample size have completed 90-day follow-up assessments. [redacted] was used in sample size calculation.

The intention-to-treat principle will be applied to the primary analysis and, therefore, to safeguard against dilution of the treatment effect associated with an approximate 5% non adherence rate (due to loss to follow-up, consent withdrawal, treatment crossovers and stroke mimics), the above sample size was inflated by a factor of 1.108 (5). Therefore, a total sample size of 948 was planned for this study.

The study enrollment was terminated early in December 2016, after 313 patients were randomized. The analysis will be carried out based on all available data from patients randomized prior to termination. With this sample size, power calculations show that the study will be substantially underpowered to conduct superiority tests (**Table 1**). Therefore, all efficacy analyses will include estimation of treatment effects with the associated two-sided 95% confidence intervals.

Table 1 Estimated Power for the Final Sample Size (N=313)			
Assumed Effect Size	7%	8%	9%
Statistical Power [a]	25%	31%	38%
[a] Based on he t-test for the observed proportions of mRS 0-1 at Day 90, using type I error of 0.025, one-sided. Assumptions are based on a blinded evaluation of non-adherences in the Jan 2017 Development Safety Update Report (DSUR) datacut: - Control response rate among confirmed strokes is 74%. - 10% of randomized subjects are stroke mimics and 80% of those stroke mimics will have favorable outcome. - 1% of subjects received concomitant tPA - 7.4% subjects without Day 90 outcome (290 effective sample size)			

2.4 ANALYSIS TIMING

One interim analysis of the primary efficacy outcome was planned for clear futility once 50% of the subjects were enrolled. The interim analysis was to be conducted according to the beta-spending approach (9) with an O'Brien - Fleming-type boundary. Because of the early termination of the study, the interim analysis will not be performed.

3. STUDY CONDUCT

3.1 RANDOMIZATION ISSUES

Due to the emergency nature of randomization in acute stroke clinical trials, treatment assignments must be made in an expeditious manner that also ensures even distribution in the treatment groups. A "step forward" randomization regimen will be used to ensure that a randomized treatment assignment is available upon patients' arrival at the hospital and treatment can initiate immediately upon signing the informed consent and eligibility confirmation (16).

This procedure will be implemented using an Interactive Web Response System (IWRS). Before the first patient is enrolled at a site, the site will register with IWRS as part of site activation and "Use Next" drug kit IDs will be allocated for the first potential patient. This assigned kit will be used to treat the first patient enrolled at the site. Next, the study site enters identification information of the patient AFTER he/she is treated (within 8 hours of initiation of the patient's study treatment). IWRS will then assign "Use Next" drug kit IDs for the next patient that becomes eligible at

that site. Randomization is triggered as soon as the "Use Next" drug kits are used to treat an eligible patient.

Allocation of the "Use Next" kits will be determined by a dynamic randomization algorithm. Particularly, allocation will be balanced within the site using the urn method (17). In case of perfect balance within site, the overall allocation will be balanced using the biased coin method (14, 15). More details are available in Appendix C.

3.2 DATA MONITORING

An independent Data Monitoring Committee (iDMC) will provide ongoing review of safety outcomes and review efficacy and safety outcomes to inform a futility interim analysis after 50% of patients have completed or withdrawn from the study.

The iDMC will be composed of external advisors. The iDMC will perform functions such as:

- Review all accumulated safety data at periodic safety reviews
- Make recommendation to the Sponsor as to whether it is appropriate to discontinue the study after assessing the interim efficacy and safety data in aggregate

An independent Data Coordinating Center (iDCC) will be responsible for the preparation and review of unmasked data.

Details on iDMC membership, activities and timing are available in the Charter for the iDMC. Elaboration of the analyses that were to be conducted by the iDCC at the Interim Analysis is contained in the Interim SAP that was developed and signed-off for this study.

3.2.1 Safety Review Timing

Scheduled iDMC meetings to review safety data were planned to occur after 5% (50), 25% (237), 50% (474), and 75% (711) of patients had been enrolled or approximately annually. The last iDMC meeting prior to study termination was held on 03 May 2016, and included 225 enrolled subjects. Safety monitoring reviews included an unblinded evaluation of all adverse events (AEs), serious adverse events (SAEs), adverse events of special interest (AESI) including sICH, and any

other protocol-specified tests deemed critical to the safety evaluation of the study. However, the sponsor, investigators and other responsible for study conduct remained blinded.

4. STATISTICAL METHODS

Descriptive statistics will be produced for all endpoints. Data listings will be generated for specific endpoints as appropriate.

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 (CIs) will be constructed as two-sided 95% CIs.

4.1 ANALYSIS POPULATIONS

4.1.1 Intent-to-Treat Population

All randomized patients are included in the intent-to-treat (ITT) population to adhere to the ITT principle. For efficacy analyses, treatment groups will be assigned as randomized (IV Alteplase 0.9 mg/kg + Aspirin Placebo, IV Alteplase Placebo + Aspirin 325 mg).

4.1.2 Per-Protocol Population

The per-protocol (PP) population is defined as the subset of the ITT population excluding major protocol violators deemed to have the potential to affect patient outcome in terms of efficacy. No analyses will be performed for the PP population given the early enrollment termination. However, major protocol deviations will be identified in a blinded fashion prior to database lock and will be summarized. These deviations will be determined based on the medical monitors' records, as well as programmatically, using the following criteria at a minimum:

- Did not receive alteplase treatment as randomized
- Received any additional reperfusion therapy (e.g. IV or IA alteplase and/or endovascular therapy)

4.1.3 Safety Population

The Safety population is defined as all patients who received any amount of study drug. For safety analyses, treatment groups will be assigned according to the IV alteplase/alteplase placebo actually received. Thus, patients who receive active IV alteplase will be assigned to IV Alteplase 0.9 mg/kg + Aspirin Placebo group, and patients who receive IV alteplase placebo or did not receive the IV study drug (very rare) will be assigned to IV Alteplase Placebo + Aspirin 325 mg group. Group assignment will not take into account the actual dose of aspirin; however, it is expected that the majority of cases will follow the randomized treatment. Cases who did not receive treatment as randomized will be listed as described in Section 4.6.1.

4.2 ANALYSIS OF STUDY CONDUCT

The number and percentage of patients who have signed informed consent, been randomized in IWRS, received treatment, and completed through Day 30 and Day 90 (ie, completed the study) will be tabulated overall and for each treatment group. Reasons for not completing the study will also be tabulated overall and by treatment group using numbers and percentages.

A summary of the duration of the patient participation in the study will be produced. Duration will be calculated as the later date of the last efficacy or last safety assessment minus the first study drug administration date + 1.

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

Major protocol deviations will be listed and summarized by treatment group.

For those patients who are considered to have failed screening, the reason(s) for failure will be provided in a listing.

4.3 ANALYSIS OF TREATMENT GROUP COMPARABILITY

Clinically important demographic and disease characteristics at screening will be summarized overall and for each treatment group. Summaries will be produced for the ITT population.

Continuous variables will be summarized among patients with non-missing values, by the mean, standard deviation, median, first and third quartiles, minimum and maximum values. The number of patients with non-missing values will be displayed as well. Categorical variables will be summarized using numbers and percentages of patients in each category; unknown or missing will be included as a separate category.

The variables to be summarized include but are not restricted to the following:

- Demographics: sex, ethnicity, race(s), age (continuous and categorized as < 65 vs. ≥65)
- Last known well time to treatment (continuous and categorized as 0-2 hours, > 2-3 hours and >3 hours)
- Stroke characteristics: previous stroke (Y/N), RISS or non-RISS, stroke etiology, location of stroke
- NIHSS: total score (as continuous and categorical)
- Baseline substance use status: smoking and drug/substance abuse
- Medical History (MedDRA), in particular hypertension, diabetes mellitus, atrial fibrillation, and prior stroke
- Baseline Vital Signs: systolic and diastolic blood pressure and pulse
- Use of Aspirin / antiplatelet drugs within 7 days prior to screening/stroke onset
- Use of Heparin / anticoagulant drugs within 7 days prior to screening/stroke onset
- Prior medications
- Baseline neuroimaging (central reading)
- Baseline Lab Parameters: hematology (complete blood count without differential), chemistry (serum glucose), coagulation (international normalized ratio, partial thromboplastin time)

Baseline for NIHSS, vital signs and lab parameters will be defined as the last available assessment prior to initiation of any study medication.

4.4 EFFICACY ANALYSES

All efficacy analyses will be to estimate treatment effects and provide the associated two-sided 95% confidence intervals.

Endpoints based on mRS will generally use imputation to achieve the complete ITT set, with the exception of the repeated measures modeling. Other efficacy variables will use all available data in the ITT population.

Assessment of efficacy will be conducted based on endpoints collected during the Day 90 visit. The protocol allows this visit to be scheduled within 14 days of the target 90 days post-treatment; however, a wider window of 30 days will be adopted for the purpose of efficacy analysis.

More details of imputation approach for mRS are provided in Section 4.7 and Appendix D.

4.4.1 Primary Efficacy Endpoint

The primary efficacy analysis will estimate the effect of treatment with IV alteplase therapy and with the standard medical care (aspirin) in AIS patients with mild symptoms. The primary efficacy outcome is derived from the mRS.

The 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'. The mRS is collected at 30 and 90 days after start of study drug administration. If death occurs prior to an assessment day (Day 30 or Day 90), the mRS will be considered available and will be set to 6.

The proportion of patients within each treatment group with a favorable outcome, defined by mRS of 0 or 1 at 90 days post-randomization, will be estimated in the IV alteplase arm and in the standard medical care arm. Reasons for not performing the assessment of mRS at Day 90 will be summarized.

The difference in proportions between the two groups will be estimated using the risk difference. The risk difference will be obtained from a linear model with the binary mRS 0-1 outcome as the response, treatment, and age, time from last known well to treatment, and baseline NIHSS as continuous covariates. Quadratic terms for the continuous covariates may be added to the model, if the Wald p-value for the quadratic term is <0.1. No interactions among the covariates will be included in this model.

A common approach for implementation of this model is binary regression, a GLM with the Bernoulli distribution for the outcome and the identity link function. This

model has a possibility of non-convergence when the estimated response probability falls outside of the (0,1) interval. To circumvent the non-convergence problem, an approach of using the ordinary least squares with a robust standard error is used in this study. It was shown that if a robust standard error is used with the ordinary least squares estimation, the estimate of the risk difference is equal or similar to the estimate from the GLM with an identity link for Bernoulli outcome approach (22, 23).

The primary efficacy analysis will include all ITT patients, with patients grouped according to the treatment assigned at randomization. Missing or out of window mRS at Day 90 will be imputed with LOCF or late Day 90 mRS, if available, or simulated with a hot deck method (see Section 4.7 for handling of missing mRS).

As a supportive analysis of the primary efficacy endpoint, unadjusted or crude risk difference will be estimated along with the two-sided 95% CI using normal approximation. The standard error for the unadjusted risk difference will be derived from the group standard errors, assuming independence:

$$SE(D) = \sqrt{\frac{p(1-p)}{N_1} + \frac{q(1-q)}{N_2}}$$

where p and q are the observed group proportions and N_1 and N_2 are the group sample sizes.

The treatment effect for the binary mRS outcome will also be evaluated using the odds ratio statistic, derived from either a logistic regression, or a repeated measures model. These are considered exploratory analyses and described in Section 4.4.3.1.

4.4.2 Secondary Efficacy Endpoints

Secondary efficacy outcomes include mRS (full scale) and global favorable recovery at Day 90. These results will further describe the treatment effect.

4.4.2.1 Ordinal Modified Rankin Scale

As a secondary endpoint, mRS at Day 90 will be further explored on the range of the values (0 to 6) where the values of 5 and 6 are grouped. The distribution of full range of mRS scores at Day 90 (imputed) will be summarized by treatment group. Both tabular displays and stacked bar charts will be prepared.

The comparison will be achieved by fitting a proportional odds model with mRS score at Day 90 as the dependent variable, and treatment group, pre-treatment NIHSS score, age, and last known well time to treatment as the continuous predictors (1, 8). Quadratic terms for the continuous covariates may be added to the model, if the Wald p-value for the quadratic term is <0.1. No interactions among the covariates will be included in this model. The adjusted odds ratio with its two-sided 95% CI will be shown. Direction of the odds ratio will be chosen to reflect change in the odds of achieving a lower (better) mRS if treated with IV alteplase.

The proportional odds assumption will be tested by the score test, and the p-value will be reported. If the proportional odds assumption does not hold, the resulting odds ratio will not be presented. The alternative analysis planned for this situation will provide the adjusted mean difference between mRS scores with two-sided 95% CI. Methodology for this estimation is similar to the primary outcome, but using the ordinal mRS as the dependent variable.

4.4.2.2 Global Outcome Measure

Global outcome measure is derived from mRS = 0 or 1, NIHSS = 0 or 1, BI ≥ 95, and GOS = 1 at Day 90, considered as a whole. If death occurs prior to Day 90, all four binary outcomes will be considered as not being achieved in the analysis.

The NIHSS score is the total from scoring 11 items, assessed by a clinician. Items that are not applicable are not included in the total. It is not expected that any items will be applicable but missing; in such cases, the total score will be missing. The total score ranges from 0 to 42. NIHSS score will be collected pre-treatment, and then after 22-36 hours from start of study drug administration, after 5 days or at discharge from hospital, and at Day 90.

The Barthel index (BI) measures activities of daily living by having an assessor rate 10 items, with possible scores of 0, 5, 10 or 15. A total score, ranging from 0 to 100, is computed as the sum of the ratings from the 10 items. As this is a rater-completed assessment, no missing data are expected. However, if any item is not scored, then a score of 0 will be given as it will be presumed the subject did not meet the defined criteria for the item (20).

Glasgow Outcome Scale (GOS) is an evaluation of the outcome of stroke, assessed on a scale ranging from 1 to 5 by a clinician. The scale will be reversed from the

original published version (7) to assign lower scores to more favorable outcomes. The score is collected once in the study at Day 90.

The observed proportion of patients with a favorable outcome within each treatment group will be provided for the NIHSS (=0 or 1), BI (≥95) and GOS (=1) components of the global outcome measure, in a similar fashion as described for the primary outcome.

Treatment comparisons will be performed considering the four binary outcome measures as 4 dimensions of the outcome endpoint (12). These dimensions will be assumed to have a common odds ratio adjusted for pre-treatment NIHSS score, age, and last known well time to treatment. Quadratic terms for the continuous covariates may be added to the model, if the Wald p-value for the quadratic term is <0.1. No interactions among the covariates will be included in this model. Estimation will be performed using generalized estimating equations for a logistic model with correlation between the four outcomes of the same patient. Details of this model can be found in Appendix D. The estimated common odds ratio and its two-sided 95% CI will be reported.

Each individual outcome measure will be evaluated separately: odds ratios with two-sided 95% CIs will be derived from a logistic model with the same covariates as used for the global outcome measure to facilitate interpretation of the results.

LOCF and hot deck imputation will be used for the missing mRS component, similarly to the primary analysis. No imputation is planned for the other missing components, other than the non-achievement imputation in the event of death. However, incomplete component sets can still contribute to the model and will be included.

4.4.3 Exploratory Efficacy Endpoints

4.4.3.1 Exploratory Analyses for the Primary Endpoint

The primary analysis of the mRS 0-1 outcome will be complemented with exploratory supportive evaluations of treatment group differences.

The following analyses will be conducted to construct risk differences for mRS 0-1. Missing values for day 90 mRS will be imputed in the same manner as for the primary efficacy analysis unless otherwise specified.

- Unadjusted risk difference.
- Adjusted risk difference, from a linear regression with treatment, age, onset time to treatment, baseline NIHSS, and propensity score as covariates. The propensity score is derived from a logistic regression for treatment group with imbalanced covariates included in the model as covariates. This analysis will be triggered by presence of baseline imbalance. The criteria for baseline imbalance and details of this model are described in Appendix E.

The following analyses will be conducted to construct the odds ratio for mRS 0-1 such that the direction of the odds ratio reflects change in the likelihood of achieving a favorable outcome if treated with IV alteplase:

- Logistic regression, adjusting for pre-treatment NIHSS score, age, and last known well time to treatment as continuous covariates. Quadratic terms for the continuous covariates may be added to the model, if the Wald p-value for the quadratic term is <0.1. No interactions will be included in this model.
- Repeated measures model
  mRS outcomes at Day 30 and Day 90 will be modeled jointly by a logistic regression, using the GEE approach to account for the correlation between Day 30 and Day 90 outcomes of the same subject. Detailed description of this model is provided in Appendix D. No imputation will be performed for missing mRS for this particular analysis.

4.4.3.2 Additional Exploratory Analyses

To characterize treatment response among the subset of subjects with confirmed strokes, adjusted risk difference for the primary efficacy outcome will be repeated on the subset of patients with stroke mimics excluded. In addition, analyses as specified in section 4.4.2 for the secondary efficacy outcome measures will be produced for confirmed strokes.

4.4.3.3 Modified Rankin Scale

The mRS score will be used to evaluate the proportion of patients with a score = 0 vs 1 or higher. Observed percentages and adjusted risk difference will be presented. The adjustment will be performed in the same way as for the primary endpoint. Adjusted odds ratio will be presented in a separate analysis for this outcome in the same fashion as for mRS 0-1.

4.4.3.4 National Institutes of Health Stroke Scale

Descriptive statistics (n, mean, standard deviation, median, quartiles, minimum, and maximum) will be provided for NIHSS score by treatment group, both at Day 5 / Hospital discharge and at Day 90. Reasons for not performing the assessment of NIHSS at Day 90 will be summarized.

The NIHSS score will be used to evaluate the proportion of patients with a score = 0 vs 1 or higher, and separately, 0-1 vs. 2 or higher. Observed percentages and adjusted risk difference will be presented. The adjustment will be performed in the same way as for the primary endpoint. Adjusted odds ratio will be presented in a separate analysis for this outcome in the same fashion as for mRS 0-1.

4.4.3.5 Barthel Index

The BI total score will be used to evaluate the proportion of patients with complete independence (defined as a score = 100) and will be summarized by treatment group. Observed percentages and adjusted risk difference will be presented. The adjustment will be performed in the same way as for the primary endpoint.Reasons for not performing the assessment of BI will be summarized.

4.4.3.6 Glasgow Outcome Scale

Raw GOS score will be summarized by treatment group, and include the percentage of patients having each GOS score, and the proportion of patients with good recovery. Observed percentages and adjusted risk difference will be presented. The adjustment will be performed in the same way as for the primary endpoint. Reasons for not performing the assessment of GOS will be summarized.

4.4.4 Subgroup Analyses

For the primary endpoint, consistency of treatment effect of alteplase will be evaluated by various pre-specified baseline covariates. The primary pre-specified variables and their analytic thresholds are defined by:

- Age group (< 65 vs. ≥ 65)
- Pre-treatment NIHSS score group (0-2 vs. 3-5)
- Last known well time to treatment group (0-2 hours vs. > 2 hours)
- Stroke subgroup (RISS vs. non-RISS)

Adjusted odds ratios by subgroup will be obtained from multivariable logistic regressions with the main effects for treatment, age, last known well time to treatment, and NIHSS, and fit separately for each level of the subgroup.

Interactions of IV alteplase treatment effect with each of the subgroup variables will be explored by adding interactions of the subgroup variables with treatment to the logistic regression. The interactions will be included one at a time; thus, there will be 4 separate models to test each interaction. Results from these models will not be reported, except when the interaction p-values is smaller than 0.1, in which case the p-values will be footnoted in the forest plot.

A forest plot will be constructed to visually illustrate the subgroup analyses. The plot will show the odds ratios described above with two-sided 95% CIs, corresponding to each level of these subgroups. The plot will also show the common odds ratio estimate in different color or line pattern, as a reference. A subgroup analysis will be conducted only if there is at least 10 subjects per outcome (mRS 0-1, mRS >1), and the standard error of the odds ratio estimate is stable with the addition of the covariates.

Analysis of the efficacy endpoints is summarized in **Table 2**.

Table 2

Summary of Efficacy Analyses

Analysis	Statistical Method	Missing Data Method	Analysis Set(s)
Primary			
mRS 0-1 Day 90	Risk difference via linear regression, CI	LOCF + hot deck	ITT
Secondary			
mRS ordinal Day 90	Proportional odds model Descriptive, frequencies	LOCF + hot deck	ITT
Global outcome measure (Global test)	Logistic regression with a 4-dimensional outcome using GEE	LOCF + hot deck for mRS, non-response for death *	ITT
Exploratory			
mRS 0-1 Day 90	Logistic regression	LOCF + hot deck	ITT
	Logistic regression with Day 30 and Day 90 outcome using GEE	Repeated Measures Model (No imputation)	ITT
mRS 0 Day 90	Descriptive, the risk difference via linear regression OR via logistic regression	LOCF + hot deck	ITT
NIHSS 0, Day 90	Descriptive, the risk difference via linear regression OR via logistic regression	No imputation	ITT
NIHSS 0-1, Day 90	Descriptive, the risk difference via linear regression OR via logistic regression	No imputation	ITT
BI 100, Day 90	Descriptive, the risk difference via linear regression OR via logistic regression	No imputation	ITT

GOS 1, Day 90	Descriptive, the risk difference via linear regression OR via logistic regression	No imputation	ITT

CIs = confidence intervals.

* A full analysis set approach will be used, so that patients with missingness of some but not all Day 90 endpoint components will be included the model and contr bute to the estimation of the common OR despite missing values on some of the end point components. Death before day 90 is considerd as having not achieved the four binary end point components.

4.5 COGNITIVE, BEHAVIORAL AND PATIENT REPORTED OUTCOME ANALYSES

All the cognitive, behavioral and patient reported outcomes are considered exploratory.

4.5.1 Cognitive Assessments

A series of tests will be conducted at Day 90 to evaluate cognitive abilities of the patients. The tests and their outcomes are presented in the following table:

Table 3
Cognitive Assessments

Test	Outcome
Controlled Oral Word Association test	Number of words listed
Hopkins Verbal Learning test	Number of words recalled in 3 trials, Retention, and Recognition Discrimination Index
Digit symbol coding from the Wechsler Adult Intelligence Scale	Number of correctly coded digits
Forward and Backward Digit Span test	Maximum number of digits repeated, forward and backward scores
Benton Judgment of Line Orientation, form V	Total score
Animal Naming test	Number of unique animals named
15-item Boston Naming test	Total number of items answered correctly either spontaneously or after a stimulus cue

Outcomes from each test will be summarized by treatment group (mean, standard deviation, median, quartiles, minimum and maximum). Mean difference between treatment groups with 95% unadjusted normal CI will be presented. Reasons for the

assessment not being performed will be summarized by the number and percentage in each category.

4.5.2 Ambulatory Performance

The 10 meter walk test will be conducted at Day 90 visit to measure patient's walking speed. The speed will be measured at a comfortable pace and a fast pace, giving two attempts for each pace level. Average speed from the two attempts obtained at each pace will be summarized descriptively by treatment group. If speed can only be calculated in one of the attempts at a pace level, this result will be used.

Average speeds will be summarized by treatment group (mean, standard deviation, median, quartiles, minimum and maximum). Mean difference between treatment groups with 95% unadjusted normal CI will be presented. Reasons for the assessment not being performed will be summarized by the number and percentage in each category.

4.5.3 Patient Reported Outcomes

The patient reported outcomes (PROs) for this study include the EQ-5D, the 16 item Stroke Impact Scale (SIS-16), and the Center for Epidemiologic Studies Depression Scale (CES-D).

4.5.3.1 EQ-5D

Health-related quality of life will be assessed using the EQ-5D. The EQ-5D is a generic, preference-based health utility measure with questions about mobility, self-care, usual activities, pain/discomfort, and anxiety/depression that are used to build a composite of the patient's health status. The second component of the EQ-5D is a visual analogue scale (VAS), asking patients to rate their health from 0 to 100 where 0 represents the worst imaginable health state and 100 represents the best imaginable health state.

Each question is scored as 1 for no problem, 2 as some problem, and 3 indicating extreme problem. Ambiguous values (eg. 2 boxes are ticked for a single dimension) will be treated as missing (neither value used).

The 5 scores will then be converted into a single summary index by applying the time trade-off valuation technique for the US, published by EuroQol group. SAS

code to perform the calculation is provided in Appendix G. The resulting summary index is a continuous variable ranging from -0.11 (i.e., 3 for all questions) to 1.0 (i.e., 1 for all questions) on a scale where 0.0 = death and 1.0 = perfect health (18). If any of the 5 scores are missing, the summary index will not be calculated and set to missing.

The VAS is scored by the crossing point of the VAS with the response line, or with a horizontal line extended from the end point of the response line. Ambiguous values (eg. the line crosses the VAS twice) will be treated as missing.

Scores to each question, the summary index, and the VAS result, will be summarized descriptively, and the two-sided 95% CI of the mean difference will be presented for the index and VAS.

4.5.3.2 Stroke Impact Scale-16 (SIS-16)

Physical functioning will be evaluated using the SIS-16, which is a validated, stroke-specific, quality of life measure to assess the impact of stroke on a patient's health and life. Each of the 16 items is rated using the following scale: 1 = could not do at all; 2 = very difficult; 3 = somewhat difficult; 4 = a little difficult; 5 = not difficult at all.

The physical domain score is obtained by averaging the non-missing responses, provided that 9 or more questions are answered. The score is calculated as:

$$\text{Score} = 100 \times [(\text{Mean} - 1) / (5 - 1)]$$

which ranges from 0 to 100 (19).

Responses to individual questions and the physical domain score will be listed. The domain score will be summarized by treatment group and the two-sided 95% CI of the mean difference will be presented.

4.5.3.3 Center for Epidemiologic Studies Depression Scale (CES-D)

The CES-D is a short, self-report scale designed to measure depressive symptomatology. Responses to the 20 individual items will be scored to obtain an overall depression assessment, as follows.

1. Assign scores 0 = rarely or none of the time, 1 = some or little of the time, 2 = occasionally or moderate amount of time, 3 = most or all of the time.

2. Reverse positive items by subtracting their score from 3. Positive items are 4, 8, 12, and 16.
3. If 4 or fewer items are missing, sum the available item scores to determine the total score. If more than 4 items are missing, no score is calculated.

The total score will be used to evaluate the proportion of patients with depressive symptoms (defined as a score of $\geq$16 at 90 days) and will be summarized by treatment group.

Observed percentages and adjusted odds ratio will be presented. The adjustment will be performed in the same way as for the exploratory analysis of mRS 0-1.

4.6 SAFETY ANALYSES

MedDRA (using the latest version at the time of reporting) will be used as the thesaurus for AEs and diseases, and the World Health Organization (WHO) drug dictionary will be used for treatments and MedDRA for procedures. A glossary of these codes will be produced.

AEs with onset prior to initiation of study treatment which do not worsen after study treatment or with onset after Day 90 assessment, will be listed as non-treatment emergent and will not be included in the safety summaries.

4.6.1 Exposure to Study Medication

Exposure data will be listed for both study drugs. The listing will show the randomized treatment as well as the treatment actually received.

A summary of exposure to alteplase/alteplase placebo will be produced for the safety population. The total dose administered and duration of infusion will be summarized by active or placebo Alteplase, as actually received. For Aspirin, dose received will always be “325 mg”, and therefore will not be summarized.

Compliance with study medications will be summarized for the safety population. For Alteplase, counts of infusion altered or stopped prematurely will be tabulated along with the reason. For aspirin, the number of patients who did not take study aspirin and a summary of reasons will be presented.

4.6.2 Adverse Events

AE safety endpoints in this study are:

- Number and percentage of patients experiencing serious and non-serious AEs after receiving study drug
- Number and percentage of patients experiencing serious and non-serious AEs of special interest

AEs will be coded (using the latest MedDRA version at the time of reporting) and tabulated by system organ class (SOC); individual events within each SOC coded as preferred terms will be presented in descending frequency using the safety population. Treatment groups will be summarized according to the actual treatment received.

AEs will also be tabulated by intensity (severity) and relationship to study drug, as indicated by the investigator. Intensity of AEs will be graded according to the WHO Toxicity Grading Scale for Determining the Severity of Adverse Events (see Appendix 14 of the protocol). Incidence and severity of AEs will be described for all treated patients by treatment group and by the MedDRA classification.

A summary of incidence rates of all deaths and stroke-related or neurological deaths will be presented. Cumulative incidence of all deaths and stroke-related or neurological deaths will be presented by means of Kaplan-Meier plots if there are sufficient events (at least 5 in each group).

Timing of the AEs relative to Day 30 and Day 90 will be determined, using imputation for partial AE start and stop dates if necessary, as described in Appendix D. AEs occurring after receipt of any study medication and before Day 90 will be summarized. AEs leading to IV study drug modification will be listed. SAEs, ICH, AEs of special interest and AEs leading to study treatment discontinuation will be summarized through Day 90. In addition, non-serious AEs within 30 days will be summarized.

4.6.2.2 Adverse Events of Special Interest

Non-serious AESIs are required to be reported by the investigator to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2 of protocol for reporting instructions).

AESIs for this study include the following:

- sICH events, if not already reported as an SAE (for a list of preferred terms for ICH see Appendix 13 of protocol)
- Stroke recurrence, if not already reported as an SAE.
  Stroke recurrence is defined as a new ischemic stroke based on the AE reporting. Stroke events will be identified through a medical review of the AE preferred terms.
- Suspected Transmission of an Infectious Agent via a Medicinal Product (STIAMP) by the study drug, if not already reported as an SAE.

AESIs will be tabulated by SOC and preferred term within SOC by treatment group using the safety population.

4.6.3 Neuroimaging

Neuroimaging may consist of a non-contrast computed tomography (CT) or magnetic resonance imaging (MRI) of the brain. Non-contrast CT or MRI will be performed at baseline to ensure that the patient does not have evidence of acute intracranial hemorrhage (ICH) prior to study drug administration.

An additional non-contrast CT or MRI will be performed at 22 to 36 hours from the initiation (bolus) of study drug infusion. The purpose of this is to evaluate for ICH in the patient. Additional CT scans or MRIs should be performed based on the investigator's discretion, or if any clinical findings suggest ICH.

All CT and MRI scans will be evaluated by a central team of radiologists blinded to the clinical attributes of the case. Results from the evaluation of the CT or MRI scans by both local and central readings will be listed, as available.

4.6.3.1 Intracranial Hemorrhage

Intracranial hemorrhage events (ICH) are identified by neuroimaging and are recorded as AEs using one of the terms specified in protocol Appendix 13. The images obtained within the first 36 hours after treatment are further evaluated by central reading, which may or may not confirm ICH.

While ICH may be identified at any time during the study, the first 36 hours after treatment are the key time interval. If multiple scans indicate ICH within 36 hours, it is interpreted as a single ICH event. ICH events identified after the first 36 hours will contribute to the general AE summaries. Timing of ICH will be determined according to the rules specified in Appendix D.

Incidence of ICH within 36 hours by AE reporting will be presented by treatment group. Determination of ICH within 36 hours from local and central readings of scans will also be presented, and agreement between the local and central readings will be tabulated.

Difference in percentage of subjects with ICH within 36 hours based on central and local scan reading will be reported by treatment group. A two-sided 95% CI around this difference will be constructed using the Miettinen-Nurminen score method (20).

Additionally, central reading classifications will be used to further describe subtypes of any ICH.

The central reading will classify ICH according to the following intracerebreal subtypes:

- Hemorrhagic infarct type 1 or type 2
- Parenchymal hematoma type 1 or type 2
- Remote intraparenchymal hemorrhage type 1 or type 2

In addition, subarachnoid hemorrhage, subdural hemorrhage, or epidural hemorrhage will be noted. ICH subtypes will be tabulated by treatment group.

4.6.3.2 Symptomatic Intracranial Hemorrhage

The protocol specifies that ICH is considered symptomatic (sICH) if it is not seen on CT or MRI scan at baseline, and any neurologic decline is attributed to it by the local

investigator. Both serious sICH and non-serious sICH events are required to be reported by the investigator to the Sponsor immediately.

sICH within 36 hours is considered the primary safety outcome. sICH events are captured through AE reporting.

In addition to the primary definition of sICH, the following two definitions of sICH will be explored and reported in this study.

- sICH using Heidelberg classification: either (1) PH2 as reported by radiology core, or (2) an sICH as classified by PRISMS but also associated with 4-point total NIHSS score worsening or 2 point worsening on any single NIHSS item when comparing scores immediately pre and post scan.
- sICH using SITS-MOST definition (25): PH2 as listed by radiology core on CT scan between 22-36 hours, which is associated with deterioration of ≥4 points on the NIHSS from the lowest NIHSS value between baseline and 36 hours, but prior to repeat CT, or leading to death.

Percentage of subjects with sICH within 36 hours will be presented for each definition by treatment group. Difference between treatment group percentages will be reported along with a two-sided 95% CI constructed using the Miettinen-Nurminen score method (20).

ICH symptomatology will be tabulated for confirmed strokes and separately for stroke mimics.

4.6.4 Laboratory Data

Specimens for the laboratory tests will be analyzed by the site's local laboratory. Tests will be done at screening only and will include hematology (complete blood count without differential), serum glucose, coagulation values (INR, partial thromboplastin time), and pregnancy test.

Lab results will be converted to SI units and summarized as part of the baseline treatment group comparability described in Section 4.3; no further analysis is planned.

4.6.5 Vital Signs

Vital signs will be collected at screening, and will include measurements of pulse and systolic/diastolic blood pressure (while patient is in a supine position).

Vital signs will be summarized as part of the baseline treatment group comparability described in Section 4.3.

4.6.6 Mortality

Incidence of mortality will be calculated by treatment group and overall. Details of any deaths will be presented in the form of individual patient listings.

4.6.7 Previous and Concomitant Medications

Previous medications refer to those stopped prior to study day 1. Previous medications will be summarized by medication class and preferred term. Usage of Aspirin / antiplatelet drugs and Heparin / anticoagulant drugs up to 7 days prior to screening/stroke onset will be summarized.

Concomitant medications refer to those ongoing during the study. Concomitant medications will be summarized by medication class and preferred term. Usage of reperfusion therapy will be summarized. Reperfusion therapy includes IV or IA alteplase and/or endovascular therapy, and will be adjudicated in a medical review.

4.7 HANDLING OF DROPOUTS, MISSING/INCOMPLETE DATA, OR OUTLIERS

For the analyses of the mRS based endpoints, last observation carried forward will be used to impute missing or out of window assessments of mRS at Day 90. The mRS result carried forward needs to be on or after Day 4 to qualify for this imputation. If there is no in-window or LOCF assessment of mRS, then a late Day 90 mRS will be used if available.

ITT patients without any observed mRS past day 4 will have their Day 90 mRS simulated by a hot deck method. This will be an adjustment cells imputation (24), with the cells defined by treatment group, age (< 65 vs. ≥ 65), pre-treatment NIHSS score (0 − 2 vs. 3 − 5), and last known well time to treatment (0 − 2 hours vs. > 2 hours). For each patient with a missing mRS score, a “donor” patient will be drawn at random from the pool of patients with available mRS and who belong to the same cell as the recepient (i.e. randomized to the same treatment group and having the

same categories for age, last known well time to treatment and baseline NIHSS). The imputed mRS will then be the mRS value of the "donor".

It is expected that only a small number of patients will need the hot deck imputation; therefore, the techniques recommended in (24) for a variance estimate that incorporates the additional variance from the missing information will not be implemented.

No imputation of missing patient reported outcomes, cognitive assessments, or the 10-meter walk test will be performed except as specified in the scoring rules for the assessment. Reasons for the assessment not being performed will be summarized by the number and percentage in each category by treatment group.

4.8 INTERIM ANALYSES AND/OR SAFETY REVIEWS

One interim analysis of the primary efficacy outcome was planned for clear futility after approximately 50% of patients have completed or discontinued from the study, but this analysis was not performed due to early termination of the study. In addition, there were 4 planned safety reviews, of which two have occured after 5% (50), and 25% (237) of patients have been enrolled.

Details on the procedures for the safety reviews and the interim analysis are in the Charter for the iDMC.

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Appendix A
Protocol Synopsis

PROTOCOL SYNOPSIS

TITLE:	**A PHASE IIIB, DOUBLE-BLIND, MULTICENTER STUDY TO EVALUATE THE EFFICACY AND SAFETY OF ALTEPLASE IN PATIENTS WITH MILD STROKE (RAPIDLY IMPROVING SYMPTOMS AND MINOR NEUROLOGIC DEFICITS [PRISMS])**
PROTOCOL NUMBER:	ML29093
VERSION NUMBER:	3
EUDRACT NUMBER:	N/A
IND NUMBER:	3811
TEST PRODUCT:	Alteplase (RO5532960)
PHASE:	IIIb
INDICATION:	Acute ischemic stroke
SPONSOR:	Genentech, Inc.
DATE FINAL:	Version 1: 27 November 2013
DATE AMENDED:	Version 2: 22 August 2014 Version 3: 17 December 2014

Objectives

Primary Objectives

The primary objective of this study is to determine the efficacy of intravenous (IV) alteplase for treatment of acute ischemic stroke (AIS) in patients with mild stroke (also known as "minor neurologic deficit" and "rapidly improving stroke symptoms"), defined as a National Institutes of Health Stroke Scale (NIHSS) score ≤ 5 and not clearly disabling, within 3 hours of last known well time as measured by the proportion of patients with a modified Rankin Scale (mRS) score of 0−1 at Day 90.

Secondary Objectives

The secondary objectives of this study are as follows:

To further evaluate efficacy of IV alteplase to improve mild stroke outcomes at Day 90 via:

- Ordinal mRS
- Global favorable recovery (Global Outcome Measure derived from mRS 0-1, NIHSS 0-1, Barthel Index [BI] ≥95, and Glasgow Outcome Scale [GOS] = 1)

To evaluate safety as measured by:

- Symptomatic intracranial hemorrhage (sICH) within 36 hours – primary safety assessment
- Any intracranial hemorrhage (ICH) within 36 hours
- Mortality within 90 days

Stroke-related and neurological deaths within 90 days

Incidence, severity, and spectrum of all adverse events (AEs) and serious adverse events (SAEs)

Exploratory Objectives

The exploratory objectives for this study include subgroup analyses of the primary efficacy outcome variable in subgroups defined by age (< 65 vs. ≥ 65), pre-treatment NIHSS score (0 − 2 vs. 3 − 5), last known well time to treatment (0 − 2 hours vs. > 2− 3 hours), and stroke subgroups (rapidly improving stroke symptoms [RISS] vs. non-RISS), respectively.

The following endpoints will also be explored:

NIHSS

BI

GOS

Stroke recurrence (based on AE monitoring) within 90 days

Cognition and behavior (modified 30-minute battery: Controlled Oral Word Association test; Hopkins Verbal Learning Test-Revised [HVLT-R] trials 1, 2, and 3; digit symbol coding from the Wechsler Adult Intelligence Scale III [WAIS III]; Forward and Backward Digit Span test; Benton Judgment of Line Orientation, form V; HVLT-R trial 4 and recognition; semantic fluency [Animal Naming test]; and Boston Naming Test [BNT; 15-item short form]) at Day 90

Ambulatory performance (as measured by walking speed) at Day 90

Center for Epidemiologic Studies-Depression (CES-D*)*

Quality of life (European Quality of Life [EQ-5D] questionnaire) at Day 90

Stroke Impact Scale-16 (SIS-16) at Day 90

Study Design

Description of Study

PRISMS is a double-blind, multicenter, randomized, Phase IIIb study to evaluate the efficacy and safety of IV alteplase in AIS patients with mild strokes that do not appear to be clearly disabling. The trial will consist of a screening assessment, randomization, and treatment followed by a *Day 5 visit,* 30-day phone call*,* and a 90-day follow-up visit. Treatment should start within 3 hours from last known well time (stroke symptom onset).

Screening assessments will be used to determine the eligibility of the patient. Patients meeting eligibility criteria will be randomized in a 1:1 ratio to receive either*:*

1. One dose of alteplase 0.9 mg/kg IV (not to exceed 90 mg) and one dose of oral aspirin placebo, OR

2. One dose of IV alteplase placebo and one dose of oral aspirin 325 mg.

Number of Patients

Approximately 948 patients with AIS and mild strokes will be enrolled in the study across 75 sites in North America.

Target Population

Inclusion Criteria

Patients must meet the following criteria for study entry:

Age ≥ 18 years (no upper age limit)

Mild ischemic stroke defined as the most recent pre-treatment NIHSS score of ≤ 5 and determined as not clearly disabling by the investigator. This includes patients with persistently mild deficits as well as those who improve to a pre-treatment NIHSS score ≤ 5 (also known as RISS).

Study treatment can be initiated within 3 hours of last known well time without stroke symptoms (i.e., last seen normal).

Signed informed consent prior to initiation of any study-specific procedure or treatment. The patient or the patient's legally authorized representative must be able to provide written informed consent and understand the potential risks and benefits from study enrollment and treatment.

Exclusion Criteria

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

Computed tomography (CT) or magnetic resonance imaging (MRI) findings consisting of one of the following:

- CT with clear large hypodensity that is greater than one-third middle cerebral artery (MCA) territory (or greater than 100 cc if not in MCA territory),
- MRI with clear large hyperintensity on concurrent diffusion-weighted (DW) and fluid-attenuated inversion recovery (FLAIR) that is greater than one-third MCA territory (or greater than 100 cc if not in MCA territory),
- Imaging lesion consistent with acute hemorrhage of any degree, OR
- Evidence of intraparenchymal tumor

Disability prior to the presenting stroke (historical mRS score $\geq$ 2)

Standard contraindications to IV alteplase for patients treated within 3 hours of symptom onset, including:

- Head trauma or previous stroke within the previous 3 months
- Myocardial infarction within the previous 3 months
- Gastrointestinal or urinary tract hemorrhage within the previous 21 days
- Major surgery within the previous 14 days
- Arterial puncture at a non-compress ble site within the previous 7 days
- Any history of ICH with the exception of < 5 chronic microbleeds on MRI
- Elevated blood pressure (systolic > 185 mm Hg or diastolic > 110 mm Hg), or the use of aggressive measures (use of more than two intravenous agents to lower blood pressure) to achieve blood pressure within acceptable parameters
- Treatment with unfractionated heparin within the last 48 hours and activated partial thromboplastin time outside of the normal range as specified by the center's local laboratory
- Blood glucose < 50 mg/dL
- International normalized ratio > 1.7 (Note: This does not need to be verified prior to randomization if there is no clinical suspicion of abnormality.)
- Platelet count of < 100,000/mm3 (Note: This does not need to be verified prior to randomization if there is no clinical suspicion of abnormality.)
- Treatment with a direct thrombin inhibitor or factor Xa inh bitor (including novel oral anticoagulants [e.g., dabigatran, rivaroxaban, apixaban, edoxaban]) within the last 48 hours
- Treatment with a low-molecular-weight heparin (e.g., dalteparin, enoxaparin) within the last 48 hours

Allergic reactions to study drug or aspirin, or nonsteroidal anti-inflammatory drugs (NSAIDs)

Females of childbearing age who are known to be pregnant and/or lactating, or who have a positive pregnancy test on admission

Inability to swallow, which would prevent oral intake of aspirin or aspirin placebo tablet

Other serious, advanced, or terminal illness that would confound the clinical outcome at 90 days

Current or recent (within 3 months) participation in another investigational drug treatment protocol

Anticipated inability to obtain 3-month follow-up assessments

Previous enrollment in PRISMS

Any other condition that the investigator feels would pose a significant hazard to the patient if treatment with alteplase is initiated

Length of Study

Based on study enrollment projections, this study is estimated to take approximately 4 years to complete, from first patient in to last patient's last visit (LPLV), when the final patient completes the 90-day follow-up.

End of Study

The end of the study is defined as the date when the LPLV occurs. The LPLV is expected to occur approximately 90 days (±14 days) after the last patient is enrolled into the study.

Efficacy Outcome Measures

The primary efficacy outcome measure for this study is:

A favorable functional outcome, defined by an mRS score of 0 or 1 at Day 90 post-randomization

All mRS assessments will be made by investigators blinded to treatment assignment who are trained and certified in mRS administration

The secondary efficacy outcomes at Day 90 include:

Ordinal mRS

Global favorable recovery (Global Outcome Measure derived from mRS 0-1, NIHSS 0-1, BI ≥95, and GOS 1)

Safety Outcome Measures

The safety outcome measures for this study are as follows:

Incidence of sICH within 36 hours (primary safety assessment)

sICH is defined as any neurological decline attr buted to new ICH seen on imaging by the investigator. New ICH seen on imaging will be later confirmed on independent reading by blinded central study radiologists.

Any ICH within 36 hours

Overall mortality within 90 days

Stroke-related and neurological deaths within 90 days

Incidence, severity, and spectrum of all AEs and SAEs

Investigational Medicinal Products

Test Product (Investigational Drug)

Patients randomized to the active arm will receive treatment with 0.9 mg/kg of IV alteplase (maximum 90 mg) per standard stroke dosing.

Comparator

Patients randomized to the placebo arm will receive IV alteplase placebo consisting of 1.7 g of L-arginine, 5 mg of polysorbate 80, and 0.5 g of phosphoric acid.

Non-Investigational Medicinal Products

Comparator

To maintain standard medical care, patients randomized to the IV alteplase placebo arm will receive 325 mg of oral aspirin at the same time that they receive IV alteplase placebo.

Comparator

Patients in the IV alteplase active arm will receive an oral aspirin placebo tablet at the same time they receive IV alteplase. The placebo will be identical in appearance to active aspirin to maintain treatment blinding.

Statistical Methods

Primary Analysis

The primary efficacy analysis will test the hypothesis of superiority of IV alteplase therapy over standard medical care in AIS patients with mild deficits. The primary efficacy outcome is the

proportion of patients with a favorable outcome, defined by mRS score of 0 or 1 at 90 days post-randomization. The difference in the proportion of mRS 0−1 responders (favorable outcome) at 90 days post-randomization between the IV alteplase arm and the standard medical care arm will be compared via a Cochran-Mantel-Haenszel test, stratified by pre-treatment NIHSS score (0 − 2 vs. 3 − 5), age (< 65 vs. ≥ 65), and last known well time to treatment (0 − 2 hours vs. > 2− 3 hours). The primary efficacy analysis will include all randomized patients, with patients grouped according to the treatment assigned at randomization adhering to the intent-to-treat (ITT) principle. As a sensitivity analysis, results from the univariate Pearson's chi-square test will also be presented.

Determination of Sample Size

A sample size of 856 is required in order to achieve 80% power for the primary analysis to detect an effect size of 9% absolute difference in the proportion of patients with favorable outcomes between the alteplase and control arms. The above sample size calculation assumes a control proportion of 65% and Type I error probability of 0.025 (one-sided), and uses a group sequential design with one interim analysis for futility (non-binding), based on an O'Brien-Fleming boundary, after 50% of the anticipated sample size have completed the 90-day follow-up assessments. [redacted] was used in sample size calculation.

The ITT principle will be applied to the primary analysis and, therefore, to safeguard against dilution of the treatment effect associated with an approximate 5% non-adherence rate (due to lost to follow-up, consent withdrawal, treatment crossovers, and stroke mimics), the above sample needs to be inflated by a factor of 1/0.952 or 1.108 (Friedman et al. 1998). Therefore, a total sample size of 948 is required for this study.

Interim Analysis

One interim analysis of the primary efficacy outcome is planned for clear futility, conducted according to the beta-spending approach (Pampallona et al. 2001) with an O'Brien-Fleming-type boundary. This futility analysis will occur after approximately 50% of patients (or 474) have completed the 90-day assessment.

Appendix B
Schedule of Assessment and Study Flow Chart

ASSESSMENTS	Screening and Baseline (Visit 1)	Study Drug Administration	22–36 Hours from Start of Study Drug Administration (Visit 2)	Day 5 (or Discharge if Sooner) (Visit 3)	30 Days (±7 Days) Phone Call (Visit 4)	90 Days (±14 Days)/ Study Completion Visit (Visit 5)	Early Discontinuation (Phone call if possible)
Informed consent	X						
Review of eligibility	X						
Medical history and baseline conditions [a]	X						
Demographics	X						
Physical examination [b]	X						
Vital signs [c]	X						
Complete blood count [d]	X						
Coagulation status [e]	X						
Pregnancy test [f]	X						
Serum blood glucose [g]	X						
Imaging modality sensitive to presence of intracranial hemorrhage (either CT or MRI with DW MRI, either SW or GRE, and FLAIR images). Visit 2 MRIs should also include T1 and T2 sequences [h]	X		X				
NIHSS score [i]	X		X	X		X	
Study drug administration		X					

ASSESSMENTS	Screening and Baseline (Visit 1)	Study Drug Administration	22–36 Hours from Start of Study Drug Administration (Visit 2)	Day 5 (or Discharge if Sooner) (Visit 3)	30 Days (±7 Days) Phone Call (Visit 4)	90 Days (±14 Days)/ Study Completion Visit (Visit 5)	Early Discontinuation (Phone call if possible)
Discharge location				X			
Patient survival			X	X	X	X	x
mRS [j]					X	X	
GOS						X	
BI [k]						X	
EQ-5D [k]						X	
CES-D [k]						X	
SIS-16 [k]						X	
Cognitive assessment [l]						X	
Wa king speed [m]						X	
Concomitant medications [n]	X		X	X	X	X	X
Assessment of pregnancy					X		
Adverse event [o]	X		X	X	X	X	X

BI = Barthel Index; BNT =Boston Naming Test; CES-D = Centers for Epidemiologic Studies, Depression; CT =computed tomography; DW MRI= diffusion-weighted MRI; European Quality of Life = EQ-5D; FLAIR = fluid-attenuated inversion recovery; GOS = Glasgow Outcome Scale; GRE= gradient echo; HVLT-R=Hopkins Verbal Learning Test-Revised; ICH = intracranial hemorrhage; INR = international normalized ratio; MRI=magnetic resonance imaging; mRS modified Rankin Scale; NIHSS= NIH Stroke Scale; Stroke Impact Scale-16= SIS-16; SWMRI= suscept bility-weighted MRI; WAIS III =Wechsler Adult Intelligence Scale III.

[a] Includes smoking history and stroke history.

[b] A partial neurological examination is to be performed for each patient. The NIHSS will be a component of the neurological examination and will be performed only by practitioners who are certified to perform an NIHSS assessment. Additional, or supplemental, neurological exams will also be performed, including assessment of mental status, cranial nerves, motor function and coordination.

ASSESSMENTS	Screening and Baseline (Visit 1)	Study Drug Administration	22–36 Hours from Start of Study Drug Administration (Visit 2)	Day 5 (or Discharge if Sooner) (Visit 3)	30 Days (±7 Days) Phone Call (Visit 4)	90 Days (±14 Days)/ Study Completion Visit (Visit 5)	Early Discontinuation (Phone call if possible)

[c] Includes pulse and systolic/diastolic blood pressure (while patient is in a supine position).

[d] Complete blood count includes hemoglobin, hematocrit, white blood cell count, and platelet count.

[e] Coagulation status includes INR values of > 1.7, activated partial thromboplastin time, patient or family report of patient currently taking an oral anticoagulant (e.g., dabigatran, rivaroxaban, apixaban, edoxaban), or patient or family report of patient currently taking a low-molecular-weight heparin (e.g., dalteparin, enoxaparin) within the last 48 hours.

[f] Pregnancy status will be determined in women of childbearing potential. Serum or urine pregnancy test can be collected.

[g] Serum glucose (values of < 50 mg/dL are contraindicated for alteplase).

[h] MRI or CT scan should be performed if any clinical findings suggest ICH.

[i] NIHSS score should be assessed and recorded at the time of any clinical findings that suggest ICH.

[j] The mRS may be performed by telephone, if necessary, for visits on Days 30 and 90.

[k] The BI, CES-D, EQ-5D, and SIS-16 can be administered by phone interview if an in-person patient visit cannot be scheduled in the allotted time.

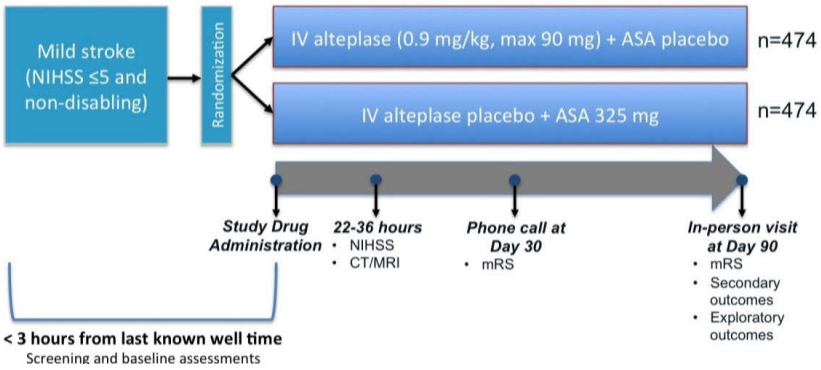
[l] Assessments include: Controlled Oral Word Association test; HVLT-R trials 1, 2, and 3; digit symbol coding from the WAIS III; Forward and Backward Digit Span test; Benton Judgment of Line Orientation, form V; HVLT-R trial 4 and recognition; semantic fluency (Animal Naming test); and BNT (15-item short form).

[m] Assessment of wa king speed requires an in-person patient visit and is best combined with administration of the BI, CES-D, EQ-5D, and SIS-16.

[n] Concomitant medications include any prescription medications or over-the-counter preparations used by the patient within 7 days prior to screening.

[o] Adverse event assessments will be performed during study drug administration; at 22−36 hours after study drug administration; on Days 5, 30, and 90 (may be done via telephone interview if necessary), and at early discontinuation. Refer to Section 5.3.1 (Adverse Event Reporting Period) for further details.

Figure 1 Study Flowchart



ASA = aspirin; CT= computed tomography; IV = intravenous; MRI = magnetic resonance imaging; mRS = modified Rankin Scale; NIHSS = National Institutes of Health Stroke Scale.

Appendix C
Randomization Scheme

Initialization of the algorithm:

- Generate a randomization list for all sites such that half the sites receive Treatment Arm A, half the sites receive Treatment Arm B as the first treatment assignment. Need to ensure that as each SITE is activated there is balance between Treatment Arm A and Treatment Arm B after every *x* sites have been activated (ie., use permuted block randomization with block size of *x*)
- At the time of drug supply receipt verification, obtain the first "USE NEXT" treatment assignment sequentially from the pre-generated randomization list. When the “USE NEXT” treatment assignment is used on a patient or wasted, then the next "USE NEXT" treatment assignment is according to the algorithm in Steps 1 and 2.

Note: If this assignment has not been done in time for the next patient’s arrival to the site, then the new patient will be randomized by a fair coin flip. This will be achieved by calling IWRS technical support.

1. Identify the site for which the “USE NEXT” kits are to be assigned (Let “j” be the site number).
2. Calculate the intermediate variables needed (see **Table 4**) and update the appropriate data set.
3. Compare the appropriate intermediate variables to the specified threshold values, site first then overall (see **Table 5**).
4. IWRS Service Provider will update the appropriate dataset with (a) USE NEXT numbers, (b) random number used by algorithm, (c) study-specific algorithm step at which treatment was determined.
5. When patients have been treated with study drug kits and registered in the system, IWRS updates the enrolled numbers and USE NEXT kit numbers.

Figure 2 displays the randomization steps for the step-forward randomization.

Table 4

Intermediate Variables for the Next Allocation Probability

Variable	Definition
S_j_A	Number of enrolled patients in Treatment Arm A at Site j
S_j_B	Number of enrolled patients in Treatment Arm B at Site j
S_j_AB	Imbalance between treatment arms in enrolled patients at Site j, S_1_A - S_1_B
S_j_Min	Minimum number of enrolled patients in the two treatment arms at Site j, S_j_Min = min(S_j_A, S_j_B)
S_j_PA	Probability for assigning the use next kits to treatment arm A in Site j, S_j_PA = (S_j_Min - S_j_A + MTI-C) / (2*S_j_Min - S_j_A - S_j_B + 2*MTI-S)
O_A	Number of enrolled (ie, treated) patients in Treatment arm A
O_B	Number of enrolled patients in Treatment arm B
O_AB	Overall imbalance between treatment arms in enrolled patients, O_A - O_B, directionality required

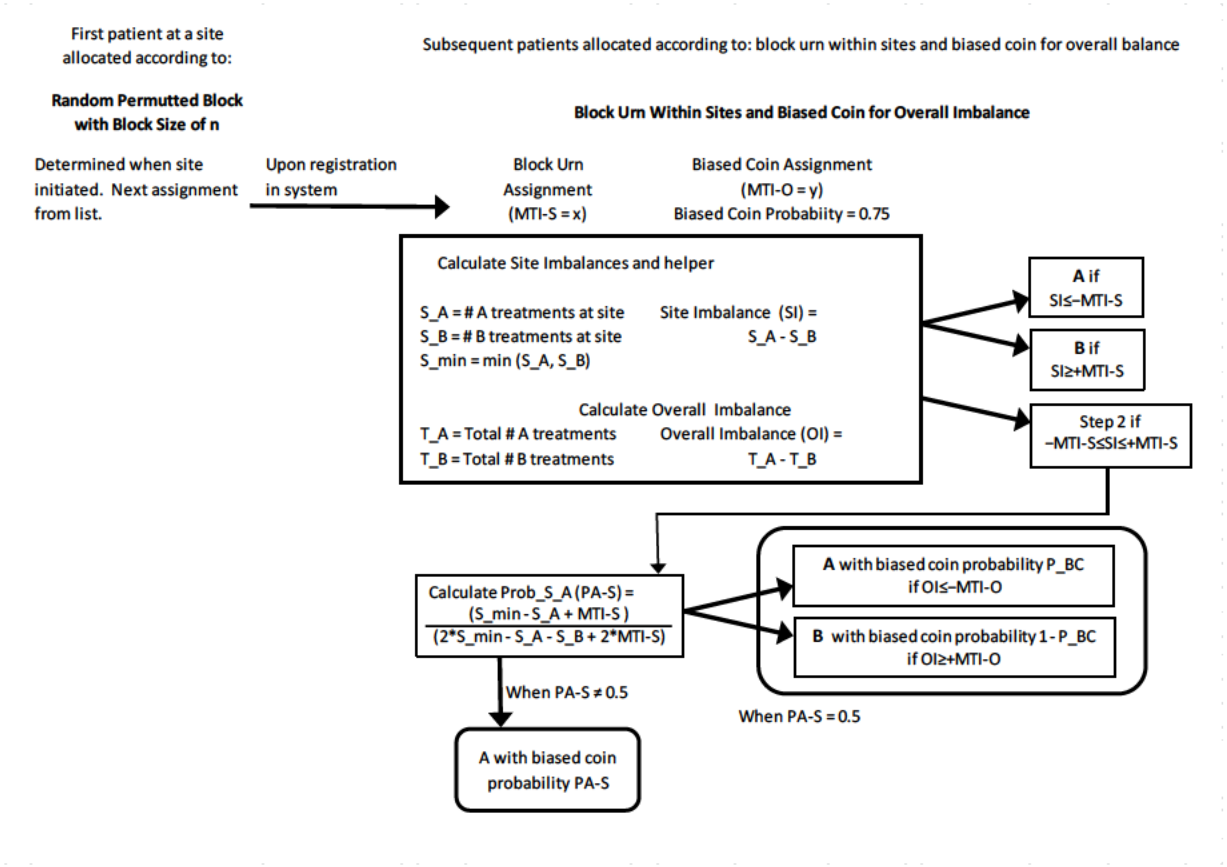
Table 5

Decisions Based on Site and Overall Imbalance

Step	Site Threshold Comparison	Type of Assignment	USE NEXT Kit Assignment
1	If S_j_AB <= –MTI-S	Deterministic	Treatment Arm A.
	If S_j_AB >= + MTI-S	Deterministic	Treatment Arm B.
	if –MTI-S < S_j_AB < + MTI-S		Go to STEP 2
2	If S_j_PA = 0.5		Check overall imbalance, O_AB
	If O_AB <= –MTI-O	Probabilistic	Treatment Arm A with probability P_BC
	If O_AB >= + MTI-O	Probabilistic	Treatment Arm A with probability 1 P_BC
	If S_j_PA ne 0.5		Treatment Arm A with probability S_j_PA

Note: MTI-S is the site threshold level (ie, x). MTI-O is the overall threshold level (ie, y). P_BC is the biased coin probability (ie, 0.75).

Figure 2 Study Randomization Schema



Appendix D
Statistical Methods Requiring Input

Handling of incomplete start and stop dates of adverse events

A missing day in a start date will be imputed with the first day of the month, but not earlier than the randomization date. A missing day in a stop date will be imputed with the last day of the month.

A missing month/year in a start date will be imputed by the month/year of randomization. A missing month in a stop date will be imputed by the month of the last contact. A completely missing stop date will be treated as an ongoing AE and will not be imputed.

Determination of ICH and sICH events

For ICH and sICH events, the following rules will be used to determine their timing relative to 36 hours:

- For ICH determination by scan reading (local or central), take the earliest post-baseline scan with bleeding identified. Use the evaluation time of that scan to compare to 36 hours.

- For ICH determination by AE reporting, take the start date of the corresponding AE. Then find all scans with the same date of local reading, where bleeding is identified. Take the earliest such scan and use the evaluation time of that scan to compare to 36 hours. If no scan is found, use 12:00 am on the AE start date to compare to 36 hours.

When there are multiple scans associated with a PH2 bleed in a subject, the first of these scans will be used to select pre- and post-scan NIHSS scores.

Logistic regression with correlated measures

This model will be used to estimate the odds ratio describing the global favorable outcome, and to estimate the odds ratio of achieving mRS 0-1 at Day 90 in the context of repeated measures.

The model is specified as follows:

- Let Y_{ik}, be the correlated binary outcomes (1 = favorable outcome, 0 = unfavorable outcome) for patient i, and let $E(Y_{ik}) = \mu_{ik}$ be the marginal means of the outcomes conditional on the patient-specific covariates, including treatment.

  For the analysis of the global favorable outcome, k=1,...,4 will index the 4 dimensions (mRS, NIHSS, BI, and GOS).

  For the analysis of mRS 0-1 k=1,2 will index the repeated evaluations of mRS (Day 30 and Day 90).

- The linear predictor will include an outcome-specific intercept α_k, treatment T_i, and the explanatory variables X_i. The link function transforming the marginal mean outcome μ_{ik} into the linear predictor will be the logit function:

  $$\text{logit}(\mu_{ik}) = \alpha_k + \beta T_i + \gamma_k X_i$$

  Importantly, it will be assumed that all outcomes share the same treatment effect β.

- The variance function of Y_{ik} will be determined by the binary distribution variance:

  $$\text{Var}(Y_{ik}) = \mu_{ik}(1 - \mu_{ik})$$

- The working correlation matrix structure for the outcomes from the same patient will be assumed to be unstructured:

  $$\text{Corr}(Y_{ik}, Y_{im}) = \rho_{km},\ k \neq m$$

  For the repeated measures model of mRS 0-1, a temporal correlation structure may be chosen instead, which takes into account the distance in time between the repeated measures:

  $$\text{Corr}(Y_{i1}, Y_{i2}) = \rho^{|d1-d2|},$$

  where d1 and d2 are study days of Day 30 and Day 90 assessments.

Estimation will be carried out by the generalized estimating equaltions (GEE).

Adjusted Risk Difference with logistic regression

The logistic regression model defined for the mRS 0-1 outcome provides estimates of the odds of a favorable outcome. These odds can then be converted to probabilities, and thus the risk difference can be obtained as a non-linear contrast

between the treatment groups. Linearization of this contrast provides an estimate of the standard error, the approximation known as the delta method.

This approach can be implemented with SAS PROC NLMIXED. Here is a sample code:

```
proc nlmixed data=MRS;
   eta=b0+b1*(TRTPN=1)+b2*AGE+b3*OTT+b4*NIHSS;
   p=1/(1+exp(-eta));
   model respn ~ binomial(1,p);
   estimate "tPA vs. Aspirin"
      1/(1+exp(-(b0+b1+b2*&m1+b3*&m2+b4*&m3)) -
      1/(1+exp(-(b0+b2*&m1+b3*&m2+b4*&m3));
run;
```

where TRTPN=1 selects tPA treatment, and macro variables m1, m2 and m3 resolve to the mean values of AGE, OTT and NIHSS, respectively.

Appendix E
Documentation of Additional Analyses Performed after Database Lock

Additional analysis for the primary outcome mRS 0-1

Baseline imbalance as measured by standardized difference in additional explanatory variables listed in **Table 6** will be explored. The standardized difference can be used to compare the mean of continuous and binary variables between treatment groups. For a continuous covariate, the standardized difference is defined as the difference in the sample means of treatment groups divided by the pooled standard error; see Austin (1) for the mathematical formula. The standardized difference compares the difference in means in units of the pooled standard deviation. Furthermore, it is not influenced by sample size and allows for the comparison of the relative balance of variables measured in different units. Although there is no universally agreed upon criterion as to what threshold of the standardized difference can be used to indicate important imbalance, a standardized difference that is less than 0.1 has been taken to indicate a negligible difference in the mean or prevalence of a covariate between treatment groups (1).

The impact of baseline imbalance if present in at least one candidate variable will be evaluated on the adjusted risk difference for the primary efficacy endpoint through a propensity score approach. Specifically, when there is at least one candidate variable that shows a standardized difference larger than 0.1, propensity scores will be generated using a logistic regression model with the treatment group as the response variable and all variables listed in **Table 6** as independent variables. The estimated propensity scores will then be included as an additional covariate in the linear regression model performed for the primary outcome. The adjusted risk difference will be reported from this model. Note that age, baseline NIHSS and onset time to treatment will not be included in the propensity score model, but instead will be explicitly adjusted for in the analysis model.

Missing covariates will be imputed so that all patients have the propensity score defined. Continuous covariates will be imputed with their mean values. Categorical covariates will be collapsed to avoid rare categories.The least frequent categories will be merged together until there are at least 10 subjects per category and the propensity score model has no estimation problems. If there is a substantial number

of missing values in a categorical variable, missing may be considered as a standalone category for the purpose of generating a propensity score.

Table 6
Baseline Characteristics to be Evaluated for Imbalance

Sex	Prior anti-platelet drug use
Race	Prior anti-coagulant drug use
Ethnicity	Baseline glucose
Smoking	Baseline systolic blood pressure
Medical History	Baseline coagulation test: INR
- Hypertension	
- Diabetes Mellitus	
- Atrial Fibrillation	
- Stroke	

Appendix F
Data Handling Rules

The data handling rules will be provided as a separate document to be developed in conjunction with any dry runs and finalized before database lock for the interim analysis and separately for the final analysis.

Appendix G
Programming Codes for Statistical Analysis

Calculation of the EQ-5D summary index

```
data LIBNAME.NEWDATANAME ; set LIBNAME.DATANAME ;

/* Generate Dummy Variables for Levels 2 and 3 of Five Dimensions */
m1 =0 ; m2 =0 ;
s1 =0 ; s2 =0 ;
u1 =0 ; u2 =0 ;
p1 =0 ; p2 =0 ;
a1 =0 ; a2 =0 ;

if mobility = 2 then m1 = 1 ;
if mobility = 3 then m2 = 1 ;
if selfcare = 2 then s1 = 1 ;
if selfcare = 3 then s2 = 1 ;
if activity = 2 then u1 = 1 ;
if activity = 3 then u2 = 1 ;
if pain = 2 then p1 = 1 ;
if pain = 3 then p2 = 1 ;
if anxiety = 2 then a1 = 1 ;
if anxiety = 3 then a2 = 1 ;

/* Generate Interaction Terms (I2, I2-squared, I3, I3-squared) */
m0 = 0 ;
s0 = 0 ;
u0 = 0 ;
p0 = 0 ;
a0 = 0 ;

if m1 = 0 and m2 = 0 then m0 = 1 ;
if s1 = 0 and s2 = 0 then s0 = 1 ;
if u1 = 0 and u2 = 0 then u0 = 1 ;
if p1 = 0 and p2 = 0 then p0 = 1 ;
if a1 = 0 and a2 = 0 then a0 = 1 ;

i2 = m1 + s1 + u1 + p1 + a1 ;
i2 = i2 - 1 ;
if i2<0 then i2 = 0 ;
i22 = i2*i2 ;
i3 = m2 + s2 + u2 + p2 + a2 ;
i3 = i3 - 1 ;
if i3<0 then i3 = 0 ;
i32 = i3*i3 ;

/* Generate D1 Term */
i1 = m0 + s0 + u0 + p0 + a0 ;
d1 = 4 - i1 ;
if d1<0 then d1 = 0 ;
```

```
/* Generate Raw Index Score */
pred =  .146016*m1  +  .557685*m2  +  .1753425*s1 + .4711896*s2  +
        .1397295*u1 +  .3742594*u2 +  .1728907*p1 + .5371011*p2  +
        .156223*a1  +  .4501876*a2 + -.1395949*d1 + .0106868*i22 +
       -.1215579*i3 + -.0147963*i32 ;

EQ_index = 1 - pred ;
if (mobility = .) or (selfcare = .) or (activity = .) or (pain = .) or
   (anxiety = .) then EQ_index = . ;

/* Drop Variables Generated by Program */
drop m1 m2 s1 s2 u1 u2 p1 p2 a1 a2 m0 s0 u0 p0 a0 i1 i2 i22 i3 i32 d1
pred ;

run ;
```

Ordinary Least Squares with Robust Standard Error

```
proc reg data=mrs;
   model mrs = trt age ott nihss / hcc hccmethod=0;
run;
```

Data Analysis Plan (DAP) Module 1 Initial Sign-Off

Study title:	A PHASE IIIB, DOUBLE-BLIND, MULTICENTER STUDY TO EVALUATE THE EFFICACY AND SAFETY OF ALTEPLASE IN PATIENTS WITH MILD STROKE (RAPIDLY IMPROVING SYMPTOMS AND MINOR NEUROLOGIC DEFICITS [PRISMS])		
Protocol #:	ML29093	DAP Version:	1.0

Author:

[redacted] [redacted] 18 May 2017

CRO Study Statistician [redacted] Signature Date

Reviewers:

[redacted] [redacted] 18 May 2017

CRO Peer Review Statistician [redacted] Signature Date

Sponsor Study Statistician [redacted] Signature Date

[redacted] [redacted] 18May2017

CRO Study Statistical Programmer [redacted] Signature Date

Approvers:

Sponsor [redacted] Signature Date

* The Biostatistics approver has ensured that key team members have been involved, contributed and reviewed the content of the List of Planned Outputs as described in the DAP Module 2 guideline.

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Author:

[redacted] CRO Study Statistician — Signature — Date

Reviewers:

[redacted] CRO Peer Review Statistician — Signature — Date

[redacted] 17 May 2017

[redacted] Sponsor Study Statistician — Signature — Date

[redacted] CRO Study Statistical Programmer — Signature — Date

Approvers:

[redacted] 17 May 2017

[redacted] Sponsor — Signature — Date

* The Biostatistics approver has ensured that key team members have been involved, contributed and reviewed the content of the List of Planned Outputs as described in the DAP Module 2 guideline.