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PROTOCOL

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)

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LEAD PRINCIPAL INVESTIGATOR: [REDACTED], M.D.

MEDICAL MONITOR: [REDACTED], M.D.

SPONSOR: Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080-4990 U.S.A

DATE FINAL: See electronic date stamp below

PROTOCOL APPROVAL

Approver's Name

Title

Date and Time (UTC)

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PROTOCOL ACCEPTANCE FORM

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South San Francisco, CA 94080-4990 U.S.A

I agree to conduct the study in accordance with the current protocol.

Principal Investigator's Name (print)

Principal Investigator's Signature

Date

Please return a copy of this form as instructed by your local study monitor and retain a copy for your study files.

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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)

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TEST PRODUCT: Alteplase (RO5532960)

PHASE: IIIb

INDICATION: Acute ischemic stroke

SPONSOR: Genentech, Inc.
1 DNA Way
South San Francisco, CA 94080-4990 U.S.A.

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) 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 improvement in mRS
 - Neurological outcome (as measured by NIHSS 0–1)
 - Independence in instrumental activities of daily (Barthel Index [BI] ≥ 95)
 - Glasgow Outcome Scale (GOS) 0–1
 - Global favorable recovery (Global Statistic with contributions from mRS 0–1, NIHSS 0–1, BI ≥ 95 , and GOS 0–1)
- 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
 - 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. \geq 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:

- Stroke recurrence (based on AE monitoring) within 90 days
- Cognition (30-minute assessment: animal naming, controlled oral word association test, digit symbol coding from the Wechsler Adult Intelligence Scale, Hopkins Verbal Learning Test, 15-item Boston Naming Test, forward and backward Digit Span test, and Rey-Osterreith Complex Figure test) at Day 90
- Ambulatory performance (as measured by walking speed) at Day 90
- Depression (Center for Epidemiologic Studies, Depression [CES-D] score) at Day 90
- 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 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 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 within 3 hours of last known well time or 2) one dose of IV alteplase placebo and one dose of oral aspirin 325 mg within 3 hours of last known well time.

Number of Patients

Approximately 948 patients with AIS and mild strokes will be enrolled in the study across 75 sites in the United States and Canada.

Target Population

Inclusion Criteria

Patients must meet the following criteria for study entry:

- Age \geq 18 years (no upper age limit)
- Mild ischemic stroke defined as the most recent pre-treatment NIHSS score of \leq 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 \leq 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:
 - Clear large hypodensity on CT (or concurrent diffusion-weighted and fluid attenuated inversion recovery hyperintensity on MRI) that is greater than one-third middle cerebral artery (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 (historical mRS score ≥ 2) prior to the presenting stroke
- 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-compressible 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 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 $< 100,000/\text{mm}^3$ (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 inhibitor (e.g., dabigatran, rivaroxaban, apixaban, edoxaban) within the last 48 hours
- Allergic reactions to study drug or aspirin
- 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 (\pm 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 are measured at Day 90 and listed below:

- Ordinal improvement in mRS
- Neurological outcome (as measured by NIHSS 0 – 1)
- Instrumental activities of daily living, as measured by the BI \geq 95
- GOS 0 – 1
- Global outcome (Global Statistic with contributions from mRS 0 – 1, NIHSS 0 – 1, BI \geq 95, and GOS 0 – 1)

Safety Outcome Measures

The safety outcome measures for this study are as follows:

- Incidence of sICH within 36 hours (see below)
 - sICH, defined as any neurological decline attributed to new ICH seen on imaging by the investigator and 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
- Incidence, severity, and spectrum of all AEs and SAEs

Investigational Medicinal Products

Test Product

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 to active aspirin in appearance 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 symptoms. The primary efficacy outcome is the proportion of patients with a favorable outcome, defined by mRS score of 0 or 1 at 90 days.

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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. \geq 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 in the primary analysis to detect effect size of 9% absolute difference in the proportion of patients with favorable outcomes between alteplase and control arms. The above power consideration assumes a control proportion of 65% and Type I error probabilities 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, 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, and treatment crossovers), 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 with O'Brien and Fleming-type boundaries. This futility analysis will occur after approximately 50% of patients (or 474) have completed the 90-day assessment.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	adverse event
AESI	adverse event of special interest
AIS	acute ischemic stroke
aPTT	activated partial thromboplastin time
BI	Barthel Index
CES-D	Center for Epidemiologic Studies, Depression
CRO	contract research organization
CT	computed tomography
DW	diffusion-weighted
EC	Ethics Committee
eCRF	electronic Case Report Form
EDC	electronic data capture
EQ-5D	European Quality of Life questionnaire
FDA	U.S. Food and Drug Administration
FLAIR	fluid attenuated inversion recovery
GOS	Glasgow Outcome Scale
GRE	gradient echo
HIPAA	Health Insurance Portability and Accountability Act
ICH	intracranial hemorrhage
ID	identification
iDCC	independent Data Coordinating Center
iDMC	independent Data Monitoring Committee
IMP	investigational medicinal product
IND	Investigational New Drug (application)
INR	international normalized ratio
IRB	Institutional Review Board
IST-3	third International Stroke Trial
ITT	intent-to-treat
IU	international units
IV	intravenous
IxRS	Interactive Voice/Web Response System
LHH	likelihood of being helped vs. harmed
LPLV	last patient's last visit
MCA	middle cerebral artery
MedDRA	Medical Dictionary for Regulatory Activities

Abbreviation	Definition
MRI	magnetic resonance imaging
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NNH	number needed to harm
NNT	number needed to treat
PRO	patient-reported outcome
RISS	rapidly improving stroke symptoms
rPH	remote parenchymal hematoma
SAE	serious adverse event
SAP	Statistical Analysis Plan
sICH	symptomatic intracranial hemorrhage
SIS-16	Stroke Impact Scale-16
STIAMP	Suspected Transmission of an Infectious Agent via a Medicinal Product
SWFI	sterile water for injection
SW	susceptibility-weighted
t-PA	tissue plasminogen activator
U.S.	United States
USPI	U.S. Package Insert
WHO	World Health Organization

1. BACKGROUND

1.1 BACKGROUND ON ACUTE ISCHEMIC STROKE

Stroke is the fourth leading cause of death and a leading cause of disability in the United States (U.S.). Nearly 800,000 strokes occur in the U.S. annually; of these, approximately 85% are ischemic in nature (Rosamond et al. 2007). Treatment options are limited, as the only approved therapy for acute ischemic stroke (AIS) is alteplase, which is to be used in eligible patients and started within 3 hours from the onset of stroke symptoms. Stroke with “only minor or rapidly improving stroke symptoms” is considered a relative exclusion by national clinical guidelines, as these patients were largely excluded from definitive trials to date (Jauch et al. 2013). Consistent with this recommendation, only 15% of alteplase recipients have mild strokes (Smith et al. 2011).

More than half of all ischemic stroke patients have mild strokes at initial presentation in population-based studies (Dhamoon et al. 2009; Reeves et al. 2013). Yet, a substantial proportion (31%–52%) of ischemic stroke patients who arrive within 2–3 hours of symptom onset are not treated with intravenous (IV) alteplase primarily due to mild symptoms at the time of the treatment decision (Smith et al. 2011; De Los Rios la Rosa et al. 2012). In a national registry, among 93,517 cases arriving to Emergency Departments within 2 hours, 29,200 (31%) were not treated solely due to mild or improving symptoms, and 80.1% of untreated cases had a National Institutes of Health Stroke Scale (NIHSS) score ≤ 5 (Smith et al. 2011).

While treatment of mild stroke is generally uncommon, leading stroke centers vary widely in their approach. For example, the proportion of mild stroke patients (NIHSS < 4) treated with reperfusion therapies at National Institutes of Health/National Institute of Neurological Disorders and Stroke (NINDS) Specialized Programs of Translational Research in Acute Stroke (SPOTRIAS) centers ranged from 2% – 16% in 2008 – 2009 (Wiley et al. 2012). This variability in approach is also evident upon discussion with former leaders of the pivotal NINDS trials; some physicians treat all eligible stroke patients regardless of severity, and others treat only a minority of patients (████████, personal communication). There is unquestionable community equipoise regarding the acute treatment of the mild stroke population.

1.2 BACKGROUND ON ALTEPLASE

1.2.1 Fibrinolysis and Tissue Plasminogen Activator

Physiologic dissolution of a thrombus depends primarily on the proteolytic action of plasmin formed from plasminogen at the site of a clot. The mechanism for this activation *in vivo* has not been definitively established. Plasminogen, the zymogen precursor of plasmin, may be activated by substances found in the plasma milieu, in tissue (tissue plasminogen activator [t-PA]), in urine (urokinase), and in bacteria (streptokinase). The generated plasmin may be inhibited by plasma proteins such as $\alpha 2$ -antiplasmin and $\alpha 2$ -macroglobulin (Robbins 1982).

Kinetic analyses suggest that plasminogen activation in the presence of fibrin occurs after the binding of plasminogen and t-PA at the clot site. t-PA has a high affinity for fibrin, thereby efficiently activating the fibrin-bound plasminogen and converting it to plasmin. Plasmin, which when bound to fibrin is protected from rapid inactivation by α_2 -antiplasmin, then proceeds to digest the fibrin clot.

See the alteplase Investigator's Brochure for additional details on nonclinical and clinical studies.

1.3 STUDY RATIONALE AND BENEFIT-RISK ASSESSMENT

Alteplase is the only approved therapeutic agent for the treatment of AIS and is widely accepted as the standard of care for eligible patients (Edlow et al. 2013; Jauch et al. 2013). 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 (Jauch, et al. 2013). 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 on 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" throughout the protocol.

To date, all completed major randomized acute stroke trials have excluded patients with mild strokes to varying degrees. In the two NINDS trials, such patients were excluded according to the protocol (largely to prevent patients with transient ischemic attacks from being enrolled if they were having rapidly improving symptoms), but did not include an explicit definition of mild stroke except for pure sensory stroke, isolated ataxia, isolated dysarthria, and isolated facial weakness (NINDS rt-PA Study Group 1995). The operational definition for stroke symptoms too mild to treat was left to the judgment of the randomizing physician (TREAT Task Force 2013). Interestingly, a post hoc analysis of the 624 patients in the NINDS trial revealed that there were 58 patients (9%) enrolled with an NIHSS score ≤ 5 , none of whom had isolated motor symptoms, isolated facial droop, isolated ataxia, dysarthria, isolated sensory, and three or fewer of each of additional isolated deficits (Khatri et al. 2010). Perhaps more significantly, 2971 patients were excluded primarily due to "rapidly improving" or "minor symptoms." However, a significant unmet medical need exists for patients with mild stroke. Available outcome data for patients with mild stroke who do not receive alteplase consistently demonstrate a high rate of suboptimal outcomes. Specifically, in two different prospective databases

of patients with mild deficits (NIHSS score ≤ 5) and no baseline disability, approximately one-third (29%–32%) were significantly disabled (as measured by modified Rankin Scale [mRS] score 2 – 6) at 90 days (Fischer et al. 2010; Khatri et al. 2012; [REDACTED], personal communication).

Existing, albeit limited, data suggest that the potential benefit-risk ratio for alteplase in mild stroke should be favorable. Gonzales et al. demonstrated better functional outcomes among mild strokes treated with IV alteplase compared to those not treated in a large, single-center, retrospective cohort; this was shown with two different thresholds of stroke severity (NIHSS ≤ 7 and NIHSS ≤ 3) (Gonzales et al. 2006). Additionally, a recent post hoc analysis of the subset of the third International Stroke Trial (IST-3) patients with mild strokes (pre-treatment NIHSS ≤ 5 who met all other NINDS rt-PA study eligibility criteria) showed a trend toward benefit for alteplase ([REDACTED], personal communication). For PRISMS, the primary efficacy outcome assumption (mRS 0 – 1) is a 9% absolute treatment effect of alteplase relative to placebo. This assumption is based on several retrospective analyses of a similar patient populations (Köhrmann et al. 2009; Fischer et al. 2010; Hassan et al. 2010b; Khatri et al. 2012) as well as the aforementioned IST-3 trial subgroup analysis ([REDACTED], personal communication).

The most prevalently reported and life-threatening adverse event (AE) associated with alteplase treatment for acute ischemic stroke is symptomatic intracranial hemorrhage (sICH). Post hoc analyses, case series, and retrospective studies in patients with mild stroke suggest an absolute sICH rate of 0 – 2% with alteplase treatment (NINDS rt-PA Study Group 2005; Baumann 2006; Gonzales et al. 2006; Köhrmann et al. 2009; Steffenhagen et al. 2009; Hassan et al. 2010a; Hassan et al. 2010b; Huisa et al. 2012). These data are consistent with analyses of IV alteplase trials of more severe patients suggesting that stroke severity, as measured by the NIHSS score, is a strong predictor of sICH risk (Khatri et al. 2007). No data exist to estimate the sICH rate for patients with mild stroke who did not receive t-PA; therefore, for this study, we are using a 0% sICH rate for the placebo arm, which is likely a conservative estimate. An absolute sICH difference of 2% at 36 hours (primary safety endpoint) has been assumed for the alteplase arm vs. the placebo control arm in the proposed study. For this study, sICH has been defined as any neurologic decline attributed to intracranial hemorrhage (ICH) seen on imaging by the local investigator within 36 hours of study drug administration, consistent with the NINDS trial definition for sICH (NINDS rt-PA Study Group 1995).

Assuming the proposed study demonstrates both a 9% absolute treatment effect rate, the number of patients needed to treat (NNT) to show benefit (i.e., mRS score of 0 – 1) for one patient is 11. Assuming a 2% absolute difference in sICH rate in patients treated with alteplase, the corresponding value for the number needed to harm (NNH), specifically for sICH, is conservatively estimated to be 50. The actual number NNH, or the number of patients who would have worse outcomes at 90 days with alteplase compared to placebo treatment, is likely to be much higher than is reflected by the sICH

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rate alone, as many patients who experience sICH have severe baseline infarcts and are destined for poor outcomes (Saver 2007).

For comparison, the absolute treatment effect for mRS score of 0–1 at 90 days was 13% for the NINDS-sponsored pivotal study for alteplase. This equates to an NNT of 7.9. In the same study, the absolute difference in sICH was 5.8% at 24 ± 12 hours pos-treatment, or an NNH of 17.2 (NINDS rt-PA Study Group 1995).

Table 1 Comparison of PRISMS and NINDS Studies

Study	Absolute Treatment Effect (%)	Absolute Difference in sICH (%)	NNT	NNH	LHH ^a
Proposed study in patients with mild stroke and RISS	9	2	11	50	0.22
NINDS-sponsored pivotal study for alteplase	13	5.8 ^b	7.9	17.2	0.46

LHH=likelihood of being helped vs. harmed; NINDS=National Institute of Neurological Disorders and Stroke; NNH=number needed to harm; NNT=number needed to treat; RISS=rapidly improving stroke symptoms; sICH=symptomatic intracranial hemorrhage.

^a LHH is the likelihood of being helped versus harmed and is formulaically the ratio between the NNT and the NNH. The LHH has been proposed as an expression to capture both the NNT and the NNH into a single value. An LHH below 1.0 indicates the expected benefits outweigh the possible harm. The lower the LHH, the more favorable the benefit-risk ratio is for the treatment in the patient population of interest (Demaerschalk 2007).

^b In the NINDS study, patients who received alteplase and developed sICH within 36 hours had a mortality rate of 45% (NINDS rt-PA Study Group 2005).

2. OBJECTIVES

2.1 PRIMARY OBJECTIVES

The primary objective of this study is to determine the efficacy of IV alteplase for treatment of AIS in patients with mild stroke (also known as “minor neurologic deficit” and “rapidly improving stroke symptoms”), defined as an NIHSS score ≤ 5 and not clearly disabling, within 3 hours of last known well time as measured by the proportion of patients with mRS 0–1 at Day 90.

2.2 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 improvement in mRS
 - Neurological outcome (as measured by NIHSS 0–1)
 - Independence in instrumental activities of daily (Barthel Index [BI] ≥ 95)
 - Glasgow Outcome Scale (GOS) 0–1

- Global favorable recovery (Global Statistic with contributions from mRS 0–1, NIHSS 0–1, BI \geq 95, and GOS 0–1)
- To evaluate safety as measured by:
 - sICH within 36 hours – primary safety assessment
 - Any ICH within 36 hours
 - Mortality within 90 days
 - Incidence, severity, and spectrum of all AEs and serious adverse events (SAEs)

2.3 EXPLORATORY OBJECTIVES

The exploratory objectives for this study include subgroup analyses of the primary efficacy outcome variable in subgroups defined by age (< 65 vs. \geq 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 (RISS vs. non-RISS), respectively.

The following endpoints will also be explored:

- Stroke recurrence (based on AE monitoring) within 90 days
- Cognition (30-minute assessment: animal naming, controlled oral word association test, digit symbol coding from the Wechsler Adult Intelligence Scale, Hopkins Verbal Learning Test, 15-item Boston Naming Test, forward and backward Digit Span test, and Rey-Osterreith Complex Figure test) at Day 90
- Ambulatory performance (as measured by walking speed) at Day 90
- Depression (Center for Epidemiologic Studies, Depression [CES-D] score) at Day 90
- Quality of life (European Quality of Life [EQ-5D] questionnaire) at Day 90
- Stroke Impact Scale-16 (SIS-16) at Day 90

3. STUDY DESIGN

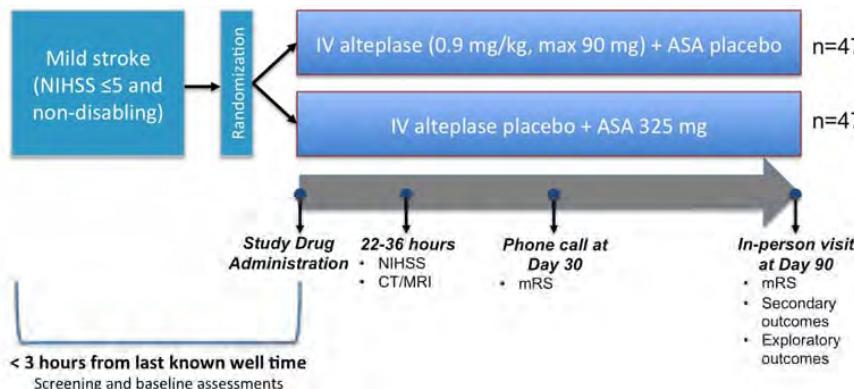
3.1 DESCRIPTION OF STUDY

PRISMS is a double-blind, multicenter, randomized, Phase IIIb study to evaluate the efficacy and safety of IV alteplase in AIS patients with mild stroke and with deficits that do not appear to be clearly disabling. The trial will consist of a screening assessment, randomization, and treatment followed by a 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 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 within 3 hours of last known well time or 2) one dose of IV alteplase placebo and one dose of oral aspirin 325 mg within 3 hours of last known well time (Figure 1).

A full schedule of assessments is provided in Appendix 1.

Figure 1 Abbreviated Study Schema



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

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 stratified by site 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 informed consent and eligibility confirmation. Further details on step forward randomization can be found in Section 4.2, Method of Treatment Assignment and Blinding.

An independent Data Monitoring Committee (iDMC) will provide ongoing review of potential safety concerns and review efficacy and safety outcomes to inform a futility interim analysis after 50% of patients have achieved the primary endpoint (following completion of the Day 90 visit). The composition of the iDMC and analysis will be documented in detail in a separate iDMC charter.

3.2 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.

3.3 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 (\pm 14 days) after the last patient is enrolled into the study.

3.3.1 Number of Patients

Approximately 948 patients with AIS and mild strokes will be enrolled in the study across 75 sites in the U.S. and Canada.

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3.4 RATIONALE FOR STUDY DESIGN

This study is designed to assess whether alteplase is superior to standard of care (i.e., aspirin) in patients with mild stroke within 3 hours of last known well time. Patients will be randomized 1:1 and receive either IV alteplase plus aspirin placebo or IV alteplase placebo plus aspirin 325 mg; hence, this is an active-comparator trial. Other than the study intervention, patients will receive standard of care for AIS at the enrolling institutions. The primary endpoint using the mRS assessment of global disability at 90 days is standard for current AIS trials.

The study design of PRISMS has the following characteristics:

- **Consistent with the currently approved indication in the alteplase USPI:** Treatment with alteplase in PRISMS will occur in otherwise eligible AIS patients within 3 hours from the last known well time.
- **Conduct of a large, multicenter study:** The Sponsor plans to enroll 948 patients across approximately 75 sites in the U.S. and Canada (for comparison, the two NINDS trials enrolled a total of 624 patients).
- **Multiple endpoints involving different events:** In addition to the primary efficacy endpoint of mRS at Day 90, which measures disability, secondary endpoints at Day 90 will include the NIHSS score (neurologic deficit), BI (instrumental activities of daily living), GOS (disability), and a Global Statistic calculation.

3.4.1 Rationale for Alteplase Dosage

The dose selected for alteplase (0.9 mg/kg, maximum 90 mg) is the approved dose for eligible patients with AIS in the U.S. and Canada and is considered the standard of care by the stroke community (Jauch et al. 2013).

3.4.2 Rationale for Patient Population

The patients for this trial will be recruited from all AIS patients presenting to Emergency Departments in whom treatment can be initiated within 3 hours of last known well time at approximately 75 clinical sites within the U.S. and Canada. As described in Section 1.3, eligible patients with mild stroke will be enrolled in this study. Currently, the USPI warns against the use of alteplase in patients with “minor neurologic deficit and rapidly improving stroke symptoms” due to lack of data. Retrospective and observational data suggest potential benefit of alteplase in these patients, with a favorable benefit-risk profile.

The definition of mild stroke for PRISMS is an NIHSS score ≤ 5 and not clearly disabling. This includes patients whose symptoms are persistently mild as well as those with RISS.

An NIHSS score ≤ 5 was chosen as one criterion in the definition of mild stroke for PRISMS for the following reasons:

1. An NIHSS score ≤ 5 is commonly used as an exclusion criterion for many current AIS studies, suggesting that these patients are underrepresented in current stroke research and are treated differently by the stroke community, as shown in Table 2.

Table 2 NIHSS Exclusion Criteria of Recently Completed and Ongoing Acute Ischemic Stroke Trials

Recently Completed AIS Trials	Exclusion	Ongoing AIS Trials	Exclusion
Combined Approach to Lysis Utilizing Eptifibatide and rt-PA in Acute Ischemic Stroke (CLEAR Stroke) Trial	NIHSS ≤ 5	ARTSS	NIHSS ≤ 9
Intravenous Thrombolysis Plus Hypothermia for Acute Treatment of Ischemic Stroke	NIHSS ≤ 6	SHINE	NIHSS < 3
Albumin in Acute Ischemic Stroke Trial (ALIAS) Part One	NIHSS ≤ 5	The Intravascular Cooling in the Treatment of Stroke 2/3 (ICTuS 2/3) Trial (ICTuS2/3)	NIHSS ≤ 6
Study of the Combination Therapy of rt-PA and Eptifibatide to Treat Acute Ischemic Stroke (CLEAR-ER)	NIHSS ≤ 5	Safety/Feasibility of Autologous Mononuclear Bone Marrow Cells in Stroke Patients	NIHSS ≤ 5
Albumin in Acute Ischemic Stroke Trial (ALIAS) Part Two	NIHSS ≤ 5	SWIFT PRIME	NIHSS ≤ 7
Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy (MR RESCUE)	NIHSS ≤ 5	PENUMBRA THERAPY	NIHSS ≤ 7
NeuroThera Efficacy and Safety Trial-3 (NEST3)	NIHSS ≤ 6	PENUMBRA 3D	NIHSS ≤ 7
		CLOTBUST-ER	NIHSS ≤ 9

AIS=acute ischemic stroke; NIHSS=National Institutes of Health Stroke Scale.

Source: www.clinicaltrials.gov. Assessed August 2013. Note that the NIHSS criteria for the trials listed are mentioned in the inclusion criteria, while the entries in the above table are written as what would be the understood and corresponding exclusion criteria.

2. The largest retrospective analysis of mild stroke from the Get With the Guidelines® registry included 29,200 stroke patients who did not receive alteplase due to mild or improving symptoms. This analysis found that 80.1% of patients with mild or rapidly improving stroke who did not receive alteplase (and had a recorded NIHSS score) had an initial NIHSS score ≤ 5 (Smith et al. 2011).

Based on these findings, an NIHSS score ≤ 5 was determined to be the most appropriate cut-off to define the patient population with mild stroke.

It is notable that the alteplase USPI (Activase USPI 2011) distinguishes patients with minor neurological deficit from those with RISS. However, PRISMS combines these two patient groups into one operationally defined patient population (mild stroke): all eligible patients with an NIHSS score ≤ 5 at the time of randomization without clearly disabling symptoms, irrespective of previous NIHSS scores. Data demonstrating low alteplase use in patients with NIHSS scores ≤ 5 do not distinguish between whether the patient's deficit was persistently mild or improved to this status (Smith et al. 2011). Furthermore, no data exist that suggest patients who improved to a mild status at the time of treatment with alteplase have different outcomes from patients whose neurologic deficit is persistently mild. To date, available data suggest that alteplase therapy would potentially be beneficial in both patients with RISS who improve to an NIHSS score ≤ 5 and patients with persistently mild symptoms with an NIHSS score ≤ 5 .

Conversely, patients who improve to an NIHSS score > 5 at the time of treatment decision will be excluded from PRISMS as they are not considered to have RISS. Current clinical practice suggests that these patients would still have a moderate or severe stroke at the time of treatment decision and should receive alteplase, if eligible, rather than be randomized to a study. Such patients should not be defined as rapidly improving, given that they have not improved to an NIHSS score low enough to merit withholding therapy. This definition of RISS is consistent with a recent consensus statement by leading stroke and emergency medicine experts (TREAT Task Force 2013).

PRISMS will also exclude patients who are assessed at the time of pre-treatment to likely be clearly disabled at 90 days, as defined by specific criteria described in the study protocol. These deficits at presentation include, but are not limited to, homonymous hemianopsia, severe limb weakness, severe aphasia, hemineglect, or cortical blindness. Patients with clearly disabling deficits will be excluded from this study irrespective of the NIHSS score at time of randomization, as current clinical practice and ethical responsibility suggest that these patients should be evaluated to receive alteplase rather than be enrolled in a randomized study where they may receive placebo.

3.4.3 Rationale for Control Group

The overall rationale for this trial is to determine whether IV alteplase improves outcomes in patients with mild stroke. The control group (i.e., mild stroke patients who receive standard of care) has been included so that outcomes in the active treatment group can be compared to those who receive standard of care. The control group will receive IV alteplase placebo and 325 mg of oral aspirin. The rationale for the control group receiving aspirin is that it would be unethical to withhold a dose of aspirin from patients who would not be receiving active study drug. The standard of care for patients suffering from AIS who are ineligible for IV alteplase therapy is oral aspirin administered within the first 24 hours after the onset of their stroke symptoms.

3.5 OUTCOME MEASURES

3.5.1 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 are measured at Day 90 and listed below:

- Ordinal improvement in mRS
- Neurological outcome (as measured by NIHSS 0–1)
- Instrumental activities of daily living, as measured by the BI \geq 95
- GOS 0–1
- Global outcome (Global Statistic with contributions from mRS 0–1, NIHSS 0–1, BI \geq 95, and GOS 0–1)

3.5.2 Safety Outcome Measures

The safety outcome measures for this study are as follows:

- Incidence of sICH within 36 hours (see below)
 - sICH, defined as any neurological decline attributed to new ICH seen on imaging by the investigator and 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
- Incidence, severity, and spectrum of all AEs and SAEs

Symptomatic Intracranial Hemorrhage

In this study, an ICH is considered symptomatic if it is not seen on computed tomography (CT) or magnetic resonance imaging (MRI) scan at baseline and any neurologic decline is attributed to it by the local investigator. To detect asymptomatic intracranial hemorrhage, CT scan is required at 22 to 36 hours. Additional CT scans should be performed at any time based on the investigator's discretion that clinical findings suggested hemorrhage. An MRI may be substituted for CT per institutional protocol. Details on MRI types are described in Section 4.5.1.6.

Intracranial hemorrhage events will be classified as hemorrhagic infarct type 1 or type 2, parenchymal hematoma type 1 or type 2, remote intraparenchymal hemorrhage, subarachnoid hemorrhage, subdural hemorrhage, or epidural hemorrhage. Descriptions of each are shown in the table below.

Table 3 Intracranial Hemorrhage Definitions

Type	Description
HI type 1	Small petechiae along margin of infarct
HI type 2	More confluent petechiae within the infarcted area but without space-occupying effect
PH type 1	Hematoma in $\leq 30\%$ of the infarcted area with some slight space-occupying effect
PH type 2	Dense hematoma $> 30\%$ total of the infarcted area with substantial space-occupying effect or any hemorrhagic area outside the infarcted area
rPH type 1	Small or medium sized blood clots located remote from the actual infarct; a mild space-occupying effect could be present. Remote primary intracerebral hemorrhage
rPH type 2	Large confluent dense blood clots in an area remote from the actual infarct; substantial space-occupying effect might be present.

HI=hemorrhagic infarct; PH=parenchymal hematoma; rPH=remote parenchymal hematoma.

Source: Wahlgren et al. 2007.

3.5.3 Exploratory Outcome Measures

The exploratory outcome measures for this study are as follows:

- Stroke recurrence (based on AE monitoring) within 90 days
- Cognition (30-minute assessment: animal naming, controlled oral word association test, digit symbol coding from the Wechsler Adult Intelligence Scale, Hopkins Verbal Learning Test, 15-item Boston Naming Test, forward and backward Digit Span test, and Rey-Osterreith Complex Figure test) at Day 90
- Ambulatory performance (as measured by walking speed) at Day 90
- Depression (CES-D score) at Day 90
- Quality of life (EQ-5D questionnaire) at Day 90
- SIS-16 at Day 90

4. MATERIALS AND METHODS

4.1 PATIENTS

This study will include adult patients with AIS.

4.1.1 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.

4.1.2 Exclusion Criteria

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

- CT or MRI findings consisting of one of the following:
 - Clear large hypodensity on CT (or concurrent diffusion-weighted [DW] and fluid attenuated inversion recovery [FLAIR] hyperintensity on MRI) that is greater than one-third middle cerebral artery (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 (historical mRS score ≥ 2) prior to the presenting stroke
- 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-compressible 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 heparin within the last 48 hours and activated partial thromboplastin time (aPTT) outside of the normal range as specified by the center's local laboratory
 - Blood glucose <50 mg/dL
 - International normalized ratio (INR) >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 inhibitor (e.g., dabigatran, rivaroxaban, apixaban, edoxaban) within the last 48 hours
- Allergic reactions to study drug or aspirin

- 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

4.2 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

Step Forward Randomization

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. This procedure will be implemented using an Interactive Voice/Web Response System (IxRS) where study sites enter identification (ID) information (see below) of a patient AFTER he/she is treated (within 8 hours of initiation of the patient's study treatment). The IxRS will then assign drug kit IDs for the next patient that becomes eligible at that site. The pharmacist will prepare "use next" labels and place them on the appropriate study drug kits based on the drug kit IDs via IxRS. When the next potentially eligible patient is identified, the investigator (or designee) will notify the pharmacist (or designee) to begin pre-mixing study drug from the alteplase/alteplase-placebo drug kit. Pre-mixing may occur prior to signing informed consent but should be performed after key eligibility criteria (e.g., NIHSS \leq 5 and clearly non-disabling, study drug initiation will occur within 3 hours of time last known well, CT/MRI negative for ICH) to minimize drug wastage. Study drug bolus will be administered after confirming that all eligibility criteria have been met and informed consent signed, and this will indicate enrollment in the trial. This approach will ensure minimal delays to treatment. Following study drug administration (within 8 hours), the study site should enter the patient's ID, including date of birth, patient initials, screening ID, and confirm drug kit IDs from the kits with which the patient is treated. Once this information is entered into IxRS, it will result in two actions:

1. IxRS will assign a patient ID to the currently treated patient, which will be correlated to the drug kit ID previously labeled as "use next," via the randomization visit record.
2. IxRS will assign a new drug kit ID for the next patient that becomes eligible at that site to be labeled as "use next."

In the case when pre-mixed drug is not used (e.g., patient is determined to be ineligible for study or declines informed consent prior to drug administration), sites will enter drug kit IDs into IxRS and indicate that the kits were not used. IxRS will then assign a new set of drug kit ID for the pharmacist to label with “use next” labels. The pharmacist will properly dispose of the pre-mixed drug and the matching aspirin/aspirin-placebo kit in accordance with institutional guidelines.

Prior to the first patient enrolled at a site, a site will register with IxRS as part of site activation, which will assign drug kit IDs ahead of time for the first potential patient. Once the first patient is enrolled at a site, the procedure described above will commence.

Study Unblinding

If premature unblinding is necessary in the case of an SAE for which patient management might be affected by knowledge of treatment assignment, the investigator will be able to break the treatment code by contacting the IxRS vendor.

Specifically, in the rare event that a patient worsens (e.g., to a moderate or severe stroke) from time of study treatment and the patient remains within a time window within institutional guidelines to initiate thrombolytic therapy, the investigator has the option to unblind the treatment to ensure the patient receives appropriate treatment. However, the following criteria must be met prior to unblinding:

1. Patient must have an NIHSS score ≥ 6 (or new clearly disabling deficit) measured twice within a 10-minute span.
2. Patient’s NIHSS increase must be ≥ 2 points from the pre-treatment NIHSS (e.g., if a patient’s pre-treatment NIHSS was 5, the worsened NIHSS must be ≥ 7).

If the investigator wishes to know the identity of the study drug for any other reason, he or she should contact the Medical Monitor directly. Treatment codes should not be broken except under circumstances deemed necessary by the site investigator. The investigator should document and provide an explanation for any premature unblinding (e.g., unblinding due to sICH, unblinding due to severe systemic bleeding, or unblinding due to clinical worsening). Following any unblinding event, the site investigator should contact the Medical Monitor within 24 hours.

For regulatory reporting purposes, and if required by local health authorities, the Sponsor will break the treatment code for all serious, unexpected suspected adverse reactions (see Section 5.7) that are considered by the investigator or Sponsor to be related to study drug.

4.3 STUDY TREATMENT

4.3.1 Formulation, Packaging, and Handling

4.3.1.1 Alteplase

Alteplase is a single polypeptide containing 527 amino acids; it has three carbohydrate side chains and is predominantly one-chain t-PA. It is a recombinant protein produced in a continuous mammalian tissue culture system and is purified by a series of chromatographic steps. The manufacturing process includes fermentation with use of the antibiotic gentamicin; however, the presence of the antibiotic is not detectable in the final product.

Alteplase is a sterile, white to pale yellow, lyophilized powder for IV administration after reconstitution with sterile water for injection (SWFI), USP, without preservatives. Phosphoric acid and/or sodium hydroxide may be used prior to lyophilization for pH adjustment. Biologic potency is determined by an in vitro clot lysis assay and is expressed in international units (IU) as tested against the World Health Organization (WHO) standard.

Each 50-mg vial of Activase contains 50 mg of alteplase, 1.7 g of L-arginine, 5 mg of polysorbate 80, and 0.5 g of phosphoric acid.

Each kit will contain 2 vials of 50-mg of alteplase labeled for "clinical trial use only."

Vials of 50-mg alteplase must be stored at 2°C–30°C and protected from light.

4.3.1.2 Alteplase Placebo

Alteplase placebo is the same formulation as the active without the alteplase protein. Each 50-cc vial contains 1.7 g of L-arginine, 5 mg of polysorbate 80, and 0.5 g of phosphoric acid.

Each kit will contain 2 vials of 50 mg of alteplase placebo labeled for "clinical trial use only."

Alteplase placebo vials should be stored at 2°C–30°C and protected from light.

4.3.1.3 Aspirin

Aspirin (325 mg) will be packaged for the study. Each kit will contain one bottle, which has 100 tablets labeled for "clinical trial use only." Only one tablet will be administered to the patient, and the remainder of the bottle will be kept at the site for drug accountability.

Aspirin tablets should be stored at 25°C with excursions permitted between 15°C–30°C.

4.3.1.4 Aspirin Placebo

Aspirin placebo will be identical to active aspirin in appearance to maintain treatment blinding. Each bottle of aspirin placebo contains 100 tablets labeled for “clinical trial use only.” Only one tablet will be administered to the patient, and the remainder of the bottle will be kept at the site for drug accountability.

Placebo tablets should be stored in the same manner as aspirin tablets: 25°C with excursions permitted between 15°C–30°C.

4.3.2 Dosage, Administration, and Compliance

4.3.2.1 Alteplase

For AIS, the recommended dose is 0.9 mg/kg infused over 60 minutes with 10% of the total dose administered as an initial IV bolus over 1 minute. The total dose should not exceed 90 mg.

Because alteplase and alteplase placebo contain no antibacterial preservatives, 50-mg vials should be reconstituted immediately before use by adding 50 mL of SWFI, USP, to the vial; this yields a concentration of 1 mg/mL. Using aseptic technique, direct the flow of SWFI directly into the lyophilized cake of study drug. It is recommended that an 18-gauge needle be used for the reconstitution of the 50-mg vial. Once reconstituted, gently swirl the vial. **Do not shake.** Slight foaming upon reconstitution is not unusual; allowing the vial to stand undisturbed for several minutes is usually sufficient to allow dissipation of any large bubbles. Before further dilution or administration, the product should be visually inspected for particulate matter and discoloration. If a precipitate is seen, the product should not be used.

After reconstitution, alteplase may be administered at 1 mg/mL. Do not further dilute (e.g., <1 mg/mL) the solution. The osmolality of this solution is approximately 215 mOsm/kg. Polyvinyl chloride bags, glass bottles, or polypropylene syringes are acceptable. Do not use filter needles for infusion. Alteplase is stable for up to 8 hours in these solutions at room temperature. Exposure to light has no effect on the stability of these solutions. Excessive agitation during dilution should be avoided; mixing should be accomplished with gentle swirling and/or slow inversion.

No other medication should be added to infusion solutions containing alteplase. Any unused infusion solution should be discarded.

Each 50-mg vial of Activase contains 50 mg of alteplase, 1.7 g of L-arginine, 5 mg of polysorbate 80, and 0.5 g of phosphoric acid.

See Appendix 2 (Alteplase Administration) and Appendix 3 (Reconstitution of 50-mg Vials) for further details.

4.3.3 Investigational Medicinal Product Accountability

All investigational medicinal products (IMPs) required for completion of this study alteplase will be provided by the Sponsor where required by local health authority regulations. The investigational site will acknowledge receipt of IMPs using the IxRS to confirm the shipment condition and content. Any damaged shipments will be replaced.

IMPs will either be disposed of at the study site according to the study site's institutional standard operating procedure or returned to the Sponsor with the appropriate documentation. The site's method of IMP destruction must be agreed to by the Sponsor. The site must obtain written authorization from the Sponsor before any IMP is destroyed, and IMP destruction must be documented on the appropriate form.

Accurate records of all IMPs received, dispensed, returned, and disposed of by the study site should be recorded on the Drug Inventory Log.

4.3.4 Investigational Medicinal Product

4.3.4.1 Alteplase and Placebo Test Product

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.

4.3.4.2 Non-investigational Medicinal Products

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 to active aspirin in appearance to maintain treatment blinding.

4.3.5 Post-Trial Access to Alteplase

The Sponsor does not intend to provide alteplase, any investigational or non-investigational products (specified in this protocol), or any other study interventions to patients after the conclusion of the study or who are withdrawn earlier.

4.4 CONCOMITANT THERAPY

4.4.1 Permitted Therapy

Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by a patient from 7 days prior to screening to the study completion/discontinuation visit. All concomitant medications should be reported to the investigator and recorded on the Concomitant Medications electronic Case Report Form (eCRF).

4.4.2 Prohibited Therapy

Available endovascular therapies have not demonstrated clinical efficacy in definitive trials to date and therefore are not permitted.

As indicated in the inclusion criteria, use of the following therapies is prohibited prior to the study:

- Treatment with heparin within the last 48 hours and aPTT outside of the normal range as specified by the center's local laboratory
- Treatment with a direct thrombin inhibitor or factor Xa inhibitor (e.g., dabigatran, rivaroxaban, apixaban, edoxaban) within the last 48 hours
- Treatment with warfarin and INR > 1.7

The above lists of medications are not necessarily comprehensive. The investigator should contact the Medical Monitor if questions arise regarding medications not listed above. For further reference about drug interaction guidance from the U.S. Food and Drug Administration (FDA), as well as a list of common cytochrome P450 interactions, the following resources are available online:

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf>

<http://medicine.iupui.edu/clinpharm/ddis/table.aspx>

4.5 STUDY ASSESSMENTS

Please see Appendix 1 for the schedule of assessments performed during the study.

4.5.1 Description of Study Assessments

4.5.1.1 Informed Consent Forms and Screening Log

Written informed consent for participation in the study must be obtained before treatment. All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before treatment. The investigator will maintain a screening log of all patients arriving in the Emergency Department within 2.5 hours of onset of ischemic stroke. The investigator will record details of all patients not enrolled despite early arrival and record reasons for non-enrollment.

4.5.1.2 Medical History and Demographic Data

Medical history includes clinically significant diseases, such as stroke history and smoking history, use of alcohol and drugs of abuse, and all medications (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies nutritional supplements) used by the patient within 7 days prior to the screening visit, and history of allergy to any food or medication.

Demographic data will include date of birth, sex, and self-reported race/ethnicity.

4.5.1.3 Neurological Examinations

A partial neurological examination is to be performed for each patient. The NIHSS will be a component of this neurological examination and will be performed only by practitioners who are certified to perform an NIHSS assessment. Changes from baseline should be recorded in patient notes. Additional or supplemental neurological exams will also be performed, including assessment of mental status, cranial nerves, motor function, and coordination.

4.5.1.4 Vital Signs

Vital signs will include measurements of oral temperature, respiratory rate, pulse, and systolic/diastolic blood pressure (while patient is in a supine position).

4.5.1.5 Laboratory Assessments

Specimens for the following laboratory tests will be sent to the study site's local laboratory for analysis:

- Platelet count
- Serum glucose
- Coagulation (INR, aPTT) if a patient is currently taking oral or parenteral anticoagulants
- Pregnancy test (serum or urine)

All women of childbearing potential (including those who have had a tubal ligation) must have a serum pregnancy test at screening.

The following laboratory test values will be reported in the eCRFs:

- Hematology (hemoglobin, hematocrit, white blood cell count, platelet count)
- Serum glucose
- Coagulation values (INR, partial thromboplastin time)
- Pregnancy test

For sampling procedures, storage conditions, and shipment instructions, see the site's local laboratory manual.

4.5.1.6 Other Disease-Specific Assessments

Neuroimaging may consist of a non-contrast CT or MRI of the brain. If an MRI is used in the acute setting (to determine study eligibility), it must include the following at a minimum: (1) DW MRI, (2) either susceptibility-weighted [SW] imaging or gradient echo [GRE] sequences, and (3) FLAIR sequences.

Non-contrast CT or MRI will be performed at baseline to ensure that the patient does not have evidence of acute ICH prior to study drug administration.

An additional non-contrast CT or MRI will be performed at 22 to 36 hours from the initiation of study drug infusion. The purpose of this is to evaluate for ICH in the patient. An NIHSS should be performed at the time this CT scan or MRI is performed, but prior to reviewing imaging results to avoid biasing the assessment. If an MRI is performed, in addition to the above requirements for the baseline visit MRI, it must also include T1 and T2 sequences.

Additional CT scans or MRIs should be performed based on the investigator's discretion, or if any clinical findings suggest ICH (such as new headache, nausea, vomiting, significant deterioration of neurological status, or rapid increase of blood pressure). If an ICH is identified, the NIHSS score should be reassessed at the time and recorded on the Adverse Event eCRF.

CT scans or MRIs performed between 22 to 36 hours, and additional imaging performed due to clinical suspicion of deterioration up until 36 hours after study drug administration, will be evaluated by a central team of radiologists blinded to the clinical attributes of the case.

4.5.2 Patient-Reported Outcomes

Patient-reported outcomes (PRO) data will be elicited from the patients in this study to more fully characterize the clinical profile of alteplase in this patient population. The PRO questionnaires will be distributed by the investigator staff and completed in their entirety by the patient or their surrogate.

This study will assess the impact of alteplase treatment on health-related quality of life, physical function, daily functioning, and symptoms of depression.

Health-related quality of life will be assessed using the EQ-5D (Appendix 4). 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.

Physical functioning will be evaluated using the SIS-16 (Appendix 5), which is a validated, stroke-specific, quality of life measure to assess the impact of stroke on a patient's health and life.

The CES-D (Appendix 6) will be used to evaluate the proportion of patients with depressive symptoms (defined as a score of ≥ 16 at 90 days).

AE reports will not be derived from PRO data by the Sponsor. However, any PRO responses suggestive of a possible AE identified during site review of the PRO data should be reported as outlined in Section 5.3.3.11.

4.6 TIMING OF STUDY ASSESSMENTS

4.6.1 Screening/Baseline (Visit 1)

The following standard of care assessments performed prior to obtaining informed consent will be used in the screening process. Once consent is obtained, the results of the standard of care assessments may be used for the study.

- Medical history
- Demographics
- Neurological examination
- Vital signs
- Complete blood count
- Coagulation status
- Pregnancy test (serum or urine)
- Serum blood glucose
- Non-contrast CT scan or MRI, including DW MRI, either SW or GRE, and FLAIR images
- NIHSS score (This must be performed by an NIHSS-certified practitioner – see Appendix 7.
- Concomitant medications
- AEs

All screening evaluations with the exception of certain laboratory results (e.g., coagulation and platelet count) must be completed and reviewed to confirm that patients have met all eligibility criteria prior to treatment. Serum glucose is required to confirm eligibility prior to enrollment; however, if the investigator feels there is clinical suspicion that would be related to an exclusion laboratory test that would preclude the patient from eligibility, the results of those laboratory tests must be obtained prior to treatment.

The investigator will maintain a screening log to record details of all patients consented to confirm eligibility or record reasons for screening failure, as applicable.

4.6.2 Visit 2 (22 to 36 Hours after Treatment Initiation)

A non-contrast cranial CT or MRI (including DW MRI, either SW or GRE, FLAIR, and T1 and T2 sequences) will be performed at 22 to 36 hours after treatment with study drug is initiated. Either a CT scan or MRI may be performed, as consistent with the institutional standard of care; the main criterion is to perform radiographic imaging of the brain by a modality sensitive to the presence of blood. An NIHSS will also be performed during this visit, but prior to review of the imaging results (or by an investigator blinded to the results). AEs and concomitant medications will also be assessed at this visit.

4.6.3 Visit 3 (Day 5 or Discharge from Hospital, if Sooner than Day 5)

An NIHSS score will be performed at Day 5 or on the day of discharge from the hospital, if sooner than Day 5. Furthermore, the discharge location of the patient will be recorded if available at this time. If discharged after Day 5, the discharge location will be subsequently recorded. Concomitant medications will also be assessed at this visit.

4.6.4 Visit 4 (Day 30 [30 ± 7 Days])

A phone call to the patient or caregiver will be made at Day 30 and an mRS will be assessed (see Appendix 8). AEs, concomitant medications, and patient survival will be assessed at this visit. Pregnancy will also be assessed for female patients of childbearing potential.

4.6.5 Visit 5 (Day 90 [± 14 Days]/Study Completion)

Primary and secondary efficacy endpoints, as well as all exploratory endpoints, will be assessed at Day 90 during an in-person visit by the patient. The following assessments will be performed:

- NIHSS score (Appendix 7)
- Patient survival
- mRS (Appendix 8)
- GOS (Appendix 9)
- BI (Appendix 10)
- EQ-5D (Appendix 4)
- SIS-16 (Appendix 5)
- CES-D (Appendix 6)
- Cognitive assessments (**Appendix 11**)
 - Animal Naming test
 - Controlled Oral Word Association test
 - Digit symbol coding from the Wechsler Adult Intelligence Scale
 - Hopkins Verbal Learning test
 - 15-item Boston Naming test

- Forward and Backward Digit Span test
- Rey-Osterrieth Complex Figure test
- Walking speed (Appendix 12)
- Concomitant medications
- AEs

4.6.6 Early Discontinuation

Concomitant medication, AEs, and patient survival will be assessed via a phone call, if possible.

4.7 PATIENT, TREATMENT, STUDY, AND SITE DISCONTINUATION

4.7.1 Patient Discontinuation

Patients have the right to voluntarily withdraw from the study at any time and for any reason. In addition, the investigator has the right to withdraw a patient from the study at any time. Reasons for withdrawal from the study may include, but are not limited to, the following:

- Patient withdrawal of consent at any time
- Any medical condition that the investigator or Sponsor determines may jeopardize the patient's safety if he or she continues in the study
- Investigator or Sponsor determines it is in the best interest of the patient
- Patient non-compliance, specifically defined as unwillingness to participate in a 3-month assessment of neurological status and function

Every effort should be made to obtain information on patients who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. However, patients will not be followed for any reason after consent has been withdrawn. Patients who withdraw from the study will not be replaced.

4.7.2 Study Treatment Discontinuation

The study intervention should be discontinued if the patient displays symptoms concerning for sICH during the infusion of the study drug. Prompt imaging should be done to assess for ICH. If ICH is seen, study drug infusion should not be resumed and the treatment assignment should be unblinded. If ICH is not seen, study drug infusion should immediately be restarted.

Patients must discontinue study treatment if they experience any of the following:

- Anaphylactoid reaction (including angioedema)
- Serious bleeding (not controllable by local pressure) at puncture site or serious bleeding in a critical location (e.g., intracranial, gastrointestinal, retroperitoneal, pericardial)

The primary reason for study treatment discontinuation should be documented on the appropriate eCRF. Patients who discontinue study treatment prematurely will not be replaced. Patients who discontinue study treatment prematurely will be followed for the course of the study based on the intent-to-treat (ITT) principle.

4.7.3 Study and Site Discontinuation

The Sponsor has the right to terminate this study at any time. The Sponsor will notify the investigator if the Sponsor decides to discontinue the study. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to patients.
- Patient enrollment is unsatisfactory
- A pre-specified boundary at the interim analysis was crossed

The Sponsor has the right to close a site at any time. Reasons for closing a site may include, but are not limited to, the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the International Conference on Harmonisation guideline for Good Clinical Practice
- No study activity (i.e., all patients have completed study assessments and all obligations have been fulfilled)

5. ASSESSMENT OF SAFETY

5.1 SAFETY PLAN

The safety profile of alteplase across indications is generally well established. The principal adverse reactions to alteplase are bleeding and allergic reactions.

5.1.1 Risk of Bleeding

Bleeding is the most frequent adverse reaction associated with alteplase in all approved indications, including AIS. This may be either superficial from punctures or damaged blood vessels or internal bleeding at any site or body cavity. Bleeding may result in life-threatening situations, permanent disability, or death.

The incidence of ICH, especially symptomatic ICH, in patients with AIS is higher in alteplase-treated patients than placebo-treated patients in published studies (for detailed information, see the Investigator's Brochure).

Management of Bleeding

Patients will be excluded for the presence of conditions related to risks of bleeding (as outlined in Section 4.1.2, Exclusion Criteria). A non-contrast cranial CT or MRI (including either SW or GRE to detect ICH) should be performed at baseline to ensure that the patient does not have evidence of ICH prior to study drug administration.

Fibrin, which is part of the hemostatic plug formed at needle puncture sites, may be lysed during alteplase therapy. Therefore, alteplase therapy requires careful attention to potential bleeding sites, e.g., catheter-insertion sites and arterial-puncture sites. Arterial and venous punctures should be minimized. Non-compressible arterial, as well as internal jugular and subclavian venous punctures, should be avoided to minimize bleeding from non-compressible sites. In the event of serious bleeding (not controllable by local pressure) or occurring in a critical location (intracranial, gastrointestinal, retroperitoneal, or pericardial), study drug should be discontinued immediately.

Supportive care and medical management of AEs are at the discretion of the investigator.

5.1.2 Risk of Allergic Reactions

Allergic-type reactions, e.g., anaphylactoid reaction, laryngeal edema, orolingual angioedema, rash, and urticaria, have been reported. When such reactions occur, they usually respond to conventional therapy.

There have been postmarketing reports of orolingual angioedema associated with the use of alteplase. Most were patients treated for AIS. Many of these patients received concomitant angiotensin-converting enzyme inhibitors, and most cases resolved with prompt treatment.

Management of Allergic Reaction

If an anaphylactic reaction occurs, the infusion of study drug should be discontinued immediately and appropriate therapy should be promptly instituted.

5.2 SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording AEs, including SAEs and non-serious adverse events of special interest (AESI), measurement of protocol-specified vital signs, and other protocol-specified tests that are deemed critical to the safety evaluation of the study.

Certain types of events require immediate reporting to the Sponsor, as outlined in Section 5.2.2.

5.2.1 Adverse Events

According to the International Conference on Harmonisation guideline for Good Clinical Practice, an AE is any untoward medical occurrence in a clinical investigation patient administered a pharmaceutical product, regardless of causal attribution. An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition), except as described in Section 5.3.3.9
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (e.g., electrocardiogram, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug
- AEs that are related to a protocol-mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies)

5.2.2 Serious Adverse Events (Immediately Reportable to the Sponsor)

An SAE is any AE that meets any of the following criteria:

- Fatal (i.e., the AE actually causes or leads to death)
- Life-threatening (i.e., the AE, in the view of the investigator, places the patient at immediate risk of death)

This does not include any AE that had it occurred in a more severe form or was allowed to continue might have caused death.

- Requires or prolongs inpatient hospitalization (see Section 5.3.3.9)
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions)
- Congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drug
- Significant medical event in the investigator's judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above)

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an adverse event (rated as mild, moderate, or severe); the event itself may be of relatively minor medical significance (such as severe headache). "Serious" is a regulatory definition and is based on patient or event outcome or action criteria usually

associated with events that pose a threat to a patient's life or vital functions. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations.

Severity and seriousness need to be independently assessed for each AE recorded on the eCRF.

SAEs 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 for reporting instructions).

5.2.3 Non-Serious Adverse Events of Special Interest (Immediately Reportable to the Sponsor)

Non-serious AESI 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 for reporting instructions). AESI for this study include the following:

- All events of SICH (use a list of preferred terms [PTs] see Appendix 13)
- In addition, Suspected Transmission of an Infectious Agent via a Medicinal Product (STIAMP) by the study drug in a Genentech-sponsored trial qualifies as an AESI and requires immediate reporting to the Sponsor.

5.3 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

The investigator is responsible for ensuring that all AEs (see Section 5.2.1 for definition) are recorded on the Adverse Event eCRF and reported to the Sponsor in accordance with instructions provided in this section and in Section 5.3.1 to Section 5.6.

For each AE recorded on the Adverse Event eCRF, the investigator will make an assessment of seriousness (see Section 5.2.2 for seriousness criteria), severity (see Section 5.3.2), and causality (see Section 5.3.3).

5.3.1 Adverse Event Reporting Period

Investigators will seek information on AEs at each patient contact. All AEs, whether reported by the patient or noted by study personnel, will be recorded in the patient's medical record and on the Adverse Event eCRF.

After informed consent has been obtained, but prior to initiation of study drug, only SAEs caused by a protocol-mandated intervention should be reported.

After initiation of study drug, all AEs, regardless of relationship to study drug, will be reported until 90 days after administration of study drug. After this period, the investigator is not required to actively monitor patients for adverse events; however, the Sponsor should be notified if the investigator becomes aware of any post-study serious adverse events or non-serious adverse events of special interest (see Section 5.6).

5.3.2 Assessment of Severity of Adverse Events

The World Health Organization (WHO) toxicity grading scale (see Appendix 14) will be used for assessing adverse event severity. Table 4 will be used for assessing severity for adverse events that are not specifically listed in the WHO toxicity grading scale.

Table 4 Adverse Event Severity Grading Scale

Grade	Severity
1	Mild; transient or mild discomfort (<48 hours); no medical intervention or therapy required
2	Moderate; mild to moderate limitation in activity; some assistance may be needed; no or minimal medical intervention or therapy required
3	Severe; marked limitation in activity; some assistance usually required; medical intervention or therapy required; hospitalization possible
4	Life-threatening; extreme limitation in activity; significant assistance required; significant medical intervention or therapy required, hospitalization or hospice care probable

Notes: Developed by the Division of Microbiology and Infectious Diseases.

Regardless of severity, some events may also meet seriousness criteria. Refer to definition of a serious adverse event (see Section 5.2.2).

5.3.3 Assessment of Causality of Adverse Events

Investigators should use their knowledge of the patient, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether or not an AE is considered to be related to the study drug, indicating "yes" or "no" accordingly. The following guidance should be taken into consideration (see also Table 5):

- Temporal relationship of event onset to the initiation of study drug
- Course of the event, considering especially the effects of dose reduction, discontinuation of study drug, or reintroduction of study drug (as applicable)
- Known association of the event with the study drug or with similar treatments
- Known association of the event with the disease under study
- Presence of risk factors in the patient or use of concomitant medications known to increase the occurrence of the event
- Presence of non-treatment-related factors that are known to be associated with the occurrence of the event

Table 5 Causal Attribution Guidance

Is the adverse event suspected to be caused by the study drug on the basis of facts, evidence, science-based rationales, and clinical judgment?	
YES	There is a plausible temporal relationship between the onset of the adverse event and administration of the study drug, and the adverse event cannot be readily explained by the patient's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study drug; and/or the adverse event abates or resolves upon discontinuation of the study drug or dose reduction and, if applicable, reappears upon re-challenge.
NO	Adverse events will be considered related, unless they fulfill the criteria as specified below. Evidence exists that the adverse event has an etiology other than the study drug (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the adverse event has no plausible temporal relationship to administration of the study drug (e.g., cancer diagnosed 2 days after first dose of study drug).

5.3.3.1 Diagnosis versus Signs and Symptoms

A diagnosis (if known) should be recorded on the Adverse Event eCRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded on the Adverse Event eCRF. If a diagnosis is subsequently established, all previously reported AEs based on signs and symptoms should be nullified and replaced by one AE report based on the single diagnosis, with a starting date that corresponds to the starting date of the first symptom of the eventual diagnosis.

5.3.3.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. However, medically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the Adverse Event eCRF. For example:

- If vomiting results in mild dehydration with no additional treatment in a healthy adult, only vomiting should be reported on the eCRF.
- If vomiting results in severe dehydration, both events should be reported separately on the eCRF.
- If a severe gastrointestinal hemorrhage leads to renal failure, both events should be reported separately on the eCRF.
- If dizziness leads to a fall and consequent fracture, all three events should be reported separately on the eCRF.
- If neutropenia is accompanied by an infection, both events should be reported separately on the eCRF.

All AEs should be recorded separately on the Adverse Event eCRF if it is unclear as to whether the events are associated.

5.3.3.3 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between patient evaluation timepoints. Such events should only be recorded once on the Adverse Event eCRF. The initial severity of the event should be recorded, and the severity should be updated to reflect the most extreme severity any time the event worsens. If the event becomes serious, the Adverse Event eCRF should be updated to reflect this.

A recurrent AE is one that resolves between patient evaluation timepoints and subsequently recurs. Each recurrence of an AE should be recorded separately on the Adverse Event eCRF.

5.3.3.4 Abnormal Laboratory Values

Not every laboratory abnormality qualifies as an AE. A laboratory test result must be reported as an AE if it meets any of the following criteria:

- Accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention (e.g., potassium supplementation for hypokalemia) or a change in concomitant therapy
- Clinically significant in the investigator's judgment

It is the investigator's responsibility to review all laboratory findings. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an AE.

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 times the upper limit of normal associated with cholestasis), only the diagnosis (i.e., cholestasis) should be recorded on the Adverse Event eCRF.

If a clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded on the Adverse Event eCRF, along with a descriptor indicating if the test result is above or below the normal range (e.g., "elevated potassium" as opposed to "abnormal potassium"). If the laboratory abnormality can be characterized by a precise clinical term per standard definitions, the clinical term should be recorded as the AE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia."

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded on the Adverse Event eCRF unless the etiology

changes. The initial severity of the event should be recorded, and the severity or seriousness should be updated any time the event worsens.

5.3.3.5 Abnormal Vital Sign Values

Not every vital sign abnormality qualifies as an AE. A vital sign result must be reported as an AE if it meets any of the following criteria:

- Accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention or a change in concomitant therapy
- Clinically significant in the investigator's judgment

It is the investigator's responsibility to review all vital sign findings. Medical and scientific judgment should be exercised in deciding whether an isolated vital sign abnormality should be classified as an AE.

If a clinically significant vital sign abnormality is a sign of a disease or syndrome (e.g., high blood pressure), only the diagnosis (i.e., hypertension) should be recorded on the Adverse Event eCRF.

Observations of the same clinically significant vital sign abnormality from visit to visit should not be repeatedly recorded on the Adverse Event eCRF unless the etiology changes. The initial severity of the event should be recorded, and the severity or seriousness should be updated any time the event worsens.

5.3.3.6 Deaths

All deaths that occur during the protocol-specified AE reporting period (Section 5.3.1) regardless of relationship to study drug, must be recorded on the Adverse Event eCRF and immediately reported to the Sponsor (see Section 5.2.2). This includes death attributed to progression of AIS.

Death should be considered an outcome and not a distinct event. The event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the Adverse Event eCRF. Generally, only one such event should be reported. The term "sudden death" should only be used for the occurrence of an abrupt and unexpected death due to presumed cardiac causes in a patient with or without preexisting heart disease, within 1 hour of the onset of acute symptoms or, in the case of an unwitnessed death, within 24 hours after the patient was last seen alive and stable. If the cause of death is unknown and cannot be ascertained at the time of reporting, "unexplained death" should be recorded on the Adverse Event eCRF. If the cause of death later becomes available (e.g., after autopsy), "unexplained death" should be replaced by the established cause of death.

If the death is attributed to progression of AIS, "acute ischemic stroke progression" should be recorded on the Adverse Event eCRF.

5.3.3.7 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the screening visit for this study. Such conditions should be recorded on the General Medical History and Baseline Conditions eCRF.

A preexisting medical condition should be recorded as an AE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on the Adverse Event eCRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., more frequent headaches").

5.3.3.8 Lack of Efficacy or Worsening of Acute Ischemic Stroke

Medical occurrences or symptoms of deterioration that are anticipated as part of AIS should be recorded as an AE if judged by the investigator to have unexpectedly worsened in severity or frequency or changed in nature at any time during the study. When recording an unanticipated worsening of acute ischemic stroke on the Adverse Event eCRF, it is important to convey the concept that the condition has changed by including applicable descriptors (e.g., "accelerated acute ischemic stroke").

5.3.3.9 Hospitalization or Prolonged Hospitalization

Any AE that results in hospitalization (i.e., in-patient admission to a hospital) or prolonged hospitalization should be documented and reported as an SAE (per the definition of an SAE in Section 5.2.2), except as outlined below.

The following hospitalization scenarios are not considered to be AEs:

- Hospitalization for respite care
- Hospitalization for a preexisting condition, provided that all of the following criteria are met:
 - The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease.
 - The patient has not suffered an AE.

5.3.3.10 Adverse Events Associated with an Overdose

An overdose is the accidental or intentional use of a drug in an amount higher than the dose being studied. An overdose or incorrect administration of study treatment is not itself an AE, but it may result in an AE. All AEs associated with an overdose or incorrect administration of study drug should be recorded on the Adverse Event eCRF. If the associated AE fulfills serious criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2).

5.3.3.11 Patient-Reported Outcome Data

AE reports will not be derived from PRO data by the Sponsor, and safety analyses will not be performed using PRO data. However, if any PRO responses suggestive of a possible AE are identified during site review of the PRO data, the investigator will determine whether the criteria for an AE have been met and, if so, will report the event on the Adverse Event eCRF.

5.4 IMMEDIATE REPORTING REQUIREMENTS FROM INVESTIGATOR TO SPONSOR

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- SAEs
- Non-serious AESI
- Pregnancies

The investigator must report new significant follow-up information for these events to the Sponsor immediately (i.e., no more than 24 hours after becoming aware of the information). New significant information includes the following:

- New signs or symptoms or a change in the diagnosis
- Significant new diagnostic test results
- Change in causality based on new information
- Change in the event's outcome, including recovery
- Additional narrative information on the clinical course of the event

Investigators must also comply with local requirements for reporting SAEs to the local health authority and Institutional Review Board/Ethics Committee (IRB/EC).

5.4.1 Emergency Medical Contacts

Medical Monitor Contact Information

PPD Medical Monitor contact information:

Medical Monitor: [REDACTED], M.D.

Telephone Nos.: [REDACTED]

Alternate Medical Monitor contact information for all sites:

Medical Monitor: [REDACTED], M.D.

Telephone No.: [REDACTED]

5.4.2 Reporting Requirements for Serious Adverse Events and Non-Serious Adverse Events of Special Interest

For reports of SAEs and non-serious AESI, investigators should record all case details that can be gathered immediately (i.e., within 24 hours) on the Adverse Event eCRF and submit the report via the electronic data capture (EDC) system. A report will be generated and sent to the Sponsor's Safety Risk Management department by the EDC system.

In the event that the EDC system is unavailable, a paper Serious Adverse Event Reporting Form and fax cover sheet should be completed and faxed to Safety Risk Management or its designee immediately (i.e., no more than 24 hours after learning of the event), using the fax numbers provided below. Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

Sites:

Fax No.: (650) 225-4682

Alternate Fax No.: (650) 225-5288

5.4.3 Reporting Requirements for Pregnancies

5.4.3.1 Pregnancies in Female Patients

Female patients of childbearing potential will be instructed to immediately inform the investigator if they become pregnant within 30 (± 7) days after the last dose of study drug. A Pregnancy Report eCRF should be completed by the investigator immediately (i.e., no more than 24 hours after learning of the pregnancy) and submitted via the EDC system. A pregnancy report will automatically be generated and sent to Safety Risk Management. Pregnancy should not be recorded on the Adverse Event eCRF. The investigator should counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Any SAEs associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported on the Adverse Event eCRF.

In the event that the EDC system is unavailable, a Clinical Trial Pregnancy Reporting Form and fax cover sheet should be completed and faxed to Safety Risk Management or its designee immediately (i.e., no more than 24 hours after learning of the pregnancy), using the fax numbers provided in Section 5.4.2. Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

5.4.3.2 Congenital Anomalies/Birth Defects and Abortions

Any congenital anomaly/birth defect in a child born to a female patient exposed to study drug should be classified as an SAE, recorded on the Adverse Event eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2). Any spontaneous abortion should be reported in the same

fashion (as the Sponsor considers spontaneous abortions to be medically significant events).

5.5 FOLLOW-UP OF PATIENTS AFTER ADVERSE EVENTS

5.5.1 Investigator Follow-Up

The investigator should follow each AE until the event has resolved to baseline grade or better and is assessed as stable by the investigator, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all SAEs considered to be related to study drug or trial-related procedures until a final outcome can be reported.

During the study period, resolution of AEs (with dates) should be documented on the Adverse Event eCRF and in the patient's medical record to facilitate source data verification. If, after follow-up, return to baseline status or stabilization cannot be established, an explanation should be recorded on the Adverse Event eCRF.

All pregnancies reported during the study should be followed until pregnancy outcome. If the EDC system is not available at the time of pregnancy outcome, follow reporting instructions provided in Section 5.4.3.

5.5.2 Sponsor Follow-Up

For SAEs, AESI, and pregnancies, the Sponsor or a designee may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

5.6 POST-STUDY ADVERSE EVENTS

At the time of study completion or study discontinuation, the investigator should instruct each patient to report to the investigator any subsequent AEs that the patient's personal physician believes could be related to prior study drug treatment or study procedures.

The investigator is not required to actively monitor patients for AEs after the end of the AE reporting period (defined as 90 days after administration of study drug). However, the Sponsor should be notified if the investigator becomes aware of any death, other SAE, or non-serious AESI occurring after the end of the AE reporting period, regardless of causality. The Sponsor should also be notified if the investigator becomes aware of the development of cancer or a congenital anomaly/birth defect in a subsequently conceived offspring of a female patient exposed to study drug.

The investigator should report these events directly to Safety Risk Management via telephone at 1-888-835-2555.

5.7

EXPEDITED REPORTING TO HEALTH AUTHORITIES, INVESTIGATORS, INSTITUTIONAL REVIEW BOARDS, AND ETHICS COMMITTEES

The Sponsor will promptly evaluate all SAEs and non-serious AESI against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators, IRBs, ECs, and applicable health authorities based on applicable legislation.

To determine reporting requirements for single AE cases, the Sponsor will assess the expectedness of these events using the following reference document:

- Alteplase Investigator's Brochure

The Sponsor will compare the severity of each event and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.

Reporting requirements will also be based on the investigator's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

6.

STATISTICAL METHODS

This study will test whether treatment of patients with mild stroke with IV alteplase will lead to a higher proportion (9% absolute difference) of patients with a favorable outcome (mRS of 0 or 1) at 90 days.

6.1

DETERMINATION OF SAMPLE SIZE

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 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, after 50% of the anticipated sample size have completed 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, and treatment crossovers), the above sample size needs to be inflated by a factor of $1/0.95^2$ or 1.108 (Friedman et al. 1998). Therefore, a total sample size of 948 is required for this study.

6.2

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 symptoms. 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. \geq 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 ITT principle. A patient is considered enrolled and randomized in the trial, and included in the ITT population, as soon as study drug begins. As a sensitivity analysis, results from the univariate Pearson's chi-square test will also be presented.

As a supportive, secondary analysis, an ordinal (or "shift") analysis of the distribution of the mRS will be analyzed via proportional odds model if the proportionality assumption holds, and by the Mann-Whitney superiority measure if the proportionality assumption does not hold. The proportionality assumption will be assessed using the score test as well as clinical judgment on the similarities of the different odds ratios. Other secondary efficacy outcomes of interest include NIHSS score, BI, GOS, and global outcome scale at 90 days. These results will be treated as supportive evidence (or lack thereof) of the treatment effect rather than conclusive evidence, the significance of each test is determined at the two-sided alpha level of 0.05. The specific statistical model for analyzing each of these outcome measures will be detailed in the Statistical Analysis Plan (SAP).

6.3 SAFETY ANALYSIS

Incidence and severity of AEs will be described for all treated patients by treatment group as received and by the Medical Dictionary for Regulatory Activities (MedDRA) classification.

Primary safety outcomes include incidence of sICH within 36 hours, any ICH within 36 hours, overall mortality, and stroke-related and neurological deaths within 90 days of study drug initiation. The cumulative incidences of each outcome will be compared via Fisher's exact test. Mortality will be analyzed by Log-Rank test and the Kaplan-Meier estimates will be plotted over the observation period of 90 days. No alpha adjustment for multiple testing on the safety endpoints is foreseen because those analyses will be exploratory.

6.4 EXPLORATORY ANALYSES

As exploratory analyses, heterogeneity 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 (<65 vs. \geq 65)
- Pre-treatment NIHSS score (0–2 vs. 3–5)
- Last known well time to treatment (0–2 vs. 2–3 hours)
- Stroke subgroup (RISS vs. non-RISS)

Additional heterogeneity analyses will be considered and detailed in the SAP. Forest plots will be constructed to illustrate subgroup analyses. In addition, the two-way interactions of IV alteplase treatment effect with age, NIHSS score, and stroke subgroups (RISS vs. non-RISS) on the primary outcome will be explored through multivariable logistic regression. Additional exploratory analyses will be conducted to explore treatment effects on quality of life endpoints and other exploratory endpoints.

6.5 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 O'Brien and Fleming-type boundaries. This futility analysis will occur after approximately 50% of patients (or 474) have completed the 90-day assessment.

An independent Data Coordinating Center (iDCC) will be responsible for the preparation and review of unmasked data. Further details will be provided in the interim SAP and the iDMC charter.

An iDMC will be organized for review of the interim analysis results. The composition of the iDMC and analysis in detail will be documented in a separate iDMC charter.

6.6 MISSING DATA HANDLING

Under the ITT principle, all patients who are randomized will be included in the analysis. The multiple-imputation method will be used for handling missing data, including the last observation among the variables employed in imputation. As supportive analyses, missing primary outcome data will be imputed by 1) using last observation carried forward and 2) assuming missing outcomes to be unfavorable.

7. DATA COLLECTION AND MANAGEMENT

7.1 DATA QUALITY ASSURANCE

The Sponsor will supply eCRF specifications for this study. A contract research organization (CRO) will be responsible for data management of this study, including quality checking of the data. Data entered manually will be collected via EDC using eCRFs. Sites will be responsible for data entry into the EDC system. In the event of discrepant data, the CRO will request data clarification from the sites, which the sites will resolve electronically in the EDC system.

The CRO will produce a Data Quality Plan that describes the quality checking to be performed on the data with the input from the Sponsor. For this study, local laboratories will be used and data will be captured in the EDC system and will then be transferred to the Sponsor. This will include the eCRF data using the Sponsor's standard procedures and processes to transfer data.

The Sponsor will perform oversight of the data management of this study, including approval of the CRO's data management plans and specifications. Data will be transferred electronically from the CRO to the Sponsor, and the Sponsor's standard procedures will be used to handle and process the electronic transfer of these data.

eCRFs and correction documentation will be maintained in the EDC system's audit trail. The EDC vendor has back-ups for data stored, and records retention for the study data will be consistent with the CRO's standard procedures.

Data from paper PRO questionnaires will be entered into the EDC system by site staff.

7.2 ELECTRONIC CASE REPORT FORMS

eCRFs are to be completed using a Sponsor-designated EDC system. Sites will receive training and have eCRF completion guidelines for the eCRF completion. eCRFs will be submitted electronically to the Sponsor and should be handled in accordance with instructions from the Sponsor.

All eCRFs should be completed by designated trained site staff. eCRFs should be reviewed and electronically signed and dated by the investigator or a designee.

At the end of the study, the investigator will receive patient data for his or her site in a readable format on a compact disc that must be kept with the study records. Acknowledgement of receipt of the compact disc is required.

7.3 SOURCE DATA DOCUMENTATION

Study monitors will perform ongoing source data verification to confirm that critical protocol data (e.g., source data) entered into the eCRFs by authorized site personnel are accurate, complete, and verifiable from source documents. Source documents (paper or electronic) are those in which patient data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, patient-reported outcomes, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, patient files, and records kept at pharmacies, laboratories, and medico-technical departments involved in a clinical trial.

Before study initiation, the types of source documents that are to be generated will be clearly defined in the Study Trial Monitoring Plan. This includes any protocol data to be entered directly into the eCRFs (i.e., no prior written or electronic record of the data) and considered source data.

Source documents that are required to verify the validity and completeness of data entered into the eCRFs must not be obliterated or destroyed and must be retained per the policy for retention of records described in Section 7.5.

To facilitate source data verification, the investigators and institutions must provide the Sponsor direct access to applicable source documents and reports for trial-related monitoring, Sponsor audits, and IRB/EC review. The investigational site must also allow inspection by applicable health authorities.

7.4 USE OF COMPUTERIZED SYSTEMS

When clinical observations are entered directly into an investigational site's computerized medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve as the source document if the system has been validated in accordance with health authority requirements pertaining to computerized systems used in clinical research. An acceptable computerized data collection system allows preservation of the original entry of data. If original data are modified, the system should maintain a viewable audit trail that shows the original data as well as the reason for the change, name of the person making the change, and date of the change.

7.5 RETENTION OF RECORDS

Records and documents pertaining to the conduct of this study and the distribution of IMP, including eCRFs, PRO data (if applicable), Informed Consent Forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for at least 15 years after completion or discontinuation of the study, or for the length of time required by relevant national or local health authorities, whichever is longer. After that period of time, the documents may be destroyed, subject to local regulations.

No records may be disposed of without the written approval of the Sponsor. Written notification should be provided to the Sponsor prior to transferring any records to another party or moving them to another location.

8. ETHICAL CONSIDERATIONS

8.1 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in full conformance with the International Conference on Harmonisation E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the International Conference on Harmonisation E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Studies conducted in the United States or under a U.S. Investigational New Drug (IND) application will comply with FDA regulations and applicable local, state, and

federal laws. Studies conducted in the EU/EEA will comply with the EU Clinical Trial Directive (2001/20/EC).

8.2 INFORMED CONSENT

The Sponsor's sample Informed Consent Form (and ancillary sample Informed Consent Forms such as a Child's Assent or Caregiver's Informed Consent Form, if applicable) will be provided to each site. If applicable, it will be provided in a certified translation of the local language. The Sponsor or its designee must review and approve any proposed deviations from the Sponsor's sample Informed Consent Forms or any alternate consent forms proposed by the site (collectively, the "Consent Forms") before IRB/EC submission. The final IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes according to local requirements.

The Consent Forms should be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate. The final revised IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes.

Patients must be re-consented to the most current version of the Consent Forms (or to a significant new information/findings addendum in accordance with applicable laws and IRB/EC policy) during their participation in the study. For any updated or revised Consent Forms, the case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

A copy of each signed Consent Form must be provided to the patient or the patient's legally authorized representative. All signed and dated Consent Forms must remain in each patient's study file or in the site file and must be available for verification by study monitors at any time.

For sites in the U.S., each Consent Form may also include patient authorization to allow use and disclosure of personal health information in compliance with the U.S. Health Insurance Portability and Accountability Act of 1996 (HIPAA). If the site utilizes a separate Authorization Form for patient authorization for use and disclosure of personal health information under the HIPAA regulations, the review, approval, and other processes outlined above apply except that IRB review and approval may not be required per study site policies.

8.3 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE

This protocol, the Informed Consent Forms, any information to be given to the patient, and relevant supporting information must be submitted to the IRB/EC by the Principal Investigator and reviewed and approved by the IRB/EC before the study is initiated. In addition, any patient recruitment materials must be approved by the IRB/EC.

The Principal Investigator is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. Investigators are also responsible for promptly informing the IRB/EC of any protocol amendments (see Section 9.5).

In addition to the requirements for reporting all AEs to the Sponsor, investigators must comply with requirements for reporting SAEs to the local health authority and IRB/EC. Investigators may receive written IND safety reports or other safety-related communications from the Sponsor. Investigators are responsible for ensuring that such reports are reviewed and processed in accordance with health authority requirements and the policies and procedures established by their IRB/EC, and archived in the site's study file.

8.4 CONFIDENTIALITY

The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient ID number. This means that patient names are not included in data sets that are transmitted to any Sponsor location.

Patient medical information obtained by this study is confidential and may only be disclosed to third parties as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law.

Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare, for treatment purposes.

Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA and other national and local health authorities, Sponsor monitors, representatives, and collaborators, and the IRB/EC for each study site, as appropriate.

8.5 FINANCIAL DISCLOSURE

Investigators will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate health authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study (i.e., LPLV).

9. STUDY DOCUMENTATION, MONITORING, AND ADMINISTRATION

9.1 STUDY DOCUMENTATION

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including but not limited to the protocol, protocol

amendments, Informed Consent Forms, and documentation of IRB/EC and governmental approval. In addition, at the end of the study, the investigator will receive the patient data, which includes an audit trail containing a complete record of all changes to data.

9.2 PROTOCOL VIOLATIONS

The investigator should document and explain any protocol violations. The investigator should promptly report any violations that might impact patient safety and data integrity to the Sponsor and to the IRB/EC in accordance with established IRB/EC policies and procedures.

9.3 SITE INSPECTIONS

Site visits will be conducted by the Sponsor or an authorized representative for inspection of study data, patients' medical records, and eCRFs. The investigator will permit national and local health authorities, Sponsor monitors, representatives, and collaborators, and the IRBs/ECs to inspect facilities and records relevant to this study.

9.4 ADMINISTRATIVE STRUCTURE

Genentech, Inc., is the Sponsor and will have oversight of study management, including project management, oversight of data management, and statistical programming. A CRO will assist with study management, including project management, medical monitoring (as needed), clinical monitoring, data monitoring, and data management. Patients will be enrolled at approximately 75 sites in the U.S. and Canada.

The CRO will manage site activation, study drug supply, patient enrollment and tracking, and clinical monitoring. Genentech will handle study drug distribution.

A Steering Committee will provide scientific oversight for this study. The Steering Committee will be composed of Genentech representatives and external advisors. The Steering Committee will perform functions such as:

- Provide recommendations regarding changes in the study conduct
- Review results of the final analyses for primary and secondary endpoints
- Participate in the drafting and publishing of final study results

An iDMC will be responsible for monitoring the overall efficacy and safety of the patients in the trial after treatment with alteplase. The iDMC will be composed of external advisors. The iDMC will perform functions such as:

- Review all accumulated safety data at interim analysis
- Review efficacy data analysis performed for interim analysis for consideration of potential discontinuation of the trial for futility
- Make recommendation to the Sponsor as to if it is appropriate to discontinue the study after assessing the interim efficacy and safety data in aggregate

- Determine if the study should continue after review of interim analysis results and to assess the sample size requirement using accumulated data, as described in the protocol

An iDCC will be responsible for the preparation and review of unmasked data.

A central IRB will be utilized when possible to ensure the timely submission and approval of site regulatory documents for sites not required to use a local IRB. The CRO will be responsible for management of the central IRB.

An EDC system will be used to capture data for the study.

An IxRS will be utilized for randomization, patient tracking, and drug inventory management for the study.

9.5 PROTOCOL AMENDMENTS

Any protocol amendments will be prepared by the Sponsor. Protocol amendments will be submitted to the IRB/EC and to regulatory authorities in accordance with local regulatory requirements.

Approval must be obtained from the IRB/EC and regulatory authorities (as locally required) before implementation of any changes, except for changes necessary to eliminate an immediate hazard to patients or changes that involve logistical or administrative aspects only (e.g., change in Medical Monitor or contact information).

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Appendix 1

Schedule of Assessments

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 (± 14) Days/ Study Completion In-Person 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						
Neurological 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					

Appendix 1 (cont.) Schedule of Assessments

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 (± 14) Days/ Study Completion In-Person Visit (Visit 5)	Early Discontinuation (Phone call, if possible)
Discharge location				x			x
Patient survival					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 assessments						x	
Walking speed ^l						x	
Concomitant medications ^m	x		x	x	x	x	x
Assessment of pregnancy					x		
Adverse event ⁿ	x		x	x	x	x	x

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; ICH=intracranial hemorrhage; MRI=magnetic resonance imaging; mRS modified Rankin Scale; NIHSS=NIH Stroke Scale; Stroke Impact Scale-16=SIS-16; SWMRI=susceptibility-weighted MRI.

Appendix 1 (cont.) Schedule of Assessments

- ^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.
- ^c Includes oral temperature, respiratory rate, 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, prothrombin time, activated partial thromboplastin time, and patient or family report of patient currently taking oral anticoagulants (e.g., warfarin, dabigatran, rivaroxaban, apixaban, edoxaban).
- ^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 Assessment of walking speed requires an in-person patient visit and is best combined with administration of the BI, CES-D, EQ-5D, and SIS-16.
- ^m Concomitant medications includes any prescription medications or over-the-counter preparations used by the patient within 7 days prior to screening.
- ⁿ Adverse event assessments at Screening/Baseline, 22–36 hours after study drug administration, Days 5, 30, and 90 may be done via telephone interview if necessary, and early discontinuation.

Appendix 2

Alteplase Administration

Administration of bolus



Step 1:

Inspect solution

After reconstitution to 1 mg/mL, inspect solution for particulate matter and discoloration prior to administration

Step 2:

Discard excess

To ensure proper dosing, discard excess by removing from vial any quantity of drug in excess of that specified for patient treatment. When drawing off excess solution, be sure to insert the needle into the peripheral area of the vial top, away from the puncture site caused by the transfer device

Step 3:

Prepare bolus

Withdraw 10% of the 0.9-mg/kg dose in one of the following ways:

- Remove from vial using a syringe and needle,
- Remove from port (second injection site) on infusion line after infusion set is primed, or
- Program infusion pump to deliver bolus at infusion initiation

Step 4:

Administer bolus

Administer initial IV bolus over 1 minute

Administration of remainder of dose

Infuse the remaining 90% of 0.9-mg/kg dose over 60 minutes.

50-mg vials—Administer using either a polyvinyl chloride bag or glass vial and infusion set.

No medication should be added to the infusion solutions that contain alteplase.

For specifics regarding dosing and administration, please see the Activase full Prescribing Information.

Alteplase—Genentech, Inc.
Protocol ML29093, Version 1

Appendix 3 Reconstitution of 50-mg Vials

A single 50-mg vial of Activase may be used to treat patients weighing up to 122 lbs or 55.5 kilograms. Reconstitute Activase immediately before administration, using aseptic technique at all times—including thorough hand washing and the use of gloves.



Step 1:

Assemble the 50-mg vial of Activase, the vial of Sterile Water for Injection (SWFI), USP (included with the Activase package), a 50-mL syringe, a large-bore needle (eg, 18-gauge), and alcohol wipes. Also, prepare a polyvinyl chloride bag or glass vial along with an infusion set

Step 2:

Remove the protective cap from the top of the Activase vial and the vial of SWFI. Swab the top of each vial with an alcohol wipe to reduce the risk of contamination

Step 3:

Withdraw 50-mL of SWFI, without preservatives. Diluent is included. DO NOT USE Bacteriostatic Water for Injection, USP

Step 4:

Inject the 50-mL into the 50-mg Activase vial using a large-bore needle and syringe, directing the stream into the lyophilized cake. DO NOT USE IF VACUUM IS NOT PRESENT. The syringe should not be primed with air during preparation and should be inserted into the Activase vial stopper. Slight foaming upon reconstitution is normal. Let the solution stand undisturbed for several minutes to allow any large bubbles to dissipate

Step 5:

Mix with a gentle swirl or slow inversion. DO NOT SHAKE. Visually inspect the Activase solution for particulate matter and discoloration before administration

Activase is stable for up to 8 hours in these solutions at room temperature. Do not freeze solutions containing Activase. No other medications should be added to infusions containing Activase. Any unused infusion solution should be discarded.

Appendix 4

European Quality of Life-5D (EQ-5D) Questionnaire

By placing a checkmark in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about

I have some problems in walking about

I am confined to bed

Self-Care

I have no problems with self-care

I have some problems washing or dressing myself

I am unable to wash or dress myself

Usual Activities (e.g. work, study, housework, family or leisure activities)

I have no problems with performing my usual activities

I have some problems with performing my usual activities

I am unable to perform my usual activities

Pain/Discomfort

I have no pain or discomfort

I have moderate pain or discomfort

I have extreme pain or discomfort

Anxiety/Depression

I am not anxious or depressed

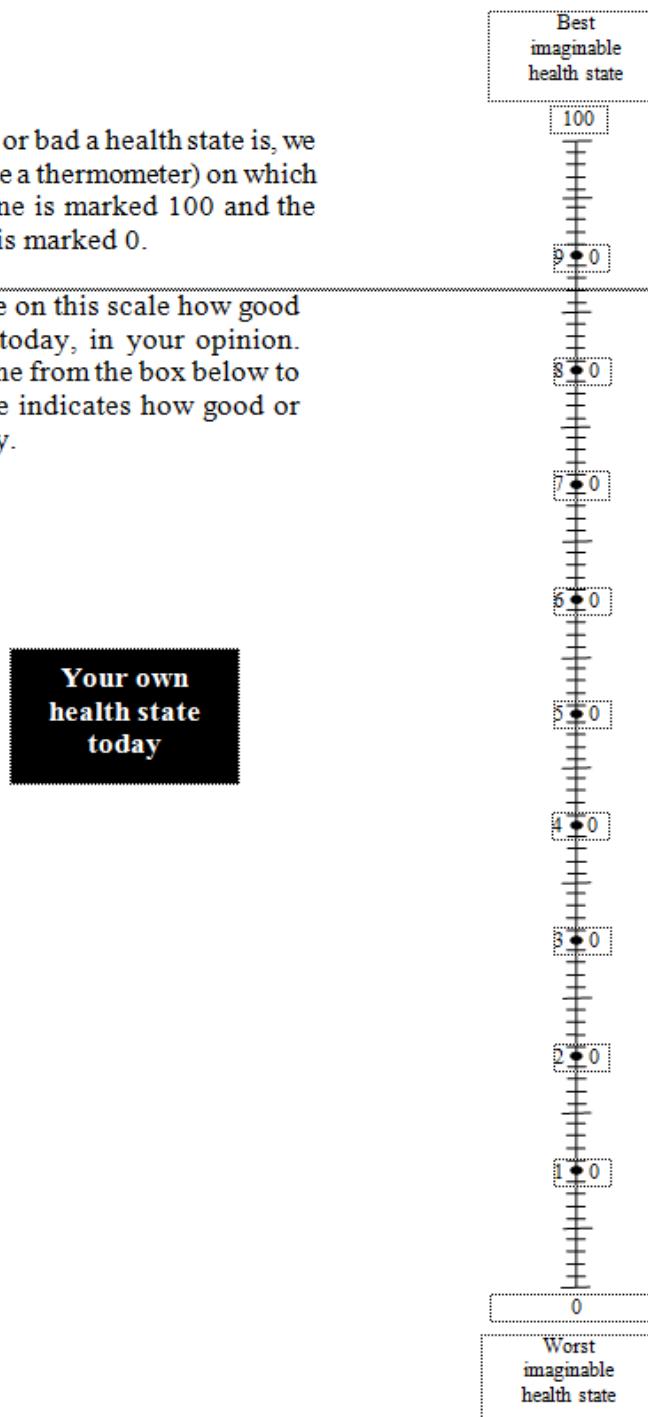
I am moderately anxious or depressed

I am extremely anxious or depressed

Appendix 4 (cont.) European Quality of Life-5D (EQ-5D) Questionnaire

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.



Appendix 5
Stroke Index Scale (SIS-16)

In the past 2 weeks, how difficult was it to...	Not difficult at all	A little difficult	Somewhat difficult	Very difficult	Could not do at all
a. Dress the top part of your body?	5	4	3	2	1
b. Bathe yourself?	5	4	3	2	1
c. Get to the toilet on time?	5	4	3	2	1
d. Control your bladder (not have an accident)?	5	4	3	2	1
e. Control your bowels (not have an accident)?	5	4	3	2	1
f. Stand without losing balance?	5	4	3	2	1
g. Go shopping?	5	4	3	2	1
h. Do heavy household chores (e.g., vacuum, laundry or yard work)?	5	4	3	2	1
i. Stay sitting without losing your balance?	5	4	3	2	1
j. Walk without losing your balance?	5	4	3	2	1
k. Move from a bed to a chair?	5	4	3	2	1
l. Walk fast?	5	4	3	2	1
m. Climb one flight of stairs?	5	4	3	2	1
n. Walk one block?	5	4	3	2	1
o. Get in and out of a car?	5	4	3	2	1
p. Carry heavy objects (e.g. bag of groceries) with your affected hand?	5	4	3	2	1

Appendix 6

Center for Epidemiologic Studies, Depression (CES-D) Scale, NIMH

Below is a list of the ways you might have felt or behaved. Please tell me how often you have felt this way during the past week.

Week	During the Past			
	Rarely or none of the time (less than 1 day)	Some or a little of the time (1-2 days)	Occasionally or a moderate amount of time (3-4 days)	Most or all of the time (5-7 days)
1. I was bothered by things that usually don't bother me.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2. I did not feel like eating; my appetite was poor.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3. I felt that I could not shake off the blues even with help from my family or friends.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4. I felt I was just as good as other people.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5. I had trouble keeping my mind on what I was doing.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6. I felt depressed.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7. I felt that everything I did was an effort.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8. I felt hopeful about the future.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9. I thought my life had been a failure.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10. I felt fearful.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11. My sleep was restless.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12. I was happy.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
13. I talked less than usual.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
14. I felt lonely.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
15. People were unfriendly.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
16. I enjoyed life.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
17. I had crying spells.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
18. I felt sad.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
19. I felt that people dislike me.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
20. I could not get "going."	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SCORING: zero for answers in the first column, 1 for answers in the second column, 2 for answers in the third column, 3 for answers in the fourth column. The scoring of positive items is reversed. Possible range of scores is zero to 60, with the higher scores indicating the presence of more symptomatology.

Appendix 7

National Institutes of Health Stroke Scale (NIHSS)

<p>Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).</p>		
1	<p>Has the subject had a stroke (either as the index event at randomization or as an outcome event)? If no, form is complete.</p>	<input type="radio"/> No <input type="radio"/> Yes
2	<p>(1a) Level of Consciousness</p> <p>The Investigator must choose a response, even if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation. Coma score "3".</p>	<input type="radio"/> 0=Alert; keenly responsive <input type="radio"/> 1=Not alert, but arousable by minor stimulation to obey, answer or respond <input type="radio"/> 2=Not alert, requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped) <input type="radio"/> 3=Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, areflexic (Complete form using coma scoring)
3	<p>(1b) LOC Questions</p> <p>The patient is asked the month and his/her age. The answer must be correct – there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues. Coma score "2".</p>	<input type="radio"/> 0=Answers both questions correctly <input type="radio"/> 1=Answers one question correctly <input type="radio"/> 2=Answers neither question correctly
4	<p>(1c) LOC Commands</p> <p>The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to them (pantomime) and score the result (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored. Coma score "2".</p>	<input type="radio"/> 0=Performs both tasks correctly <input type="radio"/> 1=Performs one task correctly <input type="radio"/> 2=Performs neither task correctly
5	<p>(2) Best Gaze</p> <p>Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI) score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness or other disorder of visual acuity or fields should be tested with reflexive movements and a choice made by the Investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy. Coma score as examined.</p>	<input type="radio"/> 0=Normal <input type="radio"/> 1=Partial gaze palsy. This score is given when gaze is abnormal in one or both eyes, but where forced deviation or total gaze paresis are not present <input type="radio"/> 2=Forced deviation, or total gaze paresis not overcome by the oculocephalic maneuver

Appendix 7 (cont.)

National Institutes of Health Stroke Scale (NIHSS)

8	<p style="text-align: center;">(3) Visual</p> <p>Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat as appropriate. Patient must be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia is found. If patient is blind from any cause score 3. Double simultaneous stimulation is performed at this point. If there is extinction patient receives a 1 and the results are used to answer question 22. Score as examined, using bilateral threat.</p>	<input type="radio"/> 0=No visual loss <input type="radio"/> 1=Partial hemianopia <input type="radio"/> 2=Complete hemianopia <input type="radio"/> 3=Bilateral hemianopia (blind including cortical blindness)
7	<p style="text-align: center;">(4) Facial Palsy</p> <p>Ask, or use pantomime to encourage the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barrier obscures the face, these should be removed to the extent possible. Coma Score "3".</p>	<input type="radio"/> 0=Normal symmetrical movement <input type="radio"/> 1=Minor paralysis (flattened nasolabial fold, asymmetry of smiling) <input type="radio"/> 2=Partial paralysis (total or near total paralysis of lower face) <input type="radio"/> 3=Complete paralysis of one or both sides (absence of facial movement in the upper and lower face)
8	<p style="text-align: center;">(5a) Motor Arm Left</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma Score "4".</p>	<input type="radio"/> 0=No drift, limb holds 90 (or 45) degrees for full 10 seconds <input type="radio"/> 1=Drift, limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed <input type="radio"/> 2=Some effort against gravity, limb cannot get to or maintain (if cued) 90 degrees <input type="radio"/> 3=No effort against gravity, limb falls <input type="radio"/> 4=No movement <input type="radio"/> Amputation, joint fusion
9	<p>Explain if amputation or joint fusion (Motor Arm Left):</p>	
10	<p style="text-align: center;">(5b) Motor Arm Right</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma score "4".</p>	<input type="radio"/> 0=No drift, limb holds 90 (or 45) degrees for full 10 seconds <input type="radio"/> 1=Drift, limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed <input type="radio"/> 2=Some effort against gravity, limb cannot get to or maintain (if cued) 90 degrees <input type="radio"/> 3=No effort against gravity, limb falls <input type="radio"/> 4=No movement <input type="radio"/> Amputation, joint fusion
11	<p>Explain if amputation or joint fusion (Motor Arm Right):</p>	

Appendix 7 (cont.)
National Institutes of Health Stroke Scale (NIHSS)

12	<p style="text-align: center;">(6a) Motor Leg Left</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm fails before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma score "4".</p>	<input type="radio"/> 0=No drift, leg holds 30 degrees position for full 5 seconds <input type="radio"/> 1=Drift, leg falls by the end of the 5 second period but does not hit bed <input type="radio"/> 2=Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity <input type="radio"/> 3=No effort against gravity; leg falls to bed immediately <input type="radio"/> 4=No movement <input type="radio"/> Amputation, joint fusion
13	Explain if amputation/ joint fusion (Motor Leg Left):	
14	<p style="text-align: center;">(6b) Motor Leg Right</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm fails before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma score "4".</p>	<input type="radio"/> 0=No drift, leg holds 30 degrees position for full 5 seconds <input type="radio"/> 1=Drift, leg falls by the end of the 5 second period but does not hit bed <input type="radio"/> 2=Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity <input type="radio"/> 3=No effort against gravity; leg falls to bed immediately <input type="radio"/> 4=No movement <input type="radio"/> Amputation, joint fusion
15	Explain if amputation/ joint fusion (Motor Leg Right):	
16	<p style="text-align: center;">(7) Limb Ataxia</p> <p>This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, insure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position. Coma score "0".</p>	<input type="radio"/> 0 = Absent <input type="radio"/> 1 = Present in one limb <input type="radio"/> 2 = Present in two limbs <input type="radio"/> UN = amputation or joint fusion
17	Explain if amputation or joint fusion (Limb Ataxia):	

Appendix 7 (cont.)

National Institutes of Health Stroke Scale (NIHSS)

18	<p style="text-align: center;">(8) Sensory</p> <p>Sensation or grimace to pin prick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas [arms (not hands), legs, trunk, face] as needed to accurately check for hemisensory loss. A score of 2, "severe or total," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will therefore probably score 1 or 0. The patient with brain stem stroke who has bilateral loss of sensation is scored '2'. If the patient does not respond and is quadriplegic score 2. Patients in coma (item 1a=3) are arbitrarily given a 2 on this item. Coma Score "2".</p>	<input type="radio"/> 0=Normal; no sensory loss <input type="radio"/> 1=Mild to moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick but patient is aware he/she is being touched <input type="radio"/> 2=Severe to total sensory loss; patient is not aware of being touched in the face, arm & leg
19	<p style="text-align: center;">(9) Best Language</p> <p>A great deal of information about comprehension will be obtained during the preceding sections of the examination. The patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet, and to read from the attached list of sentences. Comprehension is judged from responses here as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in coma (question 1a=3) will arbitrarily score 3 on this item. The examiner must choose a score in the patient with stupor or limited cooperation but a score of 3 should be used only if the patient is mute and follows no one step commands. Coma Score "3".</p>	<input type="radio"/> 0=No aphasia, normal <input type="radio"/> 1=Mild to moderate aphasia; some obvious loss of fluency or facility of comprehension without significant limitation on ideas expressed on form of expression <input type="radio"/> 2=Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener; listener carries burden of communication <input type="radio"/> 3=Mute, global aphasia; no usable speech or auditory comprehension
20	<p style="text-align: center;">(10) Dysarthria</p> <p>If patient is thought to be normal an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barrier to producing speech, may the item be not scored, and the examiner must clearly write an explanation. Do not tell the patient why he/she is being tested. Coma Score "2".</p>	<input type="radio"/> 0=Normal <input type="radio"/> 1=Mild to moderate; patient slurs at least some words and at worst, can be understood with some difficulty <input type="radio"/> 2=Severe; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/ anarthric <input type="radio"/> Intubated or other physical barrier.
21	<p>Explain if intubated or other physical barrier (Dysarthria):</p>	
22	<p style="text-align: center;">(11) Extinction and Inattention (Neglect)</p> <p>Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosognosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable. Coma Score "2".</p>	<input type="radio"/> 0=No abnormality <input type="radio"/> 1=Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities <input type="radio"/> 2=Profound hemi-inattention or hemi-inattention to more than one modality; does not recognize own hand or orients to only one side of space
23	<p>Did NIHSS increase by ≥ 2 points AND NIHSS score is now >5?</p> <p>If YES, consider treating patient with IV rtPA as standard of care and unblinding of treatment assignment if appropriate. Note that patient will continue to be followed in the PRISMS trial regardless of decision.</p>	<input type="radio"/> No <input type="radio"/> Yes
24	<p>Name of assessor:</p> <p>The assessor must be a study team member who has completed NIHSS certification.</p>	

Appendix 8

Modified Rankin Scale (mRS)

1	Modified Rankin Scale:	<input type="radio"/> (0) No symptoms at all <input type="radio"/> (1) No significant disability despite symptoms; able to carry out all usual duties and activities <input type="radio"/> (2) Slight disability; unable to carry out all previous activities but able to look after own affairs without assistance <input type="radio"/> (3) Moderate disability requiring some help, but able to walk without assistance <input type="radio"/> (4) Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance <input type="radio"/> (5) Severe disability; bedridden, incontinent, and requiring constant nursing care and attention
2	For response 2 above, specify the previous activities that can no longer be carried out:	
3	Name of assessor: The assessor must be a study team member who has completed mRS certification.	

Appendix 9 Glasgow Outcome Scale (GOS)

Note: The scale presented here is based on the original article by Jennett and Bond. It has become common practice in clinical trial administration, however, to use a modified version that places the scores in reverse order (i.e., "good recovery" = 1, "moderate disability" = 2, etc.).

Score	Description
1	DEATH
2	PERSISTENT VEGETATIVE STATE Patient exhibits no <i>obvious cortical</i> function.
3	SEVERE DISABILITY (Conscious but disabled). Patient depends upon others for daily support due to mental or physical disability or both.
4	MODERATE DISABILITY (Disabled but independent). Patient is independent as far as daily life is concerned. The disabilities found include varying degrees of dysphasia, hemiparesis, or ataxia, as well as intellectual and memory deficits and personality changes.
5	GOOD RECOVERY Resumption of normal activities even though there may be minor neurological or psychological deficits.

TOTAL (1-5): _____

References

Jennett B, Bond M. "Assessment of outcome after severe brain damage." *Lancet* 1975 Mar 1;1(7905):480-4

Provided by the Internet Stroke Center — www.strokecenter.org

Appendix 10 Barthel Index (BI)

THE BARTHEL INDEX

Patient Name: _____
 Rater Name: _____
 Date: _____

Activity	Score
FEEDING 0 = unable 5 = needs help cutting, spreading butter, etc., or requires modified diet 10 = independent	_____
BATHING 0 = dependent 5 = independent (or in shower)	_____
GROOMING 0 = needs to help with personal care 5 = independent face/hair/teeth/shaving (implements provided)	_____
DRESSING 0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	_____
BOWELS 0 = incontinent (or needs to be given enemas) 5 = occasional accident 10 = continent	_____
BLADDER 0 = incontinent, or catheterized and unable to manage alone 5 = occasional accident 10 = continent	_____
TOILET USE 0 = dependent 5 = needs some help, but can do something alone 10 = independent (on and off, dressing, wiping)	_____
TRANSFERS (BED TO CHAIR AND BACK) 0 = unable, no sitting balance 5 = major help (one or two people, physical), can sit 10 = minor help (verbal or physical) 15 = independent	_____
MOBILITY (ON LEVEL SURFACES) 0 = immobile or < 50 yards 5 = wheelchair independent, including corners, > 50 yards 10 = walks with help of one person (verbal or physical) > 50 yards 15 = independent (but may use any aid; for example, stick) > 50 yards	_____
STAIRS 0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	_____
TOTAL (0-100): _____	

Appendix 10 (cont.) Barthel Index (BI)

The Barthel ADL Index: Guidelines

1. The index should be used as a record of what a patient does, not as a record of what a patient could do.
2. The main aim is to establish degree of independence from any help, physical or verbal, however minor and for whatever reason.
3. The need for supervision renders the patient not independent.
4. A patient's performance should be established using the best available evidence. Asking the patient, friends/relatives and nurses are the usual sources, but direct observation and common sense are also important. However direct testing is not needed.
5. Usually the patient's performance over the preceding 24-48 hours is important, but occasionally longer periods will be relevant.
6. Middle categories imply that the patient supplies over 50 per cent of the effort.
7. Use of aids to be independent is allowed.

Appendix 11 **Cognitive Assessments**

A. Animal Naming Test

DIRECTIONS

1. State, "I would like to name as many animals as you can think of in one minute. I will tell you when to start and stop. Do you understand the directions?"
2. Tell the patient to start naming animals. Write down the animal names below in the order stated. Any kind of animal is acceptable. Write down repeated animals, but do not score those. After one minute, tell the patient to stop.
3. Scoring: Count each unique animal listed.

List of Animals:

Score: _____

Appendix 11 (cont.) Cognitive Assessments

B. Controlled Oral Word Association Test

Instructions:

You say:

"I will say a letter of the alphabet. Then I will want you to give me as many words that begin with that letter as quickly as you can. For example, if I say the letter "B", you might say "bad", "bottle", "bed". I do not want you to use words that are proper names such as "Boston" or "Bob". Also, do not use the same word again with a different ending such as "eat" and "eating". You will have one minute. Any questions? Begin when I say the letter. Tell me all the words that you can that start with the letter "C"

Time: 1 minute

IF THE ANSWER IS VALID MARK THE BOX LIKE THIS



Appendix 11 (cont.) Cognitive Assessments

Word Association Test - Letter "C"					
Valid		Valid			
1.	<input type="text"/>	<input type="checkbox"/> C	13.	<input type="text"/>	<input type="checkbox"/> C
2.	<input type="text"/>	<input type="checkbox"/> C	14.	<input type="text"/>	<input type="checkbox"/> C
3.	<input type="text"/>	<input type="checkbox"/> C	15.	<input type="text"/>	<input type="checkbox"/> C
4.	<input type="text"/>	<input type="checkbox"/> C	16.	<input type="text"/>	<input type="checkbox"/> C
5.	<input type="text"/>	<input type="checkbox"/> C	17.	<input type="text"/>	<input type="checkbox"/> C
6.	<input type="text"/>	<input type="checkbox"/> C	18.	<input type="text"/>	<input type="checkbox"/> C
7.	<input type="text"/>	<input type="checkbox"/> C	19.	<input type="text"/>	<input type="checkbox"/> C
8.	<input type="text"/>	<input type="checkbox"/> C	20.	<input type="text"/>	<input type="checkbox"/> C
9.	<input type="text"/>	<input type="checkbox"/> C	21.	<input type="text"/>	<input type="checkbox"/> C
10.	<input type="text"/>	<input type="checkbox"/> C	22.	<input type="text"/>	<input type="checkbox"/> C
11.	<input type="text"/>	<input type="checkbox"/> C	23.	<input type="text"/>	<input type="checkbox"/> C
12.	<input type="text"/>	<input type="checkbox"/> C	24.	<input type="text"/>	<input type="checkbox"/> C
Time Ended <input type="text"/> : <input type="text"/> HH MM					

Appendix 11 (cont.) Cognitive Assessments

C. Digit Symbol Coding

DIGIT SYMBOL SUBSTITUTION (DSS) TASK

DIGIT	1	2	3	4	5	6	7	8	9
SYMBOL	-	上	口	L	U	O	^	X	=

1	5	4	2	7	6	3	5	7	2	8	5	4	6	3	7	2	8	1	9	5	8	4	7	3
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

6	2	5	1	9	2	8	3	7	4	6	5	9	4	8	3	7	2	6	1	5	4	6	3	7
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Appendix 11 (cont.) Cognitive Assessments

D. Hopkins Verbal Learning Test (Brandt 1991)

Directions: Say, Listen carefully as I read the word list and try to memorize the words." Then read the word list aloud (one word every 2 seconds). Write down the words the patient freely recalls. Repeat word list and recall twice more. Score is the number of correct words for each trial, and the total of all three trials. (Second part of this test at end of FMSE, page 9).

Part 1: Free Recall (Form 1: four-legged animals, precious stones, human dwellings)

	Trial 1	Trial 2	Trial 3
LION			
EMERALD			
HORSE			
TENT			
SAPPHIRE			
HOTEL			
CAVE			
OPAL			
TIGER			
PEARL			
COW			
HUT			

Appendix 11 (cont.) Cognitive Assessments

E. Boston Naming Test (BNT)

(Kaplan, Goodglass & Weintraub, 1983 or 2001)

METHOD USED Short Form Even pages Odd pages All pages

Words Correct

--	--

 + Words Incorrect

--	--

 = Total words tried

--	--

TOTAL SCORE (out of 60)

--	--

Appendix 11 (cont.) Cognitive Assessments

F. Forward and Backward Digit Span Test

DIGIT SPAN (FORWARD)*

Ask patient, "Repeat each group of numbers in the same order that I say them." Read numbers at rate of one each second. If patient passes the first trial, advance to the first trial of the next item. If patient fails the first trial, administer the second trial of that item. Stop when patient fails both trials of any item. Score is best performance's maximum number of digits correct.

DIGIT FORWARD	WRITE RESPONSE	CORRECT	
8-4-7		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-7-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No
9-3-4-1		<input type="checkbox"/> Yes	<input type="checkbox"/> No
8-3-9-6		<input type="checkbox"/> Yes	<input type="checkbox"/> No
5-1-6-2-9		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-6-9-2-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No
6-4-3-5-2-7		<input type="checkbox"/> Yes	<input type="checkbox"/> No
9-1-8-4-2-7		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-1-6-9-2-8-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No
2-8-1-4-9-7-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No

DIGIT FORWARD SCORE (3-7)

DIGIT SPAN (BACKWARD)*

Say to patient, "Now I am going to say some more numbers, but this time when I stop, I want you to say them backwards. For example, if I said 7-1-9, what would you say?" Read numbers at rate of one each second. If patient passes the first trial, advance to the first trial of the next item. If patient fails the first trial, administer the second trial of that item. Stop when patient fails both trials of any item. Score is best performance's maximum number of digits correct.

DIGIT FORWARD	WRITE RESPONSE	CORRECT	
5-8		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-1		<input type="checkbox"/> Yes	<input type="checkbox"/> No
6-2-9		<input type="checkbox"/> Yes	<input type="checkbox"/> No
4-9-3		<input type="checkbox"/> Yes	<input type="checkbox"/> No
4-9-6-8		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-8-1-4		<input type="checkbox"/> Yes	<input type="checkbox"/> No
1-5-2-8-6		<input type="checkbox"/> Yes	<input type="checkbox"/> No
6-2-9-7-2		<input type="checkbox"/> Yes	<input type="checkbox"/> No
5-3-9-4-1-8		<input type="checkbox"/> Yes	<input type="checkbox"/> No
7-1-5-2-8-6		<input type="checkbox"/> Yes	<input type="checkbox"/> No

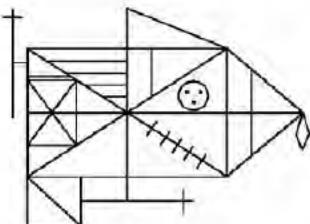
DIGIT BACKWARD SCORE (2-6)

*Wechsler, D. (1987). Wechsler Memory Scale-Revised Manual. San Antonio, TX: Psychological Corporation.

Appendix 11 (cont.) Cognitive Assessments

G. Rey-Osterrieth Complex Figure Test

Rey-Osterrieth Complex Figure Score Sheet
(Adapted from Osterrieth, 1944)



Copy Trial

- ____ 1. Cross upper left corner, outside of rectangle
- ____ 2. Large rectangle
- ____ 3. Diagonal cross
- ____ 4. Horizontal midline of (2)
- ____ 5. Vertical midline
- ____ 6. Small rectangle, within (2) to the left
- ____ 7. Small segment above (6)
- ____ 8. Four parallel lines within (2), upper left
- ____ 9. Triangle above (2), upper right
- ____ 10. Small vertical line within (2), below (9)
- ____ 11. Circle with three dots, within (2)
- ____ 12. Five parallel lines within (2) and crossing (3), lower right
- ____ 13. Sides of triangle attached to (2) on right
- ____ 14. Diamond attached to (13)
- ____ 15. Vertical line within triangle (13), parallel to the right side of (2)
- ____ 16. Horizontal line within (13), continuing (4) to the right
- ____ 17. Cross attached to lower center
- ____ 18. Square attached to (2), lower left

____ TOTAL SCORE (____ %ile)

____ TIME

minute Recall Trial

- 1. ____
- 2. ____
- 3. ____
- 4. ____
- 5. ____
- 6. ____
- 7. ____
- 8. ____
- 9. ____
- 10. ____
- 11. ____
- 12. ____
- 13. ____
- 14. ____
- 15. ____
- 16. ____
- 17. ____
- 18. ____

(____ %ile) TOTAL SCORE ____

TIME ____

SCORING: Consider each of the 18 units separately. Appraise accuracy of each unit and relative position within the whole of the design. For each unit count as follows:

Correct

Placed properly	2 points
Placed poorly	1 point

Distorted or incomplete but recognizable

Placed properly	1 point
Placed poorly	1/2 point

Absent or not recognizable 0 points

Percentile Norms for Accuracy Scores Obtained by Adults on the Copy Trial of the Complex Figure Test

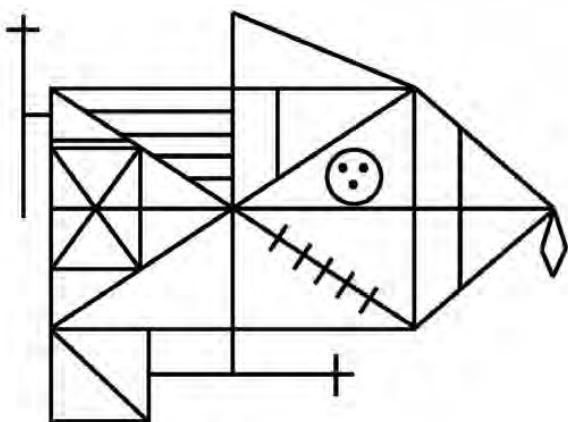
Percentile	10	20	30	40	50	60	70	80	90	100
Score	29	30	31	32	32	33	34	34	35	36

Percentile Norms for Accuracy Scores Obtained by Adults on Memory Trials of the Complex Figure Test

Percentile	10	20	30	40	50	60	70	80	90	100
Score	15	17	19	21	22	24	26	27	29	31

Appendix 11 (cont.) Cognitive Assessments

Please draw the figure below on the blank half-sheet that was given to you:



Appendix 12

10 Meter Walk Test

Description: The 10 meter walk test (10mWT) is a measure of walking speed.

Equipment: Utilize a quiet hallway or open space at least 14 meters long, digital stopwatch, clipboard, tape measure, and tape to mark standardized course layout, and 2 chairs.

Bracing and Assistive Device: The subject should be wearing comfortable flat shoes or shoes with a heel less than 1/2 inch. Have the subject perform this test with their most often used assistive device and orthotics. Note the specific type of assistive device and orthotics utilized on the source document.

Physical Assistance Provided During Testing:

Physical assistance may be provided as needed to maintain safety and stability. If additional assistance is needed, a non-blinded member of the investigation team can provide the additional assistance. No assistance can be provided for paretic limb advancement. A gait belt may be used during the test. The amount of assistance provided will be recorded with assignment of a Functional Ambulation Category (FAC). See FAC for description of procedure.

Test Administration:

Course Layout: The walking Course is established indoors on a level, non-carpeted surface measuring a length of 14 meters. The course is marked with tape at 0 meters, 2 meters, 12 meters, and 14 meters. 2 chairs can be placed near the 0 meter and 14 meter mark.



1. Instruct the subject in the goal of the 10 meter walk test while the subject is seated:
“You are going to walk a distance of about 40 feet. We will repeat this distance four times. The first two times will be completed at your comfortable pace, the final two times you will walk as quickly and safely as you can. Do you have any questions?”
2. Demonstrate where you want the subject to walk.
3. Walk the subject to the start line (0 meters). Provide the subject with the following instructions:
“You will walk at a comfortable pace to the chair.” (DO NOT refer to the tape on the floor.) The start command is **“Ready and Go”**.
4. When you and the subject are ready, say **“Ready and Go”**. If the subject starts too early, start again.

Appendix 12 (cont.) 10 Meter Walk Test

5. Instructions for Recording Time:
 - START** the stopwatch when the subject's first foot crosses the plane of the 2 meter line
 - STOP** the stopwatch when the subject's first foot crosses the plane of the 12 meter line.
 - Have the subject continue walking until he/she reaches the chair after the 14 meter line.
 - Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance
“Comfortable” Pace Trial 1 line on the 10 Meter Walk Test Source Document.
 - The subject can rest, if needed, in the chair near the 14 meter line.
6. When the subject is ready, have them repeat the SAME PROCEDURE except they will start from the 14 meter line and end at 0 meter line.
 - START** the stopwatch when the subject's first foot crosses the plane of the 12 meter line
 - STOP** the stopwatch when the subject's first foot crosses the plane of the 2 meter line.
 - Have the subject continue walking until he/she reaches the chair after the 0 meter line.
 - Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance on the “Comfortable” Pace Trial 2 line on the 10 Meter Walk Test Source Document.
 - The subject can rest, if needed, in the chair near the 0 meter line.
7. Subject Instructions for the last 2 trials:

“You are going to walk the same distances for two additional trials as quickly and as safely as you can. Do you have any questions?”
8. When the subject is ready, walk the subject to the start line (0 meters). Provide the subject with the following instructions:

“You will walk as quickly and as safely as you can to the chair.” (Use an appropriate descriptor for chair/location, but DO NOT refer to the tape on the floor.)
The start command is “Ready and Go”.
9. When you and the subject are ready, say “Ready and Go”. If the subject starts too early, have them start again.

Appendix 12 (cont.) 10 Meter Walk Test

10. Instructions for Recording Time:

- START** the stopwatch when the subject's first foot crosses the plane of the 2 meter line
- STOP** the stopwatch when the subject's first foot crosses the plane of the 12 meter line.
- Have the subject continue walking until he/she reaches the chair after the 14 meter line.
- Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance on the "As Fast As Possible" Pace Trial 1 line on the 10 Meter Walk Test Source Document.
- The subject can rest, if needed, in the chair near the 14 meter line.

11. When the subject is ready, have them repeat the SAME PROCEDURE except they will start from the 14 meter line and end at 0 meter line.

- START the stopwatch when the subject's first foot crosses the plane of the 12 meter line
- STOP the stopwatch when the subject's first foot crosses the plane of the 2 meter line.
- Have the subject continue walking until he/she reaches the chair after the 0 meter line.
- Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance on the "As Fast As Possible" Pace Trial 2 line on the 10 Meter Walk Test Source Document.
- The subject can rest, if needed, in the chair near the 0 meter line.

12. Record the assistive device used and the Functional Ambulation Category (FAC) on the 10 Meter Walk Test Source Document.

REFERENCES:

Duncan PW, Sullivan KJ, Behrman AL, et al. Protocol for the Locomotor Experience Applied Post-stroke (LEAPS) trial: A randomized controlled trial. *BMC Neurology* 2007;7(1):39.

Perry J, Garrett M, Gronley JK, et al. Classification of walking handicap in the stroke population. *Stroke* 1995, 26(6):982-89.

Richards CL, Malouin F, Wood-Dauphinee S, et al. Gait velocity as an outcome measure of locomotor recovery after stroke. In: Craik RA, Oatis C, eds. *Gait Analysis: Theory and Applications*. St Louis, Mo: Mosby; 1995:355—64.

Appendix 13

Preferred Terms for Intracranial Hemorrhage

An Intracranial Hemorrhage (ICH) is considered symptomatic if it is not seen on computed tomography (CT) or magnetic resonance imaging (MRI) scan at baseline and any neurologic decline is attributed to it by the local investigator. Below is the list of preferred terms (PT's) using the MedDRA term to describe the ICH event:

- Basal ganglia hemorrhage
- Brain stem hematoma
- Brain stem hemorrhage
- Brain stem microhemorrhage
- Central nervous system hemorrhage
- Cerebellar hematoma
- Cerebellar hemorrhage
- Cerebellar microhemorrhage
- Cerebral arteriovenous malformation hemorrhagic
- Cerebral hematoma
- Cerebral hemorrhage
- Cerebral microhemorrhage
- Extradural hematoma
- Hemorrhage intracranial
- Hemorrhagic cerebral infarction
- Hemorrhagic stroke
- Hemorrhagic transformation stroke
- Intracranial hematoma
- Intraventricular hemorrhage
- Meningorrhagia
- Pituitary hemorrhage
- Purpura cerebri
- Putamen hemorrhage
- Subarachnoid hemorrhage
- Subdural hematoma
- Subdural hemorrhage
- Thalamus hemorrhage
- Traumatic intracranial hemorrhage

Appendix 14
WHO Toxicity Grading Scale for Determining the Severity of
Adverse Events

HEMATOLOGY				
ITEM	Grade 1 Toxicity	Grade 2 Toxicity	Grade 3 Toxicity	Grade 4 Toxicity
Hemoglobin	9.5 - 10.5 gm/Dl	8.0 - 9.4 gm/Dl	6.5 - 7.9 gm/Dl	< 6.5 gm/Dl
Absolute Neutrophil Count	1000-1500/mm ³	750-999/mm ³	500-749/mm ³	<500/mm ³
Platelets	75000-99000/mm ³	50000-74999/mm ³	20000-49000/mm ³	<20000/mm ³
Prothrombin Time (PT)	1.01 - 1.25 x ULN	1.26-1.5 x ULN	1.51 -3.0 x ULN	>3 x ULN
Activated Partial Thromboplastin (APPT)	1.01 -1.66 x ULN	1.67 - 2.33 x ULN	2.34 - 3 x ULN	> 3 x ULN
Fibrinogen	0.75 - 0.99 X LLN	0.50 - 0.74 x LLN	0.25 - 0.49 x LLN	< 0.25 x LLN
Fibrin Split Product	20-40 mcg/ml	41-50 mcg/ml	51-60 mcg/ml	> 60 mcg/ml
Methemoglobin	5 - 9.9 %	10.0 - 14.9 %	15.0 - 19.9%	> 20 %
LIVER ENZYMES				
AST (SGOT)	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
ALT (SGPT)	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
GGT	1.25 -2.5 x ULN	1.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Alkaline Phosphatase	1.25 - 2. 5 x ULN	1.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN
CHEMISTRIES				
Hyponatremia	130-135 mEq/L	123-129 mEq/L	116-122 mEq/L	< 116 or mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	158-165 mEq/L	> 165 mEq/L or mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	2.0 - 2.4 mEq/L or intensive replacement Rx required or hospitalization required.	<2.0 mEq/L or paresis or ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	6.6 - 7.0 mEq/l	> 7.0 mEq/L or life-threatening arrhythmia
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or mental status changes or coma

Appendix 14 (cont.)
WHO Toxicity Grading Scale for Determining the Severity of
Adverse Events

CHEMISTRIES (continued)				
Hyperglycemia (note if fasting)	116 - 160 mg/dL	161-250 mg/dL	251 - 500 mg/dL	> 500 mg/dL or ketacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	6.9 - 6.1 mg/dL	< 6.1 mg/dL or life threatening arrhythmia or tetany
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	12.6 - 13.5 mg/dL	> 13.5 mg/dL life-threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/L	1.1 - 0.9 mEq/L	0.8 - 0.6 mEq/L	< 0.6 mEq/L or life- threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 - 1.9 mg/dL or replacement Rx required	1.0 - 1.4 mg/dL intensive Rx or hospitalization required	< 1.0 mg/dL or life- threatening arrhythmia
Hyperbilirubinemia	1.1 - 1.5 x ULN	1.6 - 2.5 x ULN	2.6 - 5 x ULN	> 5 x ULN
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Creatinine	1.1 x 1.5 x ULN	1.6 - 3.0 x ULN	3.1 - 6 x ULN	> 6 x ULN or required dialysis
URINALYSIS				
Proteinuria	1+ or <0.3% or <3g/L or 200 mg - 1 gm loss/day	2-3+ or 0.3 - 1.0% or 3-10 g/L 1- 2 gm loss/day	4+ or > 1.0% or > 10 g/L 2-3.5 gm loss/day	nephrotic syndrome or > 3.5 gm loss/day
Hematuria	microscopic only	gross, no clots	gross* clots	obstructive or required transfusion
CARDIAC DYSFUNCTION				
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; No Rx required	requires treatment
Hypertension	transient inc. > 20 mm, no Rx	recurrent, chronic, > 20 mm, Rx required	requires acute Rx; No hospitalization	requires hospitalization
Hypotension	transient orthostatic hypotension, No Rx	symptoms correctable with oral fluids Rx	requires IV fluids; no hospitalization required	requires hospitalization
Pericarditis	minimal effusion	mild/moderate asymptomatic effusion, no Rx	symptomatic effusion; pain, EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; > 3 units transfused

Appendix 14 (cont.)
WHO Toxicity Grading Scale for Determining the Severity of
Adverse Events

RESPIRATORY				
Cough	transient- no Rx	treatment associated cough local Rx	uncontrolled	
Bronchospasm, Acute	transient; no Rx < 80% - 70% FEV ₁ (or peak flow)	requires Rx normalizes with bronchodilator; FEV ₁ 25% - 50% (or peak flow retractions)	no normalization with bronchodilator; FEV ₁ 25% - 50% (or peak flow retractions)	cyanosis: FEV ₁ <25% (or peak flow) or intubated
GASTROINTESTINAL				
Stomatitis	mild discomfort; no limits on activity	some limits on eating/drinking	eating/talking very limited	requires IV fluids
Nausea	mild discomfort; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	severe discomfort; no significant intake; activities limited	minimal fluid intake
Vomiting	transient emesis	occasional/moderate vomiting	orthostatic hypotension or IV fluids required	hypotensive shock or hospitalization required for IV fluid therapy
Constipation	mild	moderate	severe	distensions w/vomiting
Diarrhea	transient 3-4 loose stools/day	5-7 loose stools/day	orthostatic hypotension or > 7 loose stools/day or required IV fluids	hypotensive shock or hospitalization for IV fluid therapy required
NEURO & NEUROMUSCULAR				
Neuro-Cerebellar	slight incoordination dysdiadochokinesia	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Mood	mild anxiety or depression	moderate anxiety or depression and therapy required	severe anxiety or depression or mania; needs assistance	acute psychosis; incapacitated, requires hospitalization
Neuro Control (ADL = activities of daily living)	mild difficulty concentrating; no Rx; mild confusion/agitation; ADL unaffected	moderate confusion/agitation; some limitation of ADL; minimal Rx	severe confusion/agitation needs assistance for ADL; therapy required	toxic psychosis; hospitalization
Muscle Strength	subjective weakness no objective symptoms/signs	mild objective signs/symptoms no decrease in function	objective weakness function limited	paralysis

Appendix 14 (cont.)
WHO Toxicity Grading Scale for Determining the Severity of
Adverse Events

OTHER PARAMETERS				
Fever: oral, \geq 12 hours	37.7 – 38.5 C or 100.0 – 101.5 F	38.6 – 39.5 C or 101.6 – 102.9 F	39.6 – 40.5 C or 103 – 105 F	\geq 40 C or \geq 105 F
Headache	mild, no Rx therapy	transient, moderate; Rx required	severe; responds to initial narcotic therapy	intractable; required repeated narcotic therapy
Fatigue	no decrease in ADL	normal activity decreased 25- 50%	normal activity decreased $>$ 50%; can't work	unable to care for self
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Local Reaction	tenderness or erythema	induration \leq 10 cm or phlebitis or inflammation	induration $>$ 10 cm or ulceration	necrosis
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation, moist desquamation, or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery

PROTOCOL

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: 2

EUDRACT NUMBER: Not applicable

IND NUMBER: 3811

TEST PRODUCT: Alteplase (RO5532960)

LEAD PRINCIPAL INVESTIGATOR: [REDACTED], M.D.

MEDICAL MONITOR: [REDACTED], M.D.

SPONSOR: Genentech, Inc.

DATE FINAL: Version 1: 27 November 2013

DATE AMENDED: Version 2: See electronic date stamp below

PROTOCOL APPROVAL

Approver's Name	Title	Date and Time (UTC)
[REDACTED]	[REDACTED]	22-Aug-2014 22:07:43

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PROTOCOL AMENDMENT, VERSION 2: RATIONALE

Protocol ML29093 has been amended to provide updates to the secondary and exploratory objectives. This included removing the following endpoints from the secondary objectives: neurological outcome (as measured by the National Institutes of Health Stroke Scale [NIHSS] 0–1), Barthel Index (BI) ≥ 95 , and Glasgow Outcome Scale (GOS)=1. The following assessments were added to the exploratory objectives: the NIHSS at Day 90, BI at Day 90, and the GOS at Day 90. The cognitive and behavioral assessments also included in the exploratory outcome measures were specified in more detail. Additionally, the Rey-Osterreith Complex Figure test was removed and the Benton Judgment of Line Orientation test, form V, was added.

Additional changes to the protocol are as follows:

- Information was added to clarify that IV alteplase and alteplase placebo may not be initiated beyond 3 hours of last known well time under any circumstances.
- Instructions were added to allow sites to administer oral aspirin/aspirin placebo up to 24 hours after last known well time (instead of within 3 hours of last known well time) if a site has institutional policies on oral medications that make it challenging to administer oral study drug within 3 hours of last known well time.
- Changes were made to ensure that the language “not clearly disabling” is consistent throughout the protocol.
- A change was made to the adverse event (AE)/serious adverse event (SAE) collection time from screening/baseline to study drug administration.
- The non-serious AE collection period was reduced to 30 days (with the exception of intracranial hemorrhage, adverse events of special interest (AESIs), and AEs leading to study withdrawal) to better align with other acute stroke studies involving alteplase and to reduce the burden of AE collection at the Day 90 visit in order to minimize missing data.
- The following exclusion criteria were updated to include:
 - 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
 - Treatment with a direct thrombin inhibitor or factor Xa inhibitor (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

- Computed tomography (CT) or magnetic resonance imaging (MRI) findings consisting of one of the following:
 - Clear large hypodensity on CT (or concurrent diffusion-weighted and fluid attenuated inversion recovery hyperintensity on MRI) that is greater than one-third middle cerebral artery (MCA) territory or greater than 100 cc if not in MCA territory

Additional minor changes have been made to improve clarity and consistency.
Substantive new information appears in *italics*. This amendment represents cumulative changes to the original protocol.

PROTOCOL AMENDMENT, VERSION 2: SUMMARY OF CHANGES

GLOBAL CHANGES

Changes were made to ensure that the language “not clearly disabling” is consistent throughout the protocol. Interactive Voice/Web Response System (IxRS) was changed to Interactive Web Response System (IWRS) throughout the protocol. All references to the Alteplase Investigator’s Brochure were changed to the Alteplase U.S. Package Insert. Additionally, all mentions of study sites in the U.S. and Canada were revised to “North America.”

PROTOCOL SYNOPSIS

The protocol synopsis has been updated to reflect the changes to the protocol, where applicable.

SECTION 1.1: BACKGROUND ON ACUTE ISCHEMIC STROKE

For example, the proportion of mild stroke patients (NIHSS ≤ 3) treated with reperfusion therapies...

TABLE 1: Comparison of PRISMS and NINDS Studies

Table 1, footnote a, has been revised to read: LHH is the likelihood of being helped versus harmed and is ~~formulically~~ the ratio between the NNT and the NNH. ...

SECTION 2.2: SECONDARY OBJECTIVES

- To further evaluate efficacy of IV alteplase to improve mild stroke outcomes at Day 90 via:
 - Ordinal improvement in mRS
 - ~~Neurological outcome (as measured by NIHSS 0–1)~~
 - ~~Independence in instrumental activities of daily (Barthel Index [BI] ≥ 95)~~
 - ~~Glasgow Outcome Scale (GOS) 0–1~~
 - Global favorable recovery (Global ~~Statistic with contributions~~ 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:

Stroke-related and neurological deaths within 90 days

SECTION 2.3: EXPLORATORY OBJECTIVES

The exploratory objectives for this study include subgroup analyses of the primary efficacy outcome variable ~~in subgroups as~~ 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 (RISS vs. non-RISS), respectively.

The following endpoints will also be explored:

- NIHSS
- BI
- GOS
- Cognition *(and behavior (modified 30-minute assessment: animal naming, 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, Hopkins Verbal Learning Test, 15 item Boston Naming Test, III [WAIS III]; Forward and Backward Digit Span test, and Rey-Osterreith Complex Figure; 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]) at Day 90*
- Center for Epidemiologic Studies-Depression (CES-D) score at Day 90

SECTION 3.1: 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 ~~stroke and with deficits~~ *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.*

~~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 within 3 hours of last known well time or 2) one dose of IV alteplase placebo and one dose of oral aspirin 325 mg within 3 hours of last known well time (Figure 1):~~

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 (Figure 1).*

Every effort should be made to ensure both study drugs are initiated within 3 hours of last known well time. If institutional policies on oral medications make it challenging to administer the oral aspirin/aspirin placebo within 3 hours of last known well time, the oral study drug may be given up to 24 hours after the last known well time. IV alteplase/IV alteplase placebo MAY NOT be initiated beyond 3 hours of last known well time under any circumstances.

SECTION 3.3.1: Number of Patients

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

SECTION 3.4: RATIONALE FOR STUDY DESIGN

This study is designed to assess whether alteplase *administered within 3 hours of last known well time* is superior to standard of care (i.e., aspirin) in patients with mild stroke *within 3 hours of last known well time*.

The study design of PRISMS has the following characteristics:

- **Conduct of a large, multicenter study:** The Sponsor plans to enroll 948 patients across approximately 75 sites in the U.S. and Canada North America. (For comparison, the two NINDS trials enrolled a total of 624 patients.)
- **Multiple endpoints involving different events:** In addition to the primary efficacy endpoint of mRS at Day 90, which measures disability, secondary endpoints at Day 90 will include the NIHSS score (neurologic deficit), BI (instrumental activities of daily living), GOS (disability), and a Global Statistic calculation an ordinal mRS assessment and global favorable recovery, as calculated using the Global Outcome Measure derived from the mRS, NIHSS, BI, and GOS.

SECTION 3.4.1: Rationale for Alteplase Dosage

The dose selected for alteplase (0.9 mg/kg, maximum 90 mg) is the approved dose for eligible patients with AIS in the U.S. and Canada North America and is considered the standard of care by the stroke community (Jauch et al. 2013).

SECTION 3.4.2: Rationale for Patient Population

The patients for this trial will be recruited from all AIS patients presenting to Emergency Departments in whom treatment can be initiated within 3 hours of last known well time at approximately 75 clinical sites within the U.S. and Canada North America. ...

To date, available data suggest that alteplase therapy would potentially be beneficial in both patients with RISS who improve to an NIHSS score ≤ 5 and patients with persistently mild symptoms deficits with an NIHSS score ≤ 5 .

PRISMS will also exclude patients who are assessed at the time of pre-treatment to likely be clearly disabled at 90 days, as defined by specific criteria described in the study protocol. These clearly disabling deficits at presentation may include, but are not limited to, homonymous hemianopsia, severe limb weakness, severe aphasia, hemineglect, or cortical blindness. Patients with clearly disabling deficits will be excluded from this study irrespective of the NIHSS score at time of randomization, as current clinical practice and ethical responsibility suggest that these patients should be evaluated to receive alteplase rather than be enrolled in a randomized study where they may receive alteplase placebo.

SECTION 3.5.1: Efficacy Outcome Measures

The secondary efficacy outcomes are measured at Day 90 and listed below:

- Ordinal improvement in mRS
- Neurological outcome (as measured by NIHSS 0–1)

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- ~~Instrumental activities of daily living, as measured by the BI ≥ 95~~
- ~~GOS 0–4~~
- Global ~~outcome~~^{favorable recovery} (Global ~~Statistic with contributions~~^{Outcome} Measure derived from mRS 0–1, NIHSS 0–1, BI ≥ 95 , and GOS 0–1)

SECTION 3.5.2: Safety Outcome Measures

- Incidence of sICH within 36 hours (*primary safety assessment*)

sICH is defined as any neurological decline attributed to new ICH seen on imaging by the investigator ~~and~~. New ICH seen on imaging will be later confirmed on independent reading by blinded central study radiologists.
- Stroke-related and neurological deaths *within 90 days*

Symptomatic Intracranial Hemorrhage

To detect ~~asymptomatic~~ intracranial hemorrhage, ~~CT~~^{neuroimaging} (CT or MRI) scan is required at 22 to 36 hours. Additional ~~CT~~^{scans}^{neuroimaging} should be performed at any time based on the investigator's discretion that clinical findings suggested hemorrhage. ~~An MRI may be substituted for CT per institutional protocol.~~

Intracranial hemorrhage events will be classified as ~~include classifications of intracerebral hemorrhage (hemorrhagic infarct type 1 or type 2, parenchymal hematoma type 1 or type 2, or remote intraparenchymal hemorrhage type 1 or type 2).~~ Descriptions of each type of intracerebral hemorrhage are shown in the table below. Additionally, subarachnoid hemorrhage, subdural hemorrhage, or epidural hemorrhage. Descriptions of each are shown in the table below. should be noted.

TABLE 3: Intracranial Intracerebral Hemorrhage Definitions

The Table 3 title has been revised.

SECTION 3.5.3: Exploratory Outcome Measures

- NIHSS at Day 90
- *Instrumental activities of daily living, as measured by the BI at Day 90*
- GOS at Day 90
- Cognition ~~(and behavior (modified 30-minute assessment: animal naming, 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, Hopkins Verbal Learning Test, 15-item Boston Naming Test, III [WAIS III]; Forward and Backward Digit Span test, and Rey-Osterreith Complex Figure; 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]) at Day 90~~

SECTION 4.1.2: Exclusion Criteria

- CT or MRI findings consisting of one of the following:

- *CT with clear large hypodensity on CT (or 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) hyperintensity on MRI) that is greater than one-third MCA territory (or greater than 100 cc if not in MCA territory),*
- Disability (~~historical mRS score ≥ 2~~) prior to the presenting stroke (*historical mRS score ≥ 2*)
- Standard contraindications to IV alteplase for patients treated within 3 hours of symptom onset, including:
 - Treatment with *unfractionated heparin* within the last 48 hours and activated partial thromboplastin time (aPTT) outside of the normal range as specified by the center's local laboratory
 - Treatment with a direct thrombin inhibitor or factor Xa inhibitor (*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*

SECTION 4.2: METHOD OF TREATMENT ASSIGNMENT AND BLINDING

Pre-mixing may occur prior to signing informed consent but should be performed after key eligibility criteria (e.g., NIHSS≤5 and *not* clearly non-disabling, study drug initiation will occur within 3 hours of time last known well, CT/MRI negative for ICH) to minimize drug wastage.

2. IWRS will assign a new *pair* of drug kit IDs for the next patient that becomes eligible at that site to be labeled as "Use Next."

In the case when pre-mixed *IV* drug is not used (e.g., patient is determined to be ineligible for study or declines informed consent prior to drug administration), sites will enter drug kit IDs into IWRS and indicate that the kits were not used. ... The pharmacist will properly dispose of the pre-mixed *IV* drug and the matching aspirin/aspirin-placebo kit in accordance with institutional guidelines.

Study Unblinding

~~If premature unblinding is necessary in the case of an SAE for which patient management might be affected by knowledge of treatment assignment, the investigator will be able to break the treatment code by contacting the IxRS vendor.~~

~~Specifically, in the rare event that a patient worsens (e.g., to a moderate or severe stroke) from time of study treatment and the patient remains within a time window within institutional guidelines to initiate thrombolytic therapy, the investigator has the option to~~

~~unblind the treatment to ensure the patient receives appropriate treatment. However, the following criteria must be met prior to unblinding:~~

1. ~~Patient must have an NIHSS score ≥ 6 (or new clearly disabling deficit) measured twice within a 10 minute span.~~
2. ~~Patient's NIHSS increase must be ≥ 2 points from the pre treatment NIHSS (e.g., if a patient's pre treatment NIHSS was 5, the worsened NIHSS must be ≥ 7).~~

If a clinical situation arises in which the investigator thinks it is necessary to unblind in the interest of patient safety, the Medical Monitor must be notified immediately to discuss the intended unblinding. Unblinding should occur only in settings where identification of the study treatment is critical for treatment decisions. IWRS will be used for the unblinding process. All unblinded patients will remain in the study and require all follow-up visits.

If the investigator wishes to know the identity of the study drug for any other reason, he or she ~~should~~must contact the Medical Monitor ~~directly~~. ... The investigator should document and provide an explanation for any premature unblinding (e.g., unblinding due to ~~stroke~~, ~~unblinding due to~~ or severe systemic bleeding *that requires blood products if IV alteplase was administered, or unblinding due to clinical worsening*). ~~Following any unblinding event, during the site investigator should contact the Medical Monitor within 24 hours~~. *IV alteplase time window that would lead to IV alteplase administration if not already given*).

SECTION 4.3.2.1: Alteplase

After reconstitution, alteplase ~~may~~should be administered at 1 mg/mL. ~~Do not~~ As an alternative, the reconstituted solution ~~may~~ be diluted further ~~dilute~~ (e.g., ≤ 1 immediately before administration in an equal volume of 0.9% sodium chloride injection, USP, or 5% dextrose injection, USP, to yield a concentration of 0.5 mg/mL) ~~the solution~~.

~~Each 50 mg vial of Activase contains 50 mg of alteplase, 1.7 g of L-arginine, 5 mg of polysorbate 80, and 0.5 g of phosphoric acid.~~

SECTION 4.3.4.2: 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.

SECTION 4.3.5: Post-Trial Access to Alteplase

The Sponsor does not intend to provide alteplase, any investigational or non-investigational products (specified in this protocol), or any other study interventions to patients after the conclusion of the study or *to patients* who are withdrawn earlier.

SECTION 4.4.2: Prohibited Therapy

As indicated in the exclusion criteria, use of the following therapies is prohibited prior to the study:

- Treatment with *unfractionated heparin* within the last 48 hours and aPTT outside of the normal range as specified by the center's local laboratory
- Treatment with a direct thrombin inhibitor or factor Xa inhibitor (*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*
- Treatment with warfarin and INR > 1.7 (Note: INR does not need to be verified prior to randomization if there is no clinical suspicion of abnormality.)

SECTION 4.5.1.1: Informed Consent Forms and Screening Log

The investigator will maintain a screening log of all patients arriving in the Emergency Department within 2.5 hours of onset of ischemic stroke: *who have an initial NIHSS score of ≤5.*

SECTION 4.5.1.4: Vital Signs

Vital signs will include measurements of ~~oral~~ temperature, ~~respiratory rate~~, pulse, and systolic/diastolic blood pressure (while patient is in a supine position).

SECTION 4.5.1.5: Laboratory Assessments

Specimens for the following laboratory tests will be sent to the study site's local laboratory for analysis:

- ~~Platelet~~ Complete blood count *without differential*
- Coagulation (INR, aPTT) *if a patient is currently taking oral or parenteral anticoagulants*
- Pregnancy test (serum or urine) *for women of childbearing potential (including those who have had a tubal ligation) must have a serum pregnancy test at screening.*

The following laboratory test values will be reported in the eCRFs:

- ~~Hematology (hemoglobin, hematocrit, white~~ Complete blood cell count, ~~platelet count~~ *without differential*
- Coagulation values (INR, ~~partial thromboplastin time~~) aPTT
- Pregnancy test *for women of childbearing potential including those who have had a tubal ligation*

SECTION 4.5.1.6: Other Disease-Specific Assessments

An additional non-contrast CT or MRI will be performed at 22 to 36 hours from the initiation (*bolus*) of study drug infusion.

CT scans or MRIs performed *at baseline*, between 22 to 36 hours, and additional imaging performed due to clinical suspicion of deterioration up until 36 hours after study drug administration, will be evaluated by a central team of radiologists blinded to the clinical attributes of the case. *These images will need to be sent to the central radiologists by the local site.*

SECTION 4.5.2: Patient-Reported Outcomes

The EQ-5D, SIS-16, and CES-D will be provided in Spanish for those patients requiring translations.

SECTION 4.6.1: Screening/Baseline (Visit 1)

- Complete blood count *without differential*
- AEs

All screening evaluations with the exception of certain laboratory results (e.g., coagulation and ~~platelet~~ *complete blood count*) must be completed and reviewed to confirm that patients have met all eligibility criteria prior to treatment.

SECTION 4.6.2: Visit 2 (22 to 36 Hours after Treatment Initiation)

A non-contrast cranial CT or MRI (including DW MRI, either SW or GRE, FLAIR, and T1 and T2 sequences) will be performed at 22 to 36 hours after treatment with study drug has been initiated- (*bolus*).

AEs¹ and concomitant medications will also be assessed at this visit.

¹AE/SAE collection should be started as soon as study drug administration (*bolus*) is initiated.

SECTION 4.6.3: Visit 3 (Day 5 or Discharge from Hospital, if Sooner than Day 5)

An NIHSS score will be performed ~~at On Day 5 (or on the day of discharge from the hospital, if sooner than Day 5. Furthermore, the)~~, NIHSS score, stroke etiology, AEs, and concomitant medications will be collected. Discharge location ~~of the patient~~ will be recorded ~~if available~~ collected at ~~this~~ the time. If discharged after Day 5, the discharge location will be subsequently recorded. Concomitant medications will also be assessed ~~at this visit of hospital discharge~~.

SECTION 4.6.4: Visit 4 (Day 30 [30±7 Days])

AEs, concomitant medications, and patient survival will be assessed ~~at~~ *during this phone visit*.

SECTION 4.6.5: Visit 5 (Day 90 [±14 Days])/Study Completion)

Primary and secondary efficacy endpoints, as well as all exploratory endpoints, will be assessed at Day 90 ~~during an in person visit by~~ *with* the patient.

- Cognitive assessments (Appendix 11)
 - *Animal Naming test*

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- *HVLT-R trials 1, 2, and 3*
- ~~Hopkins Verbal Learning test~~
- ~~15 item Boston Naming test~~
- ~~Rey-Osterrieth Complex Figure test~~
- *Benton Judgment of Line Orientation test, form V*
- *HVLT-R trial 4 and recognition*
- *Animal Naming test*
- *BNT 15-item short form*

The above cognitive assessments will be provided in Spanish for those patients requiring translations with the exception of the BNT 15-item short form.

- ~~AEs~~
- *SAEs and certain AEs (See Section 5.3.1)*

SECTION 4.7.2: Study Treatment Discontinuation

If ICH is seen, study drug infusion should not be resumed and the treatment assignment ~~should~~*may* be unblinded *if blood products (to reverse the biological activity of IV alteplase) are indicated*. If ICH is not seen, study drug infusion should immediately be restarted *up to 5.5 hours after the last known well time at the same rate as the previous infusion until the remainder of study drug is infused*.

Patients who discontinue study treatment prematurely will continue in the study and be followed ~~for the course of the study~~ through Day 90, based on the intent-to-treat (ITT) principle.

SECTION 5.2.1: Adverse Events

An AE can therefore be any of the following:

- ~~AEs that are related to a protocol mandated intervention, including those that occur prior to assignment of study treatment (e.g., screening invasive procedures such as biopsies)~~

SECTION 5.2.3: Non-Serious Adverse Events of Special Interest (Immediately Reportable to the Sponsor)

AESIs for this study include the following:

- ~~All ICH events, or SICH (use if not already reported as an SAE (for a list of preferred terms for ICH see Appendix 13)~~
- *Stroke recurrence: report if the AE resulted in a substantial disruption of a person's ability to conduct normal life functions (i.e., the AE resulted in a significant, persistent, or permanent change; impairment; or damage or disruption in the patient's body function/structure, physical activities, and/or quality of life.*

SECTION 5.3.1: Adverse Event Reporting Period

~~After informed consent has been obtained, but prior to initiation of study drug, only SAEs caused by a protocol mandated intervention should be reported.~~

After initiation of study drug, all AEs, (including serious and non-serious AEs), regardless of relationship to study drug, will be reported until 30 days after administration of study drug.

After 30 days until 90 days after administration of study drug—, the following AEs should be record on the AE CRF:

- All SAEs
- Non-serious ICHs
- Non-serious AESIs
- All AEs that resulted in withdrawal from the study

All SAEs and non-serious AESIs require immediate reporting to the Sponsor (see Section 5.4).

~~After this period 90 days, the investigator is not required to actively monitor patients for adverse events AEs; however, the Sponsor should be notified if the investigator becomes aware of any post-study serious adverse events or non-serious adverse events of special interest study SAEs (see Section 5.6).~~

SECTION 5.3.3.8: Lack of Efficacy or Worsening of Acute Ischemic Stroke

Medical occurrences or symptoms of deterioration that are considered part of the disease course or disease process should be recorded as an AE if judged by the investigator to have unexpectedly worsened in severity or frequency or unexpectedly changed in nature at any time during the study. If there is uncertainty, it should be reported as an AE. When recording unexpected worsening of AIS on the Adverse Event eCRF, it is important to convey the concept that the condition has changed by including applicable descriptors of the event. Medical occurrences or symptoms of deterioration that are anticipated as part of AIS should be recorded as an AE if judged by the investigator to have unexpectedly worsened in severity or frequency or changed in nature at any time during the study. When recording such an AE on the Adverse Event eCRF, it is important to convey the concept that the condition has changed by including applicable descriptors (e.g., "accelerated acute ischemic stroke").

SECTION 5.4.1: Emergency Medical Contacts

PPD Medical Monitor contact information:

Medical Monitor: ~~Areen Said~~ *Andy Gruber, M.D.*

SECTION 5.6: POST-STUDY ADVERSE EVENTS

However, the Sponsor should be notified if the investigator becomes aware of any death, ~~or other SAE occurring after the end of the AE reporting period. The Sponsor should also be notified if the investigator becomes aware of the development of cancer, or a congenital anomaly/birth defect in a subsequently conceived offspring of a female patient exposed to study drug.~~

SECTION 6.1: DETERMINATION OF SAMPLE SIZE

The above ~~power considerations~~ sample size calculation assumes a control proportion of 65% and Type I error probability of 0.025 (one-sided)...

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, ~~and treatment crossovers, and stroke mimics~~), the above sample size needs to be inflated by a factor of $1/0.95^2$ or 1.108 (Friedman et al. 1998).

SECTION 6.2: 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 ~~symptoms~~ ~~deficits~~. ... 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). ... A patient is considered enrolled and randomized in the trial, and included in the ITT population, as soon as study drug begins.

~~Other~~ As a secondary efficacy outcomes of interest include NIHSS score, BI, GOS, and outcome, the global outcome scale score at 90 days will be analyzed using the global odds ratio test based on the generalized estimating equations analysis (Wald-type χ^2 test) with unstructured covariance-correlation matrix.

SECTION 6.3: SAFETY ANALYSIS

~~Primary~~ Safety outcomes include incidence of sICH within 36 hours, (primary safety assessment),...

SECTION 6.4: EXPLORATORY ANALYSES

The primary pre-specified variables and their analytic thresholds are defined by:

- Last known well time to treatment (0–2 vs. >2–3 hours)

SECTION 6.5: INTERIM ANALYSIS

~~Further details will be provided in the interim SAP and the iDMC charter.~~

SECTION 6.6: MISSING DATA HANDLING

The multiple-imputation method will be used for handling missing data, including the last observation (e.g., the Day 30 mRS) among the variables used in imputation.

SECTION 9.4: ADMINISTRATIVE STRUCTURE

A CRO will assist with study management, including project management, medical monitoring (as needed), clinical monitoring, data monitoring, and data management. Patients will be enrolled at approximately 75 sites in the U.S. and Canada/North America.

The iDMC will perform functions such as:

- Determine if the study should continue after review of interim analysis results ~~and to assess the sample size requirement using accumulated data, as described in the protocol~~

SECTION 10: REFERENCES

Levine SR, Khatri P, Broderick JP, et al. *Review, historical context, and clarifications of the NINDS rt-PA stroke trials exclusion criteria part 1: rapidly improving stroke symptoms*. *Stroke* 2013;44(9):2500–05.

~~The Re-examining Acute Eligibility for Thrombolysis (TREAT) Task Force. Review, historical context, and clarifications of the NINDS rt-PA stroke trials exclusion criteria: part 1: rapidly improving stroke symptoms. Stroke 2013 Jul 11. Epub ahead of print.~~

APPENDIX 1: Schedule of Assessments

The Schedule of Assessments has been revised to reflect the changes to the protocol.

APPENDIX 11: Cognitive and Behavioral Assessments

Appendix 11G, Rey-Osterrieth Complex Figure Test, has been removed. Appendix 11E, Benton Judgment of Line Orientation test, has been added. Other assessments in this appendix were reordered to reflect the preferred order of assessment administration.

APPENDIX 13: Preferred Terms for Intracranial Hemorrhage

Appendix 13 has been revised to include updated preferred terms for intracranial hemorrhage as follows:

- ~~Central nervous system hemorrhage~~
- *Epidural hemorrhage*
- ~~Purpura cerebri~~

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PROTOCOL AMENDMENT ACCEPTANCE FORM

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: 2

EUDRACT NUMBER: Not applicable

IND NUMBER: 3811

TEST PRODUCT: Alteplase (RO5532960)

LEAD PRINCIPAL INVESTIGATOR: [REDACTED], M.D.

MEDICAL MONITOR: [REDACTED], M.D.

SPONSOR: Genentech, Inc.

I agree to conduct the study in accordance with the current protocol.

Principal Investigator's Name (print)

Principal Investigator's Signature

Date

Please return a copy of this form as instructed by your local study monitor and retain a copy for your study files.

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: 2

EUDRACT NUMBER: Not applicable

IND NUMBER: 3811

TEST PRODUCT: Alteplase (RO5532960)

PHASE: IIIb

INDICATION: Acute ischemic stroke

SPONSOR: Genentech, Inc.

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 \geq 18 years (no upper age limit)
- Mild ischemic stroke defined as the most recent pre-treatment NIHSS score of \leq 5 and determined to be 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 \leq 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),*

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- 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 ≥ 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-compressible 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 $< 100,000/\text{mm}^3$ (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 inhibitor (*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
- 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.

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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 attributed 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

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. 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 $1/0.95^2$ 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.

LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	adverse event
AESI	adverse event of special interest
AIS	acute ischemic stroke
aPTT	activated partial thromboplastin time
BI	Barthel Index
BNT	<i>Boston Naming Test</i>
CES-D	Center for Epidemiologic Studies, Depression
CRO	contract research organization
CT	computed tomography
DW	diffusion-weighted
EC	Ethics Committee
eCRF	electronic Case Report Form
EDC	electronic data capture
EEA	European Economic Area
EQ-5D	European Quality of Life questionnaire
EU	European Union
FDA	U.S. Food and Drug Administration
FLAIR	fluid-attenuated inversion recovery
GOS	Glasgow Outcome Scale
GRE	gradient echo
HIPAA	Health Insurance Portability and Accountability Act
HVLT-R	<i>Hopkins Verbal Learning Test-Revised</i>
ICH	intracranial hemorrhage
ID	identification
iDCC	independent Data Coordinating Center
iDMC	independent Data Monitoring Committee
IMP	investigational medicinal product
IND	Investigational New Drug (application)
INR	international normalized ratio
IRB	Institutional Review Board
IST-3	third International Stroke Trial
ITT	intent-to-treat
IU	international units
IV	intravenous
IWRS	Interactive Web Response System

Abbreviation	Definition
LHH	likelihood of being helped vs. harmed
LPLV	last patient's last visit
MCA	middle cerebral artery
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NNH	number needed to harm
NNT	number needed to treat
PRO	patient-reported outcome
RISS	rapidly improving stroke symptoms
rPH	remote parenchymal hematoma
SAE	serious adverse event
SAP	Statistical Analysis Plan
sICH	symptomatic intracranial hemorrhage
SIS-16	Stroke Impact Scale-16
STIAMP	Suspected Transmission of an Infectious Agent via a Medicinal Product
SWFI	sterile water for injection
SW	susceptibility-weighted
t-PA	tissue plasminogen activator
U.S.	United States
USPI	U.S. Package Insert
WAIS III	<i>Wechsler Adult Intelligence Scale III</i>
WHO	World Health Organization

1. BACKGROUND

1.1 BACKGROUND ON ACUTE ISCHEMIC STROKE

Stroke is the fourth leading cause of death and a leading cause of disability in the United States (U.S.). Nearly 800,000 strokes occur in the U.S. annually; of these, approximately 85% are ischemic in nature (Rosamond et al. 2007). Treatment options are limited, as the only approved therapy for acute ischemic stroke (AIS) is alteplase, which is to be used in eligible patients and started within 3 hours from the onset of stroke symptoms. Stroke with “only minor or rapidly improving stroke symptoms” is considered a relative exclusion by national clinical guidelines, as these patients were largely excluded from definitive trials to date (Jauch et al. 2013). Consistent with this recommendation, only 15% of alteplase recipients have mild strokes (Smith et al. 2011).

More than half of all ischemic stroke patients have mild strokes at initial presentation in population-based studies (Dhamoon et al. 2009; Reeves et al. 2013). Yet, a substantial proportion (31% – 52%) of ischemic stroke patients who arrive within 2– 3 hours of symptom onset are not treated with intravenous (IV) alteplase primarily due to mild symptoms at the time of the treatment decision (Smith et al. 2011; De Los Rios la Rosa et al. 2012). In a national registry, among 93,517 cases arriving to Emergency Departments within 2 hours, 29,200 (31%) were not treated solely due to mild or improving symptoms, and 80.1% of untreated cases had a National Institutes of Health Stroke Scale (NIHSS) score ≤ 5 (Smith et al. 2011).

While treatment of mild stroke is generally uncommon, leading stroke centers vary widely in their approach. For example, the proportion of mild stroke patients (NIHSS ≤ 3) treated with reperfusion therapies at National Institutes of Health/National Institute of Neurological Disorders and Stroke (NINDS) Specialized Programs of Translational Research in Acute Stroke (SPOTRIAS) centers ranged from 2% – 16% in 2008 – 2009 (Wiley et al. 2012). This variability in approach is also evident upon discussion with former leaders of the pivotal NINDS trials; some physicians treat all eligible stroke patients regardless of severity, and others treat only a minority of patients (████████, personal communication). There is unquestionable community equipoise regarding the acute treatment of the mild stroke population.

1.2 BACKGROUND ON ALTEPLASE

1.2.1 Fibrinolysis and Tissue Plasminogen Activator

Physiologic dissolution of a thrombus depends primarily on the proteolytic action of plasmin formed from plasminogen at the site of a clot. The mechanism for this activation in vivo has not been definitively established. Plasminogen, the zymogen precursor of plasmin, may be activated by substances found in the plasma milieu, in tissue (tissue plasminogen activator [t-PA]), in urine (urokinase), and in bacteria (streptokinase). The generated plasmin may be inhibited by plasma proteins such as $\alpha 2$ -antiplasmin and $\alpha 2$ -macroglobulin (Robbins 1982).

Kinetic analyses suggest that plasminogen activation in the presence of fibrin occurs after the binding of plasminogen and t-PA at the clot site. t-PA has a high affinity for fibrin, thereby efficiently activating the fibrin-bound plasminogen and converting it to plasmin. Plasmin, which when bound to fibrin is protected from rapid inactivation by α_2 -antiplasmin, then proceeds to digest the fibrin clot.

See the Activase[®] (alteplase) U.S. Package Insert (USPI) for additional details on nonclinical and clinical studies.

1.3 STUDY RATIONALE AND BENEFIT-RISK ASSESSMENT

Alteplase is the only approved therapeutic agent for the treatment of AIS and is widely accepted as the standard of care for eligible patients (Edlow et al. 2013; Jauch et al. 2013). The currently approved alteplase 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 (Jauch, et al. 2013). 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 on who constitutes this patient population, and the absence of definitive evidence of alteplase efficacy in this setting (Levine et al. 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” throughout the protocol.

To date, all completed major randomized acute stroke trials have excluded patients with mild strokes to varying degrees. In the two NINDS trials, such patients were excluded according to the protocol (largely to prevent patients with transient ischemic attacks from being enrolled if they were having rapidly improving symptoms), but did not include an explicit definition of mild stroke except for pure sensory stroke, isolated ataxia, isolated dysarthria, and isolated facial weakness (NINDS rt-PA Study Group 1995). The operational definition for stroke symptoms too mild to treat was left to the judgment of the randomizing physician (Levine et al. 2013). Interestingly, a post hoc analysis of the 624 patients in the NINDS trial revealed that there were 58 patients (9%) enrolled with an NIHSS score ≤ 5 , none of whom had isolated motor symptoms, isolated facial droop, isolated ataxia, dysarthria, isolated sensory, and three or fewer of each of additional isolated deficits (Khatri et al. 2010). Perhaps more significantly, 2971 patients were excluded primarily due to “rapidly improving” or “minor symptoms.” However, a significant unmet medical need exists for patients with mild stroke. Available outcome data for patients with mild stroke who do not receive alteplase consistently demonstrate a high rate of suboptimal outcomes. Specifically, in two different prospective databases of patients with mild deficits (NIHSS score ≤ 5) and no baseline disability, approximately

one-third (29%–32%) were significantly disabled (as measured by modified Rankin Scale [mRS] score 2–6) at 90 days (Fischer et al. 2010; Khatri et al. 2012; [REDACTED], personal communication).

Existing, albeit limited, data suggest that the potential benefit-risk ratio for alteplase in mild stroke should be favorable. Gonzales et al. demonstrated better functional outcomes among mild strokes treated with IV alteplase compared to those not treated in a large, single-center, retrospective cohort; this was shown with two different thresholds of stroke severity (NIHSS≤7 and NIHSS≤3) (Gonzales et al. 2006). Additionally, a recent post hoc analysis of the subset of the third International Stroke Trial (IST-3) patients with mild strokes (pre-treatment NIHSS≤5 who met all other NINDS rt-PA study eligibility criteria) showed a trend toward benefit for alteplase ([REDACTED], personal communication). For PRISMS, the primary efficacy outcome assumption (mRS 0–1) is a 9% absolute treatment effect of alteplase relative to placebo. This assumption is based on several retrospective analyses of similar patient populations (Köhrmann et al. 2009; Fischer et al. 2010; Hassan et al. 2010b; Khatri et al. 2012) as well as the aforementioned IST-3 trial subgroup analysis ([REDACTED], personal communication).

The most prevalently reported and life-threatening adverse event (AE) associated with alteplase treatment for acute ischemic stroke is symptomatic intracranial hemorrhage (sICH). Post hoc analyses, case series, and retrospective studies in patients with mild stroke suggest an absolute sICH rate of 0–2% with alteplase treatment (NINDS rt-PA Study Group 2005; Baumann 2006; Gonzales et al. 2006; Köhrmann et al. 2009; Steffenhagen et al. 2009; Hassan et al. 2010a; Hassan et al. 2010b; Huisa et al. 2012). These data are consistent with analyses of IV alteplase trials of more severe patients suggesting that stroke severity, as measured by the NIHSS score, is a strong predictor of sICH risk (Khatri et al. 2007). No data exist to estimate the sICH rate for patients with mild stroke who did not receive t-PA; therefore, for this study, we are using a 0% sICH rate for the placebo arm, which is likely a conservative estimate. An absolute sICH difference of 2% at 36 hours (primary safety endpoint) has been assumed for the alteplase arm vs. the placebo control arm in the proposed study. For this study, sICH has been defined as any neurologic decline attributed to intracranial hemorrhage (ICH) seen on imaging by the local investigator within 36 hours of study drug administration, consistent with the NINDS trial definition for sICH (NINDS rt-PA Study Group 1995).

Assuming the proposed study demonstrates both a 9% absolute treatment effect rate, the number of patients needed to treat (NNT) to show benefit (i.e., mRS score of 0–1) for one patient is 11. Assuming a 2% absolute difference in sICH rate in patients treated with alteplase, the corresponding value for the number needed to harm (NNH), specifically for sICH, is conservatively estimated to be 50. The actual number NNH, or the number of patients who would have worse outcomes at 90 days with alteplase compared to placebo treatment, is likely to be much higher than is reflected by the sICH rate alone, as many patients who experience sICH have severe baseline infarcts and are destined for poor outcomes (Saver 2007).

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For comparison, the absolute treatment effect for mRS score of 0–1 at 90 days was 13% for the NINDS-sponsored pivotal study for alteplase. This equates to an NNT of 7.9. In the same study, the absolute difference in sICH was 5.8% at 24 ± 12 hours post-treatment, or an NNH of 17.2 (NINDS rt-PA Study Group 1995).

Table 1 Comparison of PRISMS and NINDS Studies

Study	Absolute Treatment Effect (%)	Absolute Difference in sICH (%)	NNT	NNH	LHH ^a
Proposed study in patients with mild stroke and RISS	9	2	11	50	0.22
NINDS-sponsored pivotal study for alteplase	13	5.8 ^b	7.9	17.2	0.46

LHH=likelihood of being helped vs. harmed; NINDS=National Institute of Neurological Disorders and Stroke; NNH=number needed to harm; NNT=number needed to treat; RISS=rapidly improving stroke symptoms; sICH=symptomatic intracranial hemorrhage.

^a LHH is the likelihood of being helped versus harmed and is the ratio between the NNT and the NNH. The LHH has been proposed as an expression to capture both the NNT and the NNH into a single value. An LHH below 1.0 indicates the expected benefits outweigh the possible harm. The lower the LHH, the more favorable the benefit-risk ratio is for the treatment in the patient population of interest (Demaerschalk 2007).

^b In the NINDS study, patients who received alteplase and developed sICH within 36 hours had a mortality rate of 45% (NINDS rt-PA Study Group 2005).

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of this study is to determine the efficacy of IV alteplase for treatment of AIS in patients with mild stroke (also known as “minor neurologic deficit” and “rapidly improving stroke symptoms”), defined as an NIHSS score ≤ 5 and not clearly disabling, within 3 hours of last known well time as measured by the proportion of patients with mRS score of 0–1 at Day 90.

2.2 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:
 - sICH within 36 hours (primary safety assessment)
 - Any ICH within 36 hours

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

2.3 EXPLORATORY OBJECTIVES

The exploratory objectives for this study include subgroup analyses of the primary efficacy outcome variable as 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 (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 test, form V; HVLT-R trial 4 and recognition; semantic fluency [Animal Naming test]; 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

3. STUDY DESIGN

3.1 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).

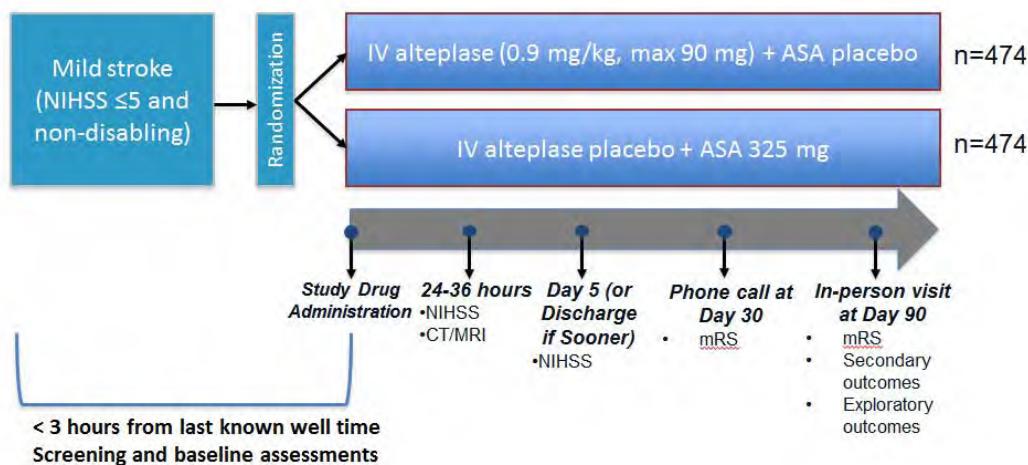
Screening assessments will be used to determine the eligibility of the patient. Patients meeting eligibility criteria will be randomized in a 1:1 ratio to receive either:

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 (Figure 1).*

Every effort should be made to ensure both study drugs are initiated within 3 hours of last known well time. If institutional policies on oral medications make it challenging to administer the oral aspirin/aspirin placebo within 3 hours of last known well time, the oral study drug may be given up to 24 hours after the last known well time. IV alteplase/IV alteplase placebo MAY NOT be initiated beyond 3 hours of last known well time under any circumstances.

A full schedule of assessments is provided in [Appendix 1](#).

Figure 1 Abbreviated Study Schema



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

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 stratified by site 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 informed consent and eligibility confirmation. Further details on step forward randomization can be found in Section [4.2](#), Method of Treatment Assignment and Blinding.

An independent Data Monitoring Committee (iDMC) will provide ongoing review of potential safety concerns and review efficacy and safety outcomes to inform a futility interim analysis after 50% of patients have achieved the primary endpoint (following completion of the Day 90 visit). The composition of the iDMC and analysis will be documented in detail in a separate iDMC charter.

3.2 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.

3.3 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 (\pm 14 days) after the last patient is enrolled into the study.

3.3.1 Number of Patients

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

3.4 RATIONALE FOR STUDY DESIGN

This study is designed to assess whether alteplase *administered within 3 hours of last known well time* is superior to standard of care (i.e., aspirin) in patients with mild stroke. Patients will be randomized 1:1 to receive either IV alteplase plus aspirin placebo or IV alteplase placebo plus aspirin 325 mg; hence, this is an active-comparator trial. Other than the study intervention, patients will receive standard of care for AIS at the enrolling institutions. The primary endpoint using the mRS assessment of global disability at 90 days is standard for current AIS trials.

The study design of PRISMS has the following characteristics:

- **Consistent with the currently approved indication in the alteplase USPI:** Treatment with alteplase in PRISMS will occur in otherwise eligible AIS patients within 3 hours from the last known well time.
- **Conduct of a large, multicenter study:** The Sponsor plans to enroll 948 patients across approximately 75 sites in *North America*. (For comparison, the two NINDS trials enrolled a total of 624 patients.)
- **Multiple endpoints involving different events:** In addition to the primary efficacy endpoint of mRS at Day 90, which measures disability, secondary endpoints at Day 90 will include *an ordinal mRS assessment and global favorable recovery, as calculated using the Global Outcome Measure derived from the mRS, NIHSS, BI, and GOS*.

3.4.1 Rationale for Alteplase Dosage

The dose selected for alteplase (0.9 mg/kg, maximum 90 mg) is the approved dose for eligible patients with AIS in *North America* and is considered the standard of care by the stroke community (Jauch et al. 2013).

3.4.2 Rationale for Patient Population

The patients for this trial will be recruited from all AIS patients presenting to Emergency Departments in whom treatment can be initiated within 3 hours of last known well time at approximately 75 clinical sites within *North America*. As described in Section 1.3, eligible patients with mild stroke will be enrolled in this study. Currently, the alteplase USPI warns against the use of alteplase in patients with “minor neurologic deficit and rapidly improving stroke symptoms” due to lack of data. Retrospective and observational data suggest potential benefit of alteplase in these patients, with a favorable benefit-risk profile.

The definition of mild stroke for PRISMS is an NIHSS score ≤ 5 and not clearly disabling. This includes patients whose symptoms are persistently mild as well as those with RISS.

An NIHSS score ≤ 5 was chosen as one criterion in the definition of mild stroke for PRISMS for the following reasons:

1. An NIHSS score ≤ 5 is commonly used as an exclusion criterion for many current AIS studies, suggesting that these patients are underrepresented in current stroke research and are treated differently by the stroke community, as shown in [Table 2](#).
2. The largest retrospective analysis of mild stroke from the Get With the Guidelines® registry included 29,200 stroke patients who did not receive alteplase due to mild or improving symptoms. This analysis found that 80.1% of patients with mild or rapidly improving stroke who did not receive alteplase (and had a recorded NIHSS score) had an initial NIHSS score ≤ 5 (Smith et al. 2011).

Based on these findings, an NIHSS score ≤ 5 was determined to be the most appropriate cut-off to define the patient population with mild stroke.

Table 2 NIHSS Exclusion Criteria of Recently Completed and Ongoing Acute Ischemic Stroke Trials

Recently Completed AIS Trials	Exclusion	Ongoing AIS Trials	Exclusion
Combined Approach to Lysis Utilizing Eptifibatide and rt-PA in Acute Ischemic Stroke (CLEAR Stroke) Trial	NIHSS≤5	ARTSS	NIHSS≤9
Intravenous Thrombolysis Plus Hypothermia for Acute Treatment of Ischemic Stroke	NIHSS≤6	SHINE	NIHSS<3
Albumin in Acute Ischemic Stroke Trial (ALIAS) Part One	NIHSS≤5	The Intravascular Cooling in the Treatment of Stroke 2/3 (ICTuS 2/3) Trial (ICTuS2/3)	NIHSS≤6
Study of the Combination Therapy of rt-PA and Eptifibatide to Treat Acute Ischemic Stroke (CLEAR-ER)	NIHSS≤5	Safety/Feasibility of Autologous Mononuclear Bone Marrow Cells in Stroke Patients	NIHSS≤5
Albumin in Acute Ischemic Stroke Trial (ALIAS) Part Two	NIHSS≤5	SWIFT PRIME	NIHSS≤7
Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy (MR RESCUE)	NIHSS≤5	PENUMBRA THERAPY	NIHSS≤7
NeuroThera Efficacy and Safety Trial-3 (NEST3)	NIHSS≤6	PENUMBRA 3D	NIHSS≤7
		CLOTBUST-ER	NIHSS≤9

AIS=acute ischemic stroke; NIHSS=National Institutes of Health Stroke Scale.

Source: www.clinicaltrials.gov. Accessed August 2013. Note that the NIHSS criteria for the trials listed are mentioned in the inclusion criteria, whereas the entries in the above table were adapted to list the corresponding exclusion criteria.

It is notable that the alteplase USPI (2011) distinguishes patients with minor neurological deficit from those with RISS. However, PRISMS combines these two patient groups into one operationally defined patient population (mild stroke): all eligible patients with an NIHSS score≤5 at the time of randomization without clearly disabling symptoms, irrespective of previous NIHSS scores. Data demonstrating low alteplase use in patients with NIHSS scores≤5 do not distinguish between whether the patient's deficit was persistently mild or improved to this status (Smith et al. 2011). Furthermore, no data exist that suggest patients who improved to a mild status at the time of treatment with alteplase have different outcomes from patients whose neurologic deficit is persistently mild. To date, available data suggest that alteplase therapy would potentially be beneficial in both patients with RISS who improve to an NIHSS score≤5 and patients with persistently mild *deficits* with an NIHSS score≤5.

Conversely, patients who improve to an NIHSS score > 5 at the time of treatment decision will be excluded from PRISMS as they are not considered to have RISS. Current clinical practice suggests that these patients would still have a moderate or severe stroke at the time of treatment decision and should receive alteplase, if eligible, rather than be randomized to a study. Such patients should not be defined as rapidly improving, given that they have not improved to an NIHSS score low enough to merit withholding therapy. This definition of RISS is consistent with a recent consensus statement by leading stroke and emergency medicine experts (Levine *et al.* 2013).

PRISMS will also exclude patients who are assessed at the time of pre-treatment to be clearly disabled. These *clearly disabling* deficits at presentation *may* include, but are not limited to, homonymous hemianopsia, severe limb weakness, severe aphasia, hemineglect, or cortical blindness. Patients with clearly disabling deficits will be excluded from this study irrespective of the NIHSS score at time of randomization, as current clinical practice and ethical responsibility suggest that these patients should be evaluated to receive alteplase rather than be enrolled in a randomized study where they may receive *alteplase* placebo.

3.4.3 Rationale for Control Group

The overall rationale for this trial is to determine whether IV alteplase improves outcomes in patients with mild stroke. The control group (i.e., mild stroke patients who receive standard of care) has been included so that outcomes in the active treatment group can be compared to those who receive standard of care. The control group will receive IV alteplase placebo and 325 mg of oral aspirin. The rationale for the control group receiving aspirin is that it would be unethical to withhold a dose of aspirin from patients who would not be receiving active study drug. The standard of care for patients suffering from AIS who are ineligible for IV alteplase therapy is oral aspirin administered within the first 24 hours after the onset of their stroke symptoms.

3.5 OUTCOME MEASURES

3.5.1 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 are measured at Day 90 and listed below:

- Ordinal mRS
- Global *favorable recovery* (Global Outcome Measure derived from mRS 0–1, NIHSS 0–1, BI \geq 95, and GOS = 1)

3.5.2 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 attributed 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

Symptomatic Intracranial Hemorrhage

In this study, an ICH is considered symptomatic if it is not seen on computed tomography (CT) or magnetic resonance imaging (MRI) scan at baseline and any neurologic decline is attributed to it by the local investigator. To detect intracranial hemorrhage, *neuroimaging (CT or MRI)* scan is required at 22 to 36 hours. Additional *neuroimaging* should be performed at any time based on the investigator's discretion that clinical findings suggested hemorrhage. Details on MRI types are described in Section 4.5.1.6.

Intracranial hemorrhage events will *include classifications of intracerebral hemorrhage (hemorrhagic infarct type 1 or type 2, parenchymal hematoma type 1 or type 2, or remote intraparenchymal hemorrhage type 1 or type 2). Descriptions of each type of intracerebral hemorrhage are shown in the table below. Additionally, subarachnoid hemorrhage, subdural hemorrhage, or epidural hemorrhage should be noted.*

Table 3 Intracerebral Hemorrhage Definitions

Type	Description
HI type 1	Small petechiae along margin of infarct
HI type 2	More confluent petechiae within the infarcted area but without space-occupying effect
PH type 1	Hematoma in ≤30% of the infarcted area with some slight space-occupying effect
PH type 2	Dense hematoma >30% total of the infarcted area with substantial space-occupying effect or any hemorrhagic area outside the infarcted area
rPH type 1	Small or medium sized blood clots located remote from the actual infarct; a mild space-occupying effect could be present. Remote primary intracerebral hemorrhage
rPH type 2	Large confluent dense blood clots in an area remote from the actual infarct; substantial space-occupying effect might be present.

HI=hemorrhagic infarct; PH=parenchymal hematoma; rPH=remote parenchymal hematoma.

Source: Wahlgren et al. 2007.

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3.5.3 Exploratory Outcome Measures

The exploratory outcome measures for this study are as follows:

- *NIHSS at Day 90*
- *Instrumental activities of daily living, as measured by the BI at Day 90*
- *GOS at Day 90*
- *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 test, form V; HVLT-R trial 4 and recognition; semantic fluency [Animal Naming test]; Boston Naming Test [BNT 15-item short form]) at Day 90*
- *Ambulatory performance (as measured by walking speed) at Day 90*
- *Depression (CES-D score) at Day 90*
- *Quality of life (EQ-5D questionnaire) at Day 90*
- *SIS-16 at Day 90*

4. MATERIALS AND METHODS

4.1 PATIENTS

This study will include adult patients with AIS.

4.1.1 Inclusion Criteria

Patients must meet the following criteria for study entry:

- Age \geq 18 years (no upper age limit)
- Mild ischemic stroke defined as the most recent pre-treatment NIHSS score of ≤ 5 and determined to be 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.

4.1.2 Exclusion Criteria

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

- CT or 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 ≥ 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-compressible 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 (aPTT) outside of the normal range as specified by the center's local laboratory
 - Blood glucose <50 mg/dL
 - International normalized ratio (INR) >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 inhibitor (*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
- 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

4.2 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

Step Forward Randomization

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 process 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. This procedure will be implemented using an Interactive Web Response System (IWRS) where study sites enter identification (ID) information of a patient AFTER he/she is treated (within 8 hours of initiation of the patient's study treatment). The IWRS will then assign drug kit IDs for the next patient that becomes eligible at that site. The pharmacist will prepare "Use Next" labels and place them on the appropriate study drug kits based on the drug kit IDs via IWRS. When the next potentially eligible patient is identified, the investigator (or designee) will notify the pharmacist (or designee) to begin pre-mixing study drug from the alteplase/alteplase-placebo drug kit. Pre-mixing may occur prior to signing informed consent but should be performed after key eligibility criteria (e.g., NIHSS≤5 and *not* clearly disabling, study drug initiation will occur within 3 hours of time last known well, CT/MRI negative for ICH) to minimize drug wastage. Study drug bolus will be administered after confirming that all eligibility criteria have been met and informed consent signed, and this will indicate enrollment in the trial. This approach will ensure minimal delays to treatment. Following study drug administration (within 8 hours), the study site should enter the patient's ID, including date of birth, patient initials, screening ID, and confirm drug kit IDs from the kits with which the patient is treated. Once this information is entered into IWRS, it will result in two actions:

1. IWRS will assign a patient ID to the currently treated patient, which will be correlated to the drug kit ID previously labeled as "Use Next," via the randomization visit record.
2. IWRS will assign a new *pair of* drug kit IDs for the next patient that becomes eligible at that site to be labeled as "Use Next."

In the case when pre-mixed *IV* drug is not used (e.g., patient is determined to be ineligible for study or declines informed consent prior to drug administration), sites will

enter drug kit IDs into IWRS and indicate that the kits were not used. IWRS will then assign a new set of drug kit IDs for the pharmacist to label with “Use Next” labels. The pharmacist will properly dispose of the pre-mixed *IV* drug and the matching aspirin/aspirin-placebo kit in accordance with institutional guidelines.

Prior to the first patient enrolled at a site, a site will register with IWRS as part of site activation, which will assign drug kit IDs ahead of time for the first potential patient. Once the first patient is enrolled at a site, the procedure described above will commence.

Study Unblinding

If a clinical situation arises in which the investigator thinks it is necessary to unblind in the interest of patient safety, the Medical Monitor must be notified immediately to discuss the intended unblinding. Unblinding should occur only in settings where identification of the study treatment is critical for treatment decisions. IWRS will be used for the unblinding process. All unblinded patients will remain in the study and require all follow-up visits.

If the investigator wishes to know the identity of the study drug for any other reason, he or she *must* contact the Medical Monitor. Treatment codes should not be broken except under circumstances deemed necessary by the site investigator. The investigator should document and provide an explanation for any premature unblinding (e.g., unblinding due to SICH or severe systemic bleeding *that requires blood products if IV alteplase was administered*, or unblinding due to clinical worsening *during the IV alteplase time window that would lead to IV alteplase administration if not already given*).

For regulatory reporting purposes, and if required by local health authorities, the Sponsor will break the treatment code for all serious, unexpected suspected adverse reactions (see Section 5.7) that are considered by the investigator or Sponsor to be related to study drug.

4.3 STUDY TREATMENT

4.3.1 Formulation, Packaging, and Handling

4.3.1.1 Alteplase

Alteplase is a single polypeptide containing 527 amino acids; it has three carbohydrate side chains and is predominantly one-chain t-PA. It is a recombinant protein produced in a continuous mammalian tissue culture system and is purified by a series of chromatographic steps. The manufacturing process includes fermentation with use of the antibiotic gentamicin; however, the presence of the antibiotic is not detectable in the final product.

Alteplase is a sterile, white to pale yellow, lyophilized powder for IV administration after reconstitution with sterile water for injection (SWFI), USP, without preservatives.

Phosphoric acid and/or sodium hydroxide may be used prior to lyophilization for

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pH adjustment. Biologic potency is determined by an in vitro clot lysis assay and is expressed in international units (IU) as tested against the World Health Organization (WHO) standard.

Each 50-mg vial of Activase contains 50 mg of alteplase, 1.7 g of L-arginine, 5 mg of polysorbate 80, and 0.5 g of phosphoric acid.

Each kit will contain 2 vials of 50-mg of alteplase labeled for “clinical trial use only.”

Vials of 50-mg alteplase must be stored at 2°C–30°C and protected from light.

4.3.1.2 Alteplase Placebo

Alteplase placebo is the same formulation as the active without the alteplase protein. Each 50-cc vial contains 1.7 g of L-arginine, 5 mg of polysorbate 80, and 0.5 g of phosphoric acid.

Each kit will contain 2 vials of 50 mg of alteplase placebo labeled for “clinical trial use only.”

Alteplase placebo vials should be stored at 2°C–30°C and protected from light.

4.3.1.3 Aspirin

Aspirin (325 mg) will be packaged for the study. Each kit will contain one bottle, which has 100 tablets labeled for “clinical trial use only.” Only one tablet will be administered to the patient, and the remainder of the bottle will be kept at the site for drug accountability.

Aspirin tablets should be stored at 25°C with excursions permitted between 15°C–30°C.

4.3.1.4 Aspirin Placebo

Aspirin placebo will be identical in appearance to active aspirin to maintain treatment blinding. Each bottle of aspirin placebo contains 100 tablets labeled for “clinical trial use only.” Only one tablet will be administered to the patient, and the remainder of the bottle will be kept at the site for drug accountability.

Placebo tablets should be stored in the same manner as aspirin tablets: 25°C with excursions permitted between 15°C–30°C.

4.3.2 Dosage, Administration, and Compliance

4.3.2.1 Alteplase

For AIS, the recommended dose is 0.9 mg/kg infused over 60 minutes with 10% of the total dose administered as an initial IV bolus over 1 minute. The total dose should not exceed 90 mg.

Because alteplase and alteplase placebo contain no antibacterial preservatives, 50-mg vials should be reconstituted immediately before use by adding 50 mL of SWFI, USP, to the vial; this yields a concentration of 1 mg/mL. Using aseptic technique, direct the flow of SWFI directly into the lyophilized cake of study drug. It is recommended that an 18-gauge needle be used for the reconstitution of the 50-mg vial. Once reconstituted, gently swirl the vial. **Do not shake.** Slight foaming upon reconstitution is not unusual; allowing the vial to stand undisturbed for several minutes is usually sufficient to allow dissipation of any large bubbles. Before further dilution or administration, the product should be visually inspected for particulate matter and discoloration. If a precipitate is seen, the product should not be used.

After reconstitution, alteplase should be administered at 1 mg/mL. As an alternative, the reconstituted solution may be diluted further immediately before administration in an equal volume of 0.9% sodium chloride injection, USP, or 5% dextrose injection, USP, to yield a concentration of 0.5 mg/mL. The osmolality of this solution is approximately 215 mOsm/kg. Polyvinyl chloride bags, glass bottles, or polypropylene syringes are acceptable. Do not use filter needles for infusion. Alteplase is stable for up to 8 hours in these solutions at room temperature. Exposure to light has no effect on the stability of these solutions. Excessive agitation during dilution should be avoided; mixing should be accomplished with gentle swirling and/or slow inversion.

No other medication should be added to infusion solutions containing alteplase. Any unused infusion solution should be discarded.

See [Appendix 2](#) (Alteplase Administration) and [Appendix 3](#) (Reconstitution of 50-mg Vials) for further details.

4.3.3 Investigational Medicinal Product Accountability

All investigational medicinal products (IMPs) required for completion of this study will be provided by the Sponsor where required by local health authority regulations. The investigational site will acknowledge receipt of IMPs using the IWRs to confirm the shipment condition and content. Any damaged shipments will be replaced.

IMPs will either be disposed of at the study site according to the study site's institutional standard operating procedure or returned to the Sponsor with the appropriate documentation. The site's method of IMP destruction must be agreed to by the Sponsor. The site must obtain written authorization from the Sponsor before any IMP is destroyed, and IMP destruction must be documented on the appropriate form.

Accurate records of all IMPs received, dispensed, returned, and disposed of by the study site should be recorded on the Drug Inventory Log.

4.3.4 Investigational Medicinal Product

4.3.4.1 Alteplase and Placebo Test Product

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.

4.3.4.2 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.

4.3.5 Post-Trial Access to Alteplase

The Sponsor does not intend to provide alteplase, any investigational or non-investigational products (specified in this protocol), or any other study interventions to patients after the conclusion of the study or *to patients* who are withdrawn earlier.

4.4 CONCOMITANT THERAPY

4.4.1 Permitted Therapy

Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by a patient from 7 days prior to screening to the study completion/discontinuation visit. All concomitant medications should be reported to the investigator and recorded on the Concomitant Medications electronic Case Report Form (eCRF).

4.4.2 Prohibited Therapy

Available endovascular therapies have not demonstrated clinical efficacy in definitive trials to date and therefore are not permitted.

As indicated in the exclusion criteria, use of the following therapies is prohibited prior to the study:

- Treatment with *unfractionated* heparin within the last 48 hours and aPTT outside of the normal range as specified by the center's local laboratory

- Treatment with a direct thrombin inhibitor or factor Xa inhibitor (*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*
- Treatment with warfarin and INR >1.7 (Note: INR does not need to be verified prior to randomization if there is no clinical suspicion of abnormality.)

The above lists of medications are not necessarily comprehensive. The investigator should contact the Medical Monitor if questions arise regarding medications not listed above. For further reference about drug interaction guidance from the U.S. Food and Drug Administration (FDA), as well as a list of common cytochrome P450 interactions, the following resources are available online:

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf>

<http://medicine.iupui.edu/clinpharm/ddis/table.aspx>

4.5 STUDY ASSESSMENTS

Please see [Appendix 1](#) for the schedule of assessments performed during the study.

4.5.1 Description of Study Assessments

4.5.1.1 Informed Consent Forms and Screening Log

Written informed consent for participation in the study must be obtained before treatment. All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before treatment. The investigator will maintain a screening log of all patients arriving in the Emergency Department within 2.5 hours of onset of ischemic stroke *who have an initial NIHSS score of ≤5*. The investigator will record details of all patients not enrolled despite early arrival and record reasons for non-enrollment.

4.5.1.2 Medical History and Demographic Data

Medical history includes clinically significant diseases, such as stroke history and smoking history, use of alcohol and drugs of abuse, and all medications (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the patient within 7 days prior to the screening visit, and history of allergy to any food or medication.

Demographic data will include date of birth, sex, and self-reported race/ethnicity.

4.5.1.3 Neurological Examinations

A partial neurological examination is to be performed for each patient. The NIHSS will be a component of this neurological examination and will be performed only by practitioners who are certified to perform an NIHSS assessment. Changes from baseline should be recorded in patient notes. Additional or supplemental neurological

exams will also be performed, including assessment of mental status, cranial nerves, motor function, and coordination.

4.5.1.4 Vital Signs

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

4.5.1.5 Laboratory Assessments

Specimens for the following laboratory tests will be sent to the study site's local laboratory for analysis:

- *Complete blood count without differential*
- Serum glucose
- Coagulation (INR, aPTT)
- Pregnancy test (serum or urine) *for women of childbearing potential including those who have had a tubal ligation*

The following laboratory test values will be reported in the eCRFs:

- *Complete blood count without differential*
- Serum glucose
- Coagulation values (INR, aPTT)
- *Pregnancy test for women of childbearing potential including those who have had a tubal ligation*

For sampling procedures, storage conditions, and shipment instructions, see the site's local laboratory manual.

4.5.1.6 Other Disease-Specific Assessments

Neuroimaging may consist of a non-contrast CT or MRI of the brain. If an MRI is used in the acute setting (to determine study eligibility), it must include the following at a minimum: (1) DW MRI, (2) either susceptibility-weighted (SW) imaging or gradient echo (GRE) sequences, and (3) FLAIR sequences.

Non-contrast CT or MRI will be performed at baseline to ensure that the patient does not have evidence of acute 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. An NIHSS should be performed at the time this CT scan or MRI is performed, but prior to reviewing imaging results to avoid biasing the assessment. If an MRI is performed, in addition to the above requirements for the baseline visit MRI, it must also include T1 and T2 sequences.

Additional CT scans or MRIs should be performed based on the investigator's discretion, or if any clinical findings suggest ICH (such as new headache, nausea, vomiting, significant deterioration of neurological status, or rapid increase of blood pressure). If an ICH is identified, the NIHSS score should be reassessed at the time and recorded on the Adverse Event eCRF.

CT scans or MRIs performed *at baseline*, between 22 to 36 hours, and additional imaging performed due to clinical suspicion of deterioration up until 36 hours after study drug administration, will be evaluated by a central team of radiologists blinded to the clinical attributes of the case. *These images will need to be sent to the central radiologists by the local site.*

4.5.2 Patient-Reported Outcomes

Patient-reported outcomes (PRO) data will be elicited from the patients in this study to more fully characterize the clinical profile of alteplase in this patient population. The PRO questionnaires will be distributed by the investigator staff and completed in their entirety by the patient or their surrogate.

This study will assess the impact of alteplase treatment on health-related quality of life, physical function, daily functioning, and symptoms of depression.

Health-related quality of life will be assessed using the EQ-5D ([Appendix 4](#)). 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.

Physical functioning will be evaluated using the SIS-16 ([Appendix 5](#)), which is a validated, stroke-specific, quality of life measure to assess the impact of stroke on a patient's health and life.

The CES-D ([Appendix 6](#)) will be used to evaluate the proportion of patients with depressive symptoms (defined as a score of ≥ 16 at 90 days).

The EQ-5D, SIS-16, and CES-D will be provided in Spanish for those patients requiring translations.

AE reports will not be derived from PRO data by the Sponsor. However, any PRO responses suggestive of a possible AE identified during site review of the PRO data should be reported as outlined in Section [5.3.3.11](#).

4.6 TIMING OF STUDY ASSESSMENTS

4.6.1 Screening/Baseline (Visit 1)

The following standard of care assessments performed prior to obtaining informed consent will be used in the screening process. Once consent is obtained, the results of the standard of care assessments may be used for the study.

- Medical history
- Demographics
- Neurological examination
- Vital signs
- Complete blood count *without differential*
- Coagulation status
- Pregnancy test (serum or urine)
- Serum blood glucose
- Non-contrast CT scan or MRI, including DW MRI, either SW or GRE, and FLAIR images
- NIHSS score (Note: This must be performed by an NIHSS-certified practitioner; see [Appendix 7](#))
- Concomitant medications

All screening evaluations with the exception of certain laboratory results (e.g., coagulation and *complete blood count*) must be completed and reviewed to confirm that patients have met all eligibility criteria prior to treatment. Serum glucose is required to confirm eligibility prior to enrollment; however, if the investigator feels there is clinical suspicion that would be related to an exclusion laboratory test that would preclude the patient from eligibility, the results of those laboratory tests must be obtained prior to treatment.

The investigator will maintain a screening log to record details of all patients consented to confirm eligibility or record reasons for screening failure, as applicable.

4.6.2 Visit 2 (22 to 36 Hours after Treatment Initiation)

A non-contrast cranial CT or MRI (including DW MRI, either SW or GRE, FLAIR, and T1 and T2 sequences) will be performed at 22 to 36 hours after treatment with study drug has been initiated (*bolus*). Either a CT scan or MRI may be performed, as consistent with the institutional standard of care; the main criterion is to perform radiographic imaging of the brain by a modality sensitive to the presence of blood. An NIHSS will also be performed during this visit, but prior to review of the imaging results (or by an

investigator blinded to the results). AEs¹ and concomitant medications will also be assessed at this visit.

4.6.3 Visit 3 (Day 5 or Discharge from Hospital, if Sooner than Day 5)

On Day 5 (or discharge from hospital, if sooner than Day 5), NIHSS score, stroke etiology, AEs, and concomitant medications will be collected. Discharge location will be collected at the time of hospital discharge.

4.6.4 Visit 4 (Day 30 [30±7 Days])

A phone call to the patient or caregiver will be made at Day 30 and an mRS will be assessed (see [Appendix 8](#)). AEs, concomitant medications, and patient survival will be assessed *during this phone visit*. Pregnancy will also be assessed for female patients of childbearing potential.

4.6.5 Visit 5 (Day 90 [±14 Days])/Study Completion

Primary and secondary efficacy endpoints, as well as all exploratory endpoints, will be assessed at Day 90 *with the patient*. The following assessments will be performed:

- NIHSS score ([Appendix 7](#))
- Patient survival
- mRS ([Appendix 8](#))
- GOS ([Appendix 9](#))
- BI ([Appendix 10](#))
- EQ-5D ([Appendix 4](#))
- SIS-16 ([Appendix 5](#))
- CES-D ([Appendix 6](#))
- Cognitive assessments ([Appendix 11](#))
 - 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 test, form V*
 - *HVLT-R trial 4 and recognition*
 - *Animal Naming test*

¹ AE/SAE collection should be started as soon as study drug administration (bolus) is initiated.

- BNT 15-item short form

The above cognitive assessments will be provided in Spanish for those patients requiring translations with the exception of the BNT 15-item short form.

- Walking speed ([Appendix 12](#))
- Concomitant medications
- SAEs and certain AEs (See Section [5.3.1](#))

4.6.6 Early Discontinuation

Concomitant medication, AEs, and patient survival will be assessed via a phone call, if possible.

4.7 PATIENT, TREATMENT, STUDY, AND SITE DISCONTINUATION

4.7.1 Patient Discontinuation

Patients have the right to voluntarily withdraw from the study at any time and for any reason. In addition, the investigator has the right to withdraw a patient from the study at any time. Reasons for withdrawal from the study may include, but are not limited to, the following:

- Patient withdrawal of consent at any time
- Any medical condition that the investigator or Sponsor determines may jeopardize the patient's safety if he or she continues in the study
- Investigator or Sponsor determines it is in the best interest of the patient
- Patient non-compliance, specifically defined as unwillingness to participate in a 3-month assessment of neurological status and function

Every effort should be made to obtain information on patients who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. However, patients will not be followed for any reason after consent has been withdrawn. Patients who withdraw from the study will not be replaced.

4.7.2 Study Treatment Discontinuation

The study intervention should be discontinued if the patient displays symptoms concerning sICH during the infusion of the study drug. Prompt imaging should be done to assess for ICH. If ICH is seen, study drug infusion should not be resumed and the treatment assignment *may be unblinded if blood products (to reverse the biological activity of IV alteplase) are indicated*. If ICH is not seen, study drug infusion should immediately be restarted *up to 5.5 hours after the last known well time at the same rate as the previous infusion until the remainder of study drug is infused*.

Patients must discontinue study treatment if they experience any of the following:

- Anaphylactoid reaction (including angioedema)
- Serious bleeding (not controllable by local pressure) at puncture site or serious bleeding in a critical location (e.g., intracranial, gastrointestinal, retroperitoneal, pericardial)

The primary reason for study treatment discontinuation should be documented on the appropriate eCRF. Patients who discontinue study treatment prematurely will not be replaced. Patients who discontinue study treatment prematurely will *continue in the study and be followed through Day 90*, based on the intent-to-treat (ITT) principle.

4.7.3 Study and Site Discontinuation

The Sponsor has the right to terminate this study at any time. The Sponsor will notify the investigator if the Sponsor decides to discontinue the study. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to patients.
- Patient enrollment is unsatisfactory
- A pre-specified boundary at the interim analysis was crossed

The Sponsor has the right to close a site at any time. Reasons for closing a site may include, but are not limited to, the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the International Conference on Harmonisation guideline for Good Clinical Practice
- No study activity (i.e., all patients have completed study assessments and all obligations have been fulfilled)

5. ASSESSMENT OF SAFETY

5.1 SAFETY PLAN

The safety profile of alteplase across indications is generally well established. The principal adverse reactions to alteplase are bleeding and allergic reactions.

5.1.1 Risk of Bleeding

Bleeding is the most frequent adverse reaction associated with alteplase in all approved indications, including AIS. This may be either superficial from punctures or damaged blood vessels or internal bleeding at any site or body cavity. Bleeding may result in life-threatening situations, permanent disability, or death.

- The incidence of ICH, especially symptomatic ICH, in patients with AIS is higher in alteplase-treated patients than placebo-treated patients in published studies (for detailed information, see the alteplase USPI).

Management of Bleeding

Patients will be excluded for the presence of conditions related to risks of bleeding (as outlined in Section 4.1.2, Exclusion Criteria). A non-contrast cranial CT or MRI (including either SW or GRE to detect ICH) should be performed at baseline to ensure that the patient does not have evidence of ICH prior to study drug administration.

Fibrin, which is part of the hemostatic plug formed at needle puncture sites, may be lysed during alteplase therapy. Therefore, alteplase therapy requires careful attention to potential bleeding sites, e.g., catheter-insertion sites and arterial-puncture sites. Arterial and venous punctures should be minimized. Non-compressible arterial, as well as internal jugular and subclavian venous punctures, should be avoided to minimize bleeding from non-compressible sites. In the event of serious bleeding (not controllable by local pressure) or occurring in a critical location (intracranial, gastrointestinal, retroperitoneal, or pericardial), study drug should be discontinued immediately.

Supportive care and medical management of AEs are at the discretion of the investigator.

5.1.2 Risk of Allergic Reactions

Allergic-type reactions, e.g., anaphylactoid reaction, laryngeal edema, orolingual angioedema, rash, and urticaria, have been reported. When such reactions occur, they usually respond to conventional therapy.

There have been postmarketing reports of orolingual angioedema associated with the use of alteplase. Most were patients treated for AIS. Many of these patients received concomitant angiotensin-converting enzyme inhibitors, and most cases resolved with prompt treatment.

Management of Allergic Reaction

If an anaphylactic reaction occurs, the infusion of study drug should be discontinued immediately and appropriate therapy should be promptly instituted.

5.2 SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording AEs, including SAEs and non-serious adverse events of special interest (AESIs), measurement of protocol-specified vital signs, and other protocol-specified tests that are deemed critical to the safety evaluation of the study.

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Certain types of events require immediate reporting to the Sponsor, as outlined in Sections [5.2.2](#) and [5.2.3](#).

5.2.1 Adverse Events

According to the International Conference on Harmonisation guideline for Good Clinical Practice, an AE is any untoward medical occurrence in a clinical investigation patient administered a pharmaceutical product, regardless of causal attribution. An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition), except as described in Section [5.3.3.9](#)
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (e.g., electrocardiogram, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug

5.2.2 Serious Adverse Events (Immediately Reportable to the Sponsor)

An SAE is any AE that meets any of the following criteria:

- Fatal (i.e., the AE actually causes or leads to death)
- Life-threatening (i.e., the AE, in the view of the investigator, places the patient at immediate risk of death)

This does not include any AE that, had it occurred in a more severe form or was allowed to continue, might have caused death.

- Requires or prolongs inpatient hospitalization (see Section [5.3.3.9](#))
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions)
- Congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drug
- Significant medical event in the investigator's judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above)

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an adverse event (rated as mild, moderate, or severe); the event itself may be of relatively minor medical significance (such as severe headache). "Serious" is a regulatory definition and is based on patient or event outcome or action criteria usually

associated with events that pose a threat to a patient's life or vital functions. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations.

Severity and seriousness need to be independently assessed for each AE recorded on the eCRF.

SAEs 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 for reporting instructions).

5.2.3 Non-Serious Adverse Events of Special Interest (Immediately Reportable to the Sponsor)

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 for reporting instructions). AESIs for this study include the following:

- *ICH events, if not already reported as an SAE (for a list of preferred terms for ICH see Appendix 13)*
- *Stroke recurrence: report if the AE resulted in a substantial disruption of a person's ability to conduct normal life functions (i.e., the AE resulted in a significant, persistent, or permanent change; impairment; or damage or disruption in the patient's body function/structure, physical activities, and/or quality of life).*
- In addition, Suspected Transmission of an Infectious Agent via a Medicinal Product (STIAMP) by the study drug in a Genentech-sponsored trial qualifies as an AESI and requires immediate reporting to the Sponsor.

5.3 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

The investigator is responsible for ensuring that all AEs (see Section 5.2.1 for definition) are recorded on the Adverse Event eCRF and reported to the Sponsor in accordance with instructions provided in this section and in Sections 5.4–5.6.

For each AE recorded on the Adverse Event eCRF, the investigator will make an assessment of seriousness (see Section 5.2.2 for seriousness criteria), severity (see Section 5.3.2), and causality (see Section 5.3.3).

5.3.1 Adverse Event Reporting Period

Investigators will seek information on AEs at each patient contact. All AEs, whether reported by the patient or noted by study personnel, will be recorded in the patient's medical record and on the Adverse Event eCRF.

After initiation of study drug, all AEs (including serious and non-serious AEs), regardless of relationship to study drug, will be reported until 30 days after administration of study drug.

After 30 days until 90 days after administration of study drug, the following AEs should be record on the AE CRF:

- *All SAEs*
- *Non-serious ICHs*
- *Non-serious AESIs*
- *All AEs that resulted in withdrawal from the study*

All SAEs and non-serious AESIs require immediate reporting to the Sponsor (see Section 5.4).

After 90 days, the investigator is not required to actively monitor patients for AEs; however, the Sponsor should be notified if the investigator becomes aware of any post-study SAEs (see Section 5.6).

5.3.2 Assessment of Severity of Adverse Events

The WHO toxicity grading scale (see [Appendix 14](#)) will be used for assessing adverse event severity. Table 4 will be used for assessing severity for adverse events that are not specifically listed in the WHO toxicity grading scale.

Table 4 Adverse Event Severity Grading Scale

Grade	Severity
1	Mild; transient or mild discomfort (<48 hours); no medical intervention or therapy required
2	Moderate; mild to moderate limitation in activity; some assistance may be needed; no or minimal medical intervention or therapy required
3	Severe; marked limitation in activity; some assistance usually required; medical intervention or therapy required; hospitalization possible
4	Life-threatening; extreme limitation in activity; significant assistance required; significant medical intervention or therapy required, hospitalization or hospice care probable

Notes: Developed by the Division of Microbiology and Infectious Diseases.

Regardless of severity, some events may also meet seriousness criteria. Refer to definition of a serious adverse event (see Section [5.2.2](#)).

5.3.3 Assessment of Causality of Adverse Events

Investigators should use their knowledge of the patient, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether or not an AE is considered to be related to the study drug, indicating "yes" or "no" accordingly. The following guidance should be taken into consideration (see also [Table 5](#)):

- Temporal relationship of event onset to the initiation of study drug

- Course of the event, considering especially the effects of dose reduction, discontinuation of study drug, or reintroduction of study drug (as applicable)
- Known association of the event with the study drug or with similar treatments
- Known association of the event with the disease under study
- Presence of risk factors in the patient or use of concomitant medications known to increase the occurrence of the event
- Presence of non-treatment-related factors that are known to be associated with the occurrence of the event

Table 5 Causal Attribution Guidance

Is the adverse event suspected to be caused by the study drug on the basis of facts, evidence, science-based rationales, and clinical judgment?	
YES	There is a plausible temporal relationship between the onset of the adverse event and administration of the study drug, and the adverse event cannot be readily explained by the patient's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study drug; and/or the adverse event abates or resolves upon discontinuation of the study drug or dose reduction and, if applicable, reappears upon re-challenge.
NO	Adverse events will be considered related, unless they fulfill the criteria as specified below. Evidence exists that the adverse event has an etiology other than the study drug (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the adverse event has no plausible temporal relationship to administration of the study drug (e.g., cancer diagnosed 2 days after first dose of study drug).

5.3.3.1 Diagnosis versus Signs and Symptoms

A diagnosis (if known) should be recorded on the Adverse Event eCRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded on the Adverse Event eCRF. If a diagnosis is subsequently established, all previously reported AEs based on signs and symptoms should be nullified and replaced by one AE report based on the single diagnosis, with a starting date that corresponds to the starting date of the first symptom of the eventual diagnosis.

5.3.3.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. However, medically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the Adverse Event eCRF. For example:

- If vomiting results in mild dehydration with no additional treatment in a healthy adult, only vomiting should be reported on the eCRF.
- If vomiting results in severe dehydration, both events should be reported separately on the eCRF.
- If a severe gastrointestinal hemorrhage leads to renal failure, both events should be reported separately on the eCRF.
- If dizziness leads to a fall and consequent fracture, all three events should be reported separately on the eCRF.
- If neutropenia is accompanied by an infection, both events should be reported separately on the eCRF.

All AEs should be recorded separately on the Adverse Event eCRF if it is unclear as to whether the events are associated.

5.3.3.3 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between patient evaluation timepoints. Such events should only be recorded once on the Adverse Event eCRF. The initial severity of the event should be recorded, and the severity should be updated to reflect the most extreme severity any time the event worsens. If the event becomes serious, the Adverse Event eCRF should be updated to reflect this.

A recurrent AE is one that resolves between patient evaluation timepoints and subsequently recurs. Each recurrence of an AE should be recorded separately on the Adverse Event eCRF.

5.3.3.4 Abnormal Laboratory Values

Not every laboratory abnormality qualifies as an AE. A laboratory test result must be reported as an AE if it meets any of the following criteria:

- Accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention (e.g., potassium supplementation for hypokalemia) or a change in concomitant therapy
- Clinically significant in the investigator's judgment

It is the investigator's responsibility to review all laboratory findings. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an AE.

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 times the upper limit of normal associated with cholestasis), only the diagnosis (i.e., cholestasis) should be recorded on the Adverse Event eCRF.

If a clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded on the Adverse Event eCRF, along with a descriptor indicating if the test result is above or below the normal range (e.g., "elevated potassium" as opposed to "abnormal potassium"). If the laboratory abnormality can be characterized by a precise clinical term per standard definitions, the clinical term should be recorded as the AE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia."

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded on the Adverse Event eCRF unless the etiology changes. The initial severity of the event should be recorded, and the severity or seriousness should be updated any time the event worsens.

5.3.3.5 Abnormal Vital Sign Values

Not every vital sign abnormality qualifies as an AE. A vital sign result must be reported as an AE if it meets any of the following criteria:

- Accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention or a change in concomitant therapy
- Clinically significant in the investigator's judgment

It is the investigator's responsibility to review all vital sign findings. Medical and scientific judgment should be exercised in deciding whether an isolated vital sign abnormality should be classified as an AE.

If a clinically significant vital sign abnormality is a sign of a disease or syndrome (e.g., high blood pressure), only the diagnosis (i.e., hypertension) should be recorded on the Adverse Event eCRF.

Observations of the same clinically significant vital sign abnormality from visit to visit should not be repeatedly recorded on the Adverse Event eCRF unless the etiology changes. The initial severity of the event should be recorded, and the severity or seriousness should be updated any time the event worsens.

5.3.3.6 Deaths

All deaths that occur during the protocol-specified AE reporting period (Section 5.3.1) regardless of relationship to study drug, must be recorded on the Adverse Event eCRF and immediately reported to the Sponsor (see Section 5.2.2). This includes death attributed to progression of AIS.

Death should be considered an outcome and not a distinct event. The event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the Adverse Event eCRF. Generally, only one such event should be reported. The term "sudden death" should only be used for the occurrence of an abrupt and unexpected death due to presumed cardiac causes in a patient with or without preexisting heart disease, within 1 hour of the onset of acute symptoms or, in the case of an unwitnessed death, within 24 hours after the patient was last seen alive and stable. If the cause of death is unknown and cannot be ascertained at the time of reporting, "unexplained death" should be recorded on the Adverse Event eCRF. If the cause of death later becomes available (e.g., after autopsy), "unexplained death" should be replaced by the established cause of death.

If the death is attributed to progression of AIS, "acute ischemic stroke progression" should be recorded on the Adverse Event eCRF.

5.3.3.7 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the screening visit for this study. Such conditions should be recorded on the General Medical History and Baseline Conditions eCRF.

A preexisting medical condition should be recorded as an AE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on the Adverse Event eCRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., more frequent headaches").

5.3.3.8 Lack of Efficacy or Worsening of Acute Ischemic Stroke

Medical occurrences or symptoms of deterioration that are considered part of the disease course or disease process should be recorded as an AE if judged by the investigator to have unexpectedly worsened in severity or frequency or unexpectedly changed in nature at any time during the study. If there is uncertainty, it should be reported as an AE. When recording unexpected worsening of AIS on the Adverse Event eCRF, it is important to convey the concept that the condition has changed by including applicable descriptors of the event.

5.3.3.9 Hospitalization or Prolonged Hospitalization

Any AE that results in hospitalization (i.e., in-patient admission to a hospital) or prolonged hospitalization should be documented and reported as an SAE (per the definition of an SAE in Section 5.2.2), except as outlined below.

The following hospitalization scenarios are not considered to be AEs:

- Hospitalization for respite care
- Hospitalization for a preexisting condition, provided that all of the following criteria are met:
 - The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease.
 - The patient has not suffered an AE.

5.3.3.10 Adverse Events Associated with an Overdose

An overdose is the accidental or intentional use of a drug in an amount higher than the dose being studied. An overdose or incorrect administration of study treatment is not itself an AE, but it may result in an AE. All AEs associated with an overdose or incorrect administration of study drug should be recorded on the Adverse Event eCRF. If the associated AE fulfills serious criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2).

5.3.3.11 Patient-Reported Outcome Data

AE reports will not be derived from PRO data by the Sponsor, and safety analyses will not be performed using PRO data. However, if any PRO responses suggestive of a possible AE are identified during site review of the PRO data, the investigator will determine whether the criteria for an AE have been met and, if so, will report the event on the Adverse Event eCRF.

5.4 IMMEDIATE REPORTING REQUIREMENTS FROM INVESTIGATOR TO SPONSOR

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- SAEs
- Non-serious AESIs
- Pregnancies

The investigator must report new significant follow-up information for these events to the Sponsor immediately (i.e., no more than 24 hours after becoming aware of the information). New significant information includes the following:

- New signs or symptoms or a change in the diagnosis
- Significant new diagnostic test results
- Change in causality based on new information
- Change in the event's outcome, including recovery
- Additional narrative information on the clinical course of the event

Investigators must also comply with local requirements for reporting SAEs to the local health authority and Institutional Review Board/Ethics Committee (IRB/EC).

5.4.1 Emergency Medical Contacts

Medical Monitor Contact Information

PPD Medical Monitor contact information:

Medical Monitor: [REDACTED], M.D.

Telephone Nos.: [REDACTED]

Alternate Medical Monitor contact information for all sites:

Medical Monitor: [REDACTED], M.D.

Telephone No.: [REDACTED]

5.4.2 Reporting Requirements for Serious Adverse Events and Non-Serious Adverse Events of Special Interest

For reports of SAEs and non-serious AESIs, investigators should record all case details that can be gathered immediately (i.e., within 24 hours) on the Adverse Event eCRF and submit the report via the electronic data capture (EDC) system. A report will be generated and sent to the Sponsor's Safety Risk Management department by the EDC system.

In the event that the EDC system is unavailable, a paper Serious Adverse Event Reporting Form and fax cover sheet should be completed and faxed to Safety Risk Management or its designee immediately (i.e., no more than 24 hours after learning of the event), using the fax numbers provided below. Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

Sites:

Fax No.: (650) 225-4682

Alternate Fax No.: (650) 225-5288

5.4.3 Reporting Requirements for Pregnancies

5.4.3.1 Pregnancies in Female Patients

Female patients of childbearing potential will be instructed to immediately inform the investigator if they become pregnant within 30 (± 7) days after the last dose of study drug. A Pregnancy Report eCRF should be completed by the investigator immediately (i.e., no more than 24 hours after learning of the pregnancy) and submitted via the EDC system. A pregnancy report will automatically be generated and sent to Safety Risk Management. Pregnancy should not be recorded on the Adverse Event eCRF. The investigator should counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Any SAEs associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported on the Adverse Event eCRF.

In the event that the EDC system is unavailable, a Clinical Trial Pregnancy Reporting Form and fax cover sheet should be completed and faxed to Safety Risk Management or its designee immediately (i.e., no more than 24 hours after learning of the pregnancy), using the fax numbers provided in Section 5.4.2. Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

5.4.3.2 Congenital Anomalies/Birth Defects and Abortions

Any congenital anomaly/birth defect in a child born to a female patient exposed to study drug should be classified as an SAE, recorded on the Adverse Event eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.2). Any spontaneous abortion should be reported in the same fashion (as the Sponsor considers spontaneous abortions to be medically significant events).

5.5 FOLLOW-UP OF PATIENTS AFTER ADVERSE EVENTS

5.5.1 Investigator Follow-Up

The investigator should follow each AE until the event has resolved to baseline grade or better and is assessed as stable by the investigator, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all SAEs considered to be related to study drug or trial-related procedures until a final outcome can be reported.

During the study period, resolution of AEs (with dates) should be documented on the Adverse Event eCRF and in the patient's medical record to facilitate source data verification. If, after follow-up, return to baseline status or stabilization cannot be established, an explanation should be recorded on the Adverse Event eCRF.

All pregnancies reported during the study should be followed until pregnancy outcome. If the EDC system is not available at the time of pregnancy outcome, follow reporting instructions provided in Section 5.4.3.

5.5.2 Sponsor Follow-Up

For SAEs, AESIs, and pregnancies, the Sponsor or a designee may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

5.6 POST-STUDY ADVERSE EVENTS

At the time of study completion or study discontinuation, the investigator should instruct each patient to report to the investigator any subsequent AEs that the patient's personal physician believes could be related to prior study drug treatment or study procedures.

The investigator is not required to actively monitor patients for AEs after the end of the AE reporting period (defined as 90 days after administration of study drug). However, the Sponsor should be notified if the investigator becomes aware of any death, development of cancer, or a congenital anomaly/birth defect in a subsequently conceived offspring of a female patient exposed to study drug.

The investigator should report these events directly to Safety Risk Management via telephone at 1-888-835-2555.

5.7 EXPEDITED REPORTING TO HEALTH AUTHORITIES, INVESTIGATORS, INSTITUTIONAL REVIEW BOARDS, AND ETHICS COMMITTEES

The Sponsor will promptly evaluate all SAEs and non-serious AESI against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators, IRBs, ECs, and applicable health authorities based on applicable legislation.

To determine reporting requirements for single AE cases, the Sponsor will assess the expectedness of these events using the following reference document:

- Alteplase USPI

The Sponsor will compare the severity of each event and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.

Reporting requirements will also be based on the investigator's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

6. STATISTICAL METHODS

This study will test whether treatment of patients with mild stroke with IV alteplase will lead to a higher proportion (9% absolute difference) of patients with a favorable outcome (mRS of 0 or 1) at 90 days.

6.1 DETERMINATION OF SAMPLE SIZE

A sample size of 856 is required in order to achieve 80% power in 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 for this 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 size needs to be inflated by a factor of $1/0.95^2$ or 1.108 (Friedman et al. 1998). Therefore, a total sample size of 948 is required for this study.

6.2 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. \geq 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 ITT principle. As a sensitivity analysis, results from the univariate Pearson's chi-square test will also be presented.

As a supportive, secondary analysis, an ordinal (or "shift") analysis of the distribution of the mRS will be analyzed via proportional odds model if the proportionality assumption holds, and by the Mann-Whitney superiority measure if the proportionality assumption does not hold. The proportionality assumption will be assessed using the score test as well as clinical judgment on the similarities of the different odds ratios. *As a secondary efficacy outcome, the global outcome score at 90 days will be analyzed using the global odds ratio test based on the generalized estimating equations analysis (Wald-type χ^2)*

test) with unstructured covariance-correlation matrix. These results will be treated as supportive evidence (or lack thereof) of the treatment effect rather than conclusive evidence. The significance of each test is determined at the two-sided alpha level of 0.05. The specific statistical model for analyzing each of these outcome measures will be detailed in the Statistical Analysis Plan (SAP).

6.3 SAFETY ANALYSIS

Incidence and severity of AEs will be described for all treated patients by treatment group as received and by the Medical Dictionary for Regulatory Activities (MedDRA) classification.

Safety outcomes include incidence of sICH within 36 hours (*primary safety assessment*), any ICH within 36 hours, overall mortality, and stroke-related and neurological deaths within 90 days of study drug initiation. The cumulative incidences of each outcome will be compared via Fisher's exact test. Mortality will be analyzed by Log-Rank test and the Kaplan-Meier estimates will be plotted over the observation period of 90 days. No alpha adjustment for multiple testing on the safety endpoints is foreseen because those analyses will be exploratory.

6.4 EXPLORATORY ANALYSES

As exploratory analyses, heterogeneity 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 (<65 vs. ≥ 65)
- Pre-treatment NIHSS score (0–2 vs. 3–5)
- Last known well time to treatment (0–2 vs. >2 –3 hours)
- Stroke subgroup (RISS vs. non-RISS)

Additional heterogeneity analyses will be considered and detailed in the SAP. Forest plots will be constructed to illustrate subgroup analyses. In addition, the two-way interactions of IV alteplase treatment effect with age, NIHSS score, and stroke subgroups (RISS vs. non-RISS) on the primary outcome will be explored through multivariable logistic regression. Additional exploratory analyses will be conducted to explore treatment effects on quality of life endpoints and other exploratory endpoints.

6.5 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.

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

An iDMC will be organized for review of the interim analysis results. The composition of the iDMC and analysis in detail will be documented in a separate iDMC charter.

6.6 MISSING DATA HANDLING

Under the ITT principle, all patients who are randomized will be included in the analysis. The multiple-imputation method will be used for handling missing data, including the last observation (*e.g.*, the Day 30 mRS) among the variables used in imputation. As supportive analyses, missing primary outcome data will be imputed by 1) using last observation carried forward and 2) assuming missing outcomes to be unfavorable.

7. DATA COLLECTION AND MANAGEMENT

7.1 DATA QUALITY ASSURANCE

The Sponsor will supply eCRF specifications for this study. A contract research organization (CRO) will be responsible for data management of this study, including quality checking of the data. Data entered manually will be collected via EDC using eCRFs. Sites will be responsible for data entry into the EDC system. In the event of discrepant data, the CRO will request data clarification from the sites, which the sites will resolve electronically in the EDC system.

The CRO will produce a Data Quality Plan that describes the quality checking to be performed on the data with the input from the Sponsor. For this study, local laboratories will be used and data will be captured in the EDC system and will then be transferred to the Sponsor. This will include the eCRF data using the Sponsor's standard procedures and processes to transfer data.

The Sponsor will perform oversight of the data management of this study, including approval of the CRO's data management plans and specifications. Data will be transferred electronically from the CRO to the Sponsor, and the Sponsor's standard procedures will be used to handle and process the electronic transfer of these data.

eCRFs and correction documentation will be maintained in the EDC system's audit trail. The EDC vendor has back-ups for data stored, and records retention for the study data will be consistent with the CRO's standard procedures.

Data from paper PRO questionnaires will be entered into the EDC system by site staff.

7.2 ELECTRONIC CASE REPORT FORMS

eCRFs are to be completed using a Sponsor-designated EDC system. Sites will receive training and have eCRF completion guidelines for the eCRF completion. eCRFs will be submitted electronically to the Sponsor and should be handled in accordance with instructions from the Sponsor.

All eCRFs should be completed by designated trained site staff. eCRFs should be reviewed and electronically signed and dated by the investigator or a designee.

At the end of the study, the investigator will receive patient data for his or her site in a readable format on a compact disc that must be kept with the study records.

Acknowledgement of receipt of the compact disc is required.

7.3 SOURCE DATA DOCUMENTATION

Study monitors will perform ongoing source data verification to confirm that critical protocol data (e.g., source data) entered into the eCRFs by authorized site personnel are accurate, complete, and verifiable from source documents. Source documents (paper or electronic) are those in which patient data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, patient-reported outcomes, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, patient files, and records kept at pharmacies, laboratories, and medico-technical departments involved in a clinical trial.

Before study initiation, the types of source documents that are to be generated will be clearly defined in the Study Trial Monitoring Plan. This includes any protocol data to be entered directly into the eCRFs (i.e., no prior written or electronic record of the data) and considered source data.

Source documents that are required to verify the validity and completeness of data entered into the eCRFs must not be obliterated or destroyed and must be retained per the policy for retention of records described in Section [7.5](#).

To facilitate source data verification, the investigators and institutions must provide the Sponsor direct access to applicable source documents and reports for trial-related monitoring, Sponsor audits, and IRB/EC review. The investigational site must also allow inspection by applicable health authorities.

7.4 USE OF COMPUTERIZED SYSTEMS

When clinical observations are entered directly into an investigational site's computerized medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve as the source document if the system has been validated in accordance with health authority requirements pertaining to computerized systems used in clinical research. An acceptable computerized data collection system allows preservation of the original entry of data. If original data are modified, the system should maintain a viewable audit trail that shows the original data as well as the reason for the change, name of the person making the change, and date of the change.

7.5 RETENTION OF RECORDS

Records and documents pertaining to the conduct of this study and the distribution of IMP, including eCRFs, PRO data (if applicable), Informed Consent Forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for at least 15 years after completion or discontinuation of the study, or for the length of time required by relevant national or local health authorities, whichever is longer. After that period of time, the documents may be destroyed, subject to local regulations.

No records may be disposed of without the written approval of the Sponsor. Written notification should be provided to the Sponsor prior to transferring any records to another party or moving them to another location.

8. ETHICAL CONSIDERATIONS

8.1 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in full conformance with the International Conference on Harmonisation E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the International Conference on Harmonisation E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Studies conducted in the United States or under a U.S. Investigational New Drug (IND) application will comply with FDA regulations and applicable local, state, and federal laws. Studies conducted in the European Union (EU)/European Economic Area (EEA) will comply with the EU Clinical Trial Directive (2001/20/EC).

8.2 INFORMED CONSENT

The Sponsor's sample Informed Consent Form (and ancillary sample Informed Consent Forms such as a Child's Assent or Caregiver's Informed Consent Form, if applicable) will be provided to each site. If applicable, it will be provided in a certified translation of the local language. The Sponsor or its designee must review and approve any proposed deviations from the Sponsor's sample Informed Consent Forms or any alternate consent forms proposed by the site (collectively, the "Consent Forms") before IRB/EC submission. The final IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes according to local requirements.

The Consent Forms should be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate. The final revised IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes.

Patients must be re-consented to the most current version of the Consent Forms (or to a significant new information/findings addendum in accordance with applicable laws and

IRB/EC policy) during their participation in the study. For any updated or revised Consent Forms, the case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

A copy of each signed Consent Form must be provided to the patient or the patient's legally authorized representative. All signed and dated Consent Forms must remain in each patient's study file or in the site file and must be available for verification by study monitors at any time.

For sites in the U.S., each Consent Form may also include patient authorization to allow use and disclosure of personal health information in compliance with the U.S. Health Insurance Portability and Accountability Act of 1996 (HIPAA). If the site utilizes a separate Authorization Form for patient authorization for use and disclosure of personal health information under the HIPAA regulations, the review, approval, and other processes outlined above apply except that IRB review and approval may not be required per study site policies.

8.3 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE

This protocol, the Informed Consent Forms, any information to be given to the patient, and relevant supporting information must be submitted to the IRB/EC by the Principal Investigator and reviewed and approved by the IRB/EC before the study is initiated. In addition, any patient recruitment materials must be approved by the IRB/EC.

The Principal Investigator is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. Investigators are also responsible for promptly informing the IRB/EC of any protocol amendments (Section 9.5).

In addition to the requirements for reporting all AEs to the Sponsor, investigators must comply with requirements for reporting SAEs to the local health authority and IRB/EC. Investigators may receive written IND safety reports or other safety-related communications from the Sponsor. Investigators are responsible for ensuring that such reports are reviewed and processed in accordance with health authority requirements and the policies and procedures established by their IRB/EC, and archived in the site's study file.

8.4 CONFIDENTIALITY

The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient ID number. This means that patient names are not included in data sets that are transmitted to any Sponsor location.

Patient medical information obtained by this study is confidential and may only be disclosed to third parties as permitted by the Informed Consent Form (or separate

authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law.

Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare, for treatment purposes.

Data generated by this study must be available for inspection upon request by representatives of the FDA and other national and local health authorities, Sponsor monitors, representatives, and collaborators, and the IRB/EC for each study site, as appropriate.

8.5 FINANCIAL DISCLOSURE

Investigators will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate health authorities.

Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study (i.e., LPLV).

9. STUDY DOCUMENTATION, MONITORING, AND ADMINISTRATION

9.1 STUDY DOCUMENTATION

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including but not limited to the protocol, protocol amendments, Informed Consent Forms, and documentation of IRB/EC and governmental approval. In addition, at the end of the study, the investigator will receive the patient data, which includes an audit trail containing a complete record of all changes to data.

9.2 PROTOCOL VIOLATIONS

The investigator should document and explain any protocol violations. The investigator should promptly report any violations that might impact patient safety and data integrity to the Sponsor and to the IRB/EC in accordance with established IRB/EC policies and procedures.

9.3 SITE INSPECTIONS

Site visits will be conducted by the Sponsor or an authorized representative for inspection of study data, patients' medical records, and eCRFs. The investigator will permit national and local health authorities, Sponsor monitors, representatives, and collaborators, and the IRBs/ECs to inspect facilities and records relevant to this study.

9.4 ADMINISTRATIVE STRUCTURE

Genentech, Inc., is the Sponsor and will have oversight of study management, including project management, oversight of data management, and statistical programming. A CRO will assist with study management, including project management, medical monitoring (as needed), clinical monitoring, data monitoring, and data management. Patients will be enrolled at approximately 75 sites in *North America*.

The CRO will manage site activation, study drug supply, patient enrollment and tracking, and clinical monitoring. Genentech will handle study drug distribution.

A Steering Committee will provide scientific oversight for this study. The Steering Committee will be composed of Genentech representatives and external advisors. The Steering Committee will perform functions such as:

- Provide recommendations regarding changes in the study conduct
- Review results of the final analyses for primary and secondary endpoints
- Participate in the drafting and publishing of final study results

An iDMC will be responsible for monitoring the overall efficacy and safety of the patients in the trial after treatment with alteplase. The iDMC will be composed of external advisors. The iDMC will perform functions such as:

- Review all accumulated safety data at interim analysis
- Review efficacy data analysis performed for interim analysis for consideration of potential discontinuation of the trial for futility
- Make recommendation to the Sponsor as to if it is appropriate to discontinue the study after assessing the interim efficacy and safety data in aggregate
- Determine if the study should continue after review of interim analysis results

An iDCC will be responsible for the preparation and review of unmasked data.

A central IRB will be utilized when possible to ensure the timely submission and approval of site regulatory documents for sites not required to use a local IRB. The CRO will be responsible for management of the central IRB.

An EDC system will be used to capture data for the study.

An IWRS will be utilized for randomization, patient tracking, and drug inventory management for the study.

9.5 PROTOCOL AMENDMENTS

Any protocol amendments will be prepared by the Sponsor. Protocol amendments will be submitted to the IRB/EC and to regulatory authorities in accordance with local regulatory requirements.

Approval must be obtained from the IRB/EC and regulatory authorities (as locally required) before implementation of any changes, except for changes necessary to eliminate an immediate hazard to patients or changes that involve logistical or administrative aspects only (e.g., change in Medical Monitor or contact information).

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Appendix 1

Schedule of Assessments

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 (± 14) Days/Study Completion (Visit 5)	Early Discontinuation (Phone call, if possible)
Informed consent	X						
Review of eligibility	X						
Medical history and baseline conditions ^a	X						
Demographics	X						
Neurological 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					

Appendix 1 (cont.) Schedule of Assessments

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 (± 14) Days/Study Completion (Visit 5)	Early Discontinuation (Phone call, if possible)
Discharge location				X			X
Patient survival					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 assessments ^l						X	
Walking 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; SW MRI=susceptibility-weighted MRI; WAIS III=Wechsler Adult Intelligence Scale III.

Appendix 1 (cont.) Schedule of Assessments

- ^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.
- ^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 walking 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.*

Appendix 2 Alteplase Administration

Administration of bolus



Step 1:

Inspect solution

After reconstitution to 1 mg/mL, inspect solution for particulate matter and discoloration prior to administration

Step 2:

Discard excess

To ensure proper dosing, discard excess by removing from vial any quantity of drug in excess of that specified for patient treatment. When drawing off excess solution, be sure to insert the needle into the peripheral area of the vial top, away from the puncture site caused by the transfer device

Step 3:

Prepare bolus

Withdraw 10% of the 0.9-mg/kg dose in one of the following ways:

- Remove from vial using a syringe and needle,
- Remove from port (second injection site) on infusion line after infusion set is primed, or
- Program infusion pump to deliver bolus at infusion initiation

Step 4:

Administer bolus

Administer initial IV bolus over 1 minute

Administration of remainder of dose

Infuse the remaining 90% of 0.9-mg/kg dose over 60 minutes.

50-mg vials—Administer using either a polyvinyl chloride bag or glass vial and infusion set.

No medication should be added to the infusion solutions that contain alteplase.

For specifics regarding dosing and administration, please see the Activase full Prescribing Information.

Appendix 3 Reconstitution of 50-mg Vials

A single 50-mg vial of Activase may be used to treat patients weighing up to 122 lbs or 55.5 kilograms. Reconstitute Activase immediately before administration, using aseptic technique at all times—including thorough hand washing and the use of gloves.



Step 1:

Assemble the 50-mg vial of Activase, the vial of Sterile Water for Injection (SWFI), USP (included with the Activase package), a 50-mL syringe, a large-bore needle (eg, 18-gauge), and alcohol wipes. Also, prepare a polyvinyl chloride bag or glass vial along with an infusion set.

Step 2:

Remove the protective cap from the top of the Activase vial and the vial of SWFI. Swab the top of each vial with an alcohol wipe to reduce the risk of contamination.

Step 3:

Withdraw 50-mL of SWFI, without preservatives. Diluent is included. DO NOT USE Bacteriostatic Water for Injection, USP

Step 4:

Inject the 50-mL into the 50-mg Activase vial using a large-bore needle and syringe, directing the stream into the lyophilized cake. DO NOT USE IF VACUUM IS NOT PRESENT. The syringe should not be primed with air during preparation and should be inserted into the Activase vial stopper. Slight foaming upon reconstitution is normal. Let the solution stand undisturbed for several minutes to allow any large bubbles to dissipate

Step 5:

Mix with a gentle swirl or slow inversion. DO NOT SHAKE. Visually inspect the Activase solution for particulate matter and discoloration before administration

Activase is stable for up to 8 hours in these solutions at room temperature. Do not freeze solutions containing Activase. No other medications should be added to infusions containing Activase. Any unused infusion solution should be discarded.

Appendix 4

European Quality of Life-5D (EQ-5D) Questionnaire

By placing a checkmark in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about

I have some problems in walking about

I am confined to bed

Self-Care

I have no problems with self-care

I have some problems washing or dressing myself

I am unable to wash or dress myself

Usual Activities (e.g. work, study, housework, family or leisure activities)

I have no problems with performing my usual activities

I have some problems with performing my usual activities

I am unable to perform my usual activities

Pain/Discomfort

I have no pain or discomfort

I have moderate pain or discomfort

I have extreme pain or discomfort

Anxiety/Depression

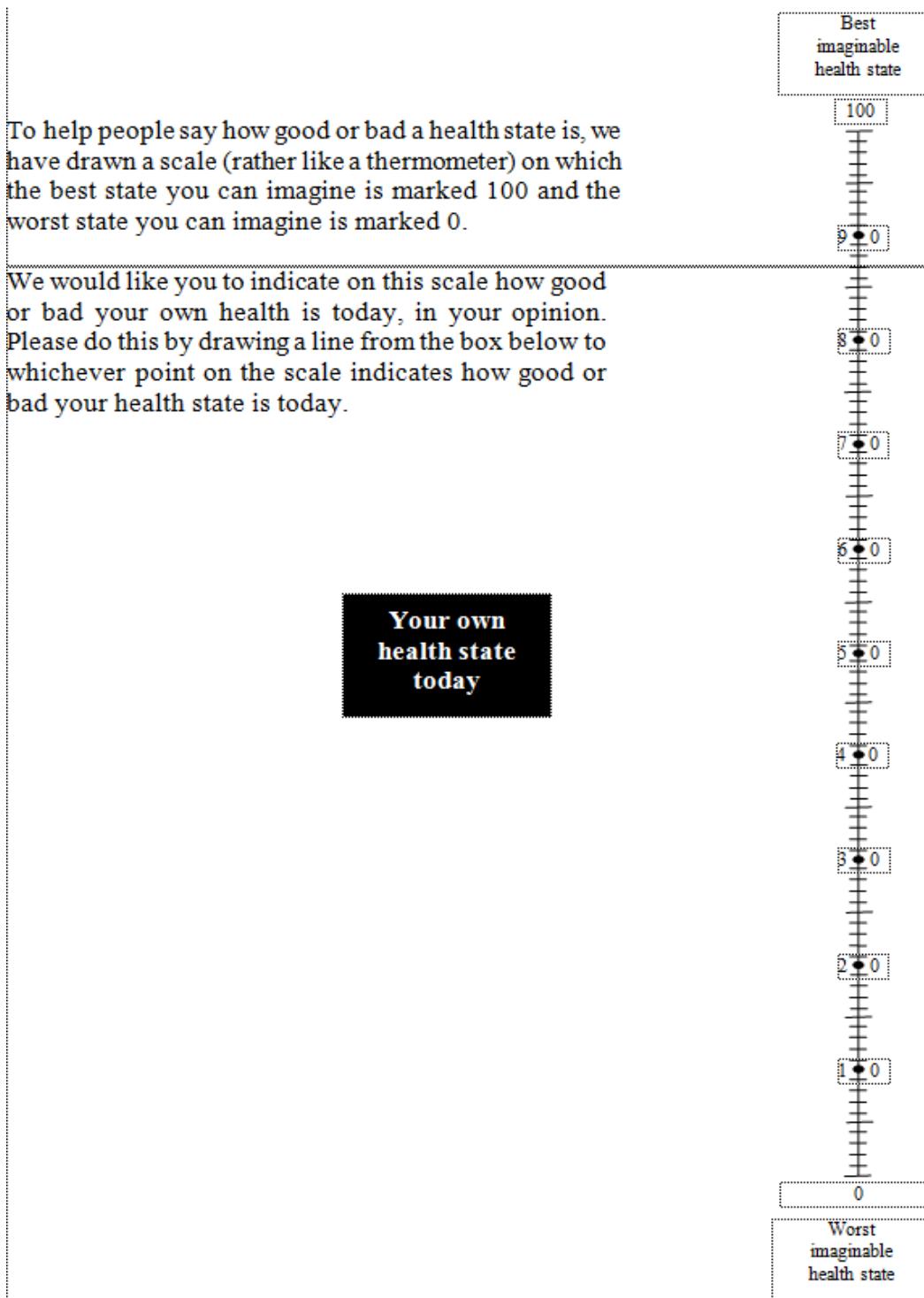
I am not anxious or depressed

I am moderately anxious or depressed

I am extremely anxious or depressed

Appendix 4 (cont.)

European Quality of Life-5D (EQ-5D) Questionnaire



Appendix 5

Stroke Index Scale (SIS-16)

In the past 2 weeks, how difficult was it to...	Not difficult at all	A little difficult	Somewhat difficult	Very difficult	Could not do at all
a. Dress the top part of your body?	5	4	3	2	1
b. Bathe yourself?	5	4	3	2	1
c. Get to the toilet on time?	5	4	3	2	1
d. Control your bladder (not have an accident)?	5	4	3	2	1
e. Control your bowels (not have an accident)?	5	4	3	2	1
f. Stand without losing balance?	5	4	3	2	1
g. Go shopping?	5	4	3	2	1
h. Do heavy household chores (e.g., vacuum, laundry or yard work)?	5	4	3	2	1
i. Stay sitting without losing your balance?	5	4	3	2	1
j. Walk without losing your balance?	5	4	3	2	1
k. Move from a bed to a chair?	5	4	3	2	1
l. Walk fast?	5	4	3	2	1
m. Climb one flight of stairs?	5	4	3	2	1
n. Walk one block?	5	4	3	2	1
o. Get in and out of a car?	5	4	3	2	1
p. Carry heavy objects (e.g. bag of groceries) with your affected hand?	5	4	3	2	1

Appendix 6

Center for Epidemiologic Studies, Depression (CES-D) Scale, NIMH

Below is a list of the ways you might have felt or behaved. Please tell me how often you have felt this way during the past week.

Week	During the Past			
	Rarely or none of the time (less than 1 day)	Some or a little of the time (1-2 days)	Occasionally or a moderate amount of time (3-4 days)	Most or all of the time (5-7 days)
1. I was bothered by things that usually don't bother me.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2. I did not feel like eating; my appetite was poor.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3. I felt that I could not shake off the blues even with help from my family or friends.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4. I felt I was just as good as other people.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5. I had trouble keeping my mind on what I was doing.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6. I felt depressed.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7. I felt that everything I did was an effort.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8. I felt hopeful about the future.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9. I thought my life had been a failure.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10. I felt fearful.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11. My sleep was restless.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12. I was happy.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
13. I talked less than usual.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
14. I felt lonely.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
15. People were unfriendly.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
16. I enjoyed life.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
17. I had crying spells.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
18. I felt sad.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
19. I felt that people dislike me.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
20. I could not get "going."	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SCORING: zero for answers in the first column, 1 for answers in the second column, 2 for answers in the third column, 3 for answers in the fourth column. The scoring of positive items is reversed. Possible range of scores is zero to 60, with the higher scores indicating the presence of more symptomatology.

Appendix 7

National Institutes of Health Stroke Scale (NIHSS)

<p>Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).</p>		
1	<p>Has the subject had a stroke (either as the index event at randomization or as an outcome event)? If no, form is complete.</p>	<input type="radio"/> No <input type="radio"/> Yes
2	<p>(1a) Level of Consciousness</p> <p>The Investigator must choose a response, even if a full evaluation is prevented by such obstacles as an endotracheal tube, language barrier, orotracheal trauma/bandages. A 3 is scored only if the patient makes no movement (other than reflexive posturing) in response to noxious stimulation. Coma score "3".</p>	<input type="radio"/> 0=Alert; keenly responsive <input type="radio"/> 1=Not alert, but arousable by minor stimulation to obey, answer or respond <input type="radio"/> 2=Not alert, requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped) <input type="radio"/> 3=Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, areflexic (Complete form using coma scoring)
3	<p>(1b) LOC Questions</p> <p>The patient is asked the month and his/her age. The answer must be correct - there is no partial credit for being close. Aphasic and stuporous patients who do not comprehend the questions will score 2. Patients unable to speak because of endotracheal intubation, orotracheal trauma, severe dysarthria from any cause, language barrier or any other problem not secondary to aphasia are given a 1. It is important that only the initial answer be graded and that the examiner not "help" the patient with verbal or non-verbal cues. Coma score "2".</p>	<input type="radio"/> 0=Answers both questions correctly <input type="radio"/> 1=Answers one question correctly <input type="radio"/> 2=Answers neither question correctly
4	<p>(1c) LOC Commands</p> <p>The patient is asked to open and close the eyes and then to grip and release the non-paretic hand. Substitute another one step command if the hands cannot be used. Credit is given if an unequivocal attempt is made but not completed due to weakness. If the patient does not respond to command, the task should be demonstrated to them (pantomime) and score the result (i.e., follows none, one or two commands). Patients with trauma, amputation, or other physical impediments should be given suitable one-step commands. Only the first attempt is scored. Coma score "2".</p>	<input type="radio"/> 0=Performs both tasks correctly <input type="radio"/> 1=Performs one task correctly <input type="radio"/> 2=Performs neither task correctly
5	<p>(2) Best Gaze</p> <p>Only horizontal eye movements will be tested. Voluntary or reflexive (oculocephalic) eye movements will be scored but caloric testing is not done. If the patient has a conjugate deviation of the eyes that can be overcome by voluntary or reflexive activity, the score will be 1. If a patient has an isolated peripheral nerve paresis (CN III, IV or VI) score a 1. Gaze is testable in all aphasic patients. Patients with ocular trauma, bandages, pre-existing blindness or other disorder of visual acuity or fields should be tested with reflexive movements and a choice made by the investigator. Establishing eye contact and then moving about the patient from side to side will occasionally clarify the presence of a partial gaze palsy. Coma score as examined.</p>	<input type="radio"/> 0=Normal <input type="radio"/> 1=Partial gaze palsy. This score is given when gaze is abnormal in one or both eyes, but where forced deviation or total gaze paresis are not present <input type="radio"/> 2=Forced deviation, or total gaze paresis not overcome by the oculocephalic maneuver

Appendix 7 (cont.)

National Institutes of Health Stroke Scale (NIHSS)

8	<p style="text-align: center;">(3) Visual</p> <p>Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat as appropriate. Patient must be encouraged, but if they look at the side of the moving finger appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia is found. If patient is blind from any cause score 3. Double simultaneous stimulation is performed at this point. If there is extinction patient receives a 1 and the results are used to answer question 22. Score as examined, using bilateral threat.</p>	<input type="radio"/> 0=No visual loss <input type="radio"/> 1=Partial hemianopia <input type="radio"/> 2=Complete hemianopia <input type="radio"/> 3=Bilateral hemianopia (blind including cortical blindness)
7	<p style="text-align: center;">(4) Facial Palsy</p> <p>Ask, or use pantomime to encourage the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barrier obscures the face, these should be removed to the extent possible. Coma Score "3".</p>	<input type="radio"/> 0=Normal symmetrical movement <input type="radio"/> 1=Minor paralysis (flattened nasolabial fold, asymmetry of smiling) <input type="radio"/> 2=Partial paralysis (total or near total paralysis of lower face) <input type="radio"/> 3=Complete paralysis of one or both sides (absence of facial movement in the upper and lower face)
8	<p style="text-align: center;">(5a) Motor Arm Left:</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma Score "4".</p>	<input type="radio"/> 0=No drift, limb holds 90 (or 45) degrees for full 10 seconds <input type="radio"/> 1=Drift, limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed <input type="radio"/> 2=Some effort against gravity, limb cannot get to or maintain (if cued) 90 degrees <input type="radio"/> 3=No effort against gravity, limb falls <input type="radio"/> 4=No movement <input type="radio"/> Amputation, joint fusion
9	<p style="text-align: center;">Explain if amputation or joint fusion (Motor Arm Left):</p>	
10	<p style="text-align: center;">(5b) Motor Arm Right</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma score "4".</p>	<input type="radio"/> 0=No drift, limb holds 90 (or 45) degrees for full 10 seconds <input type="radio"/> 1=Drift, limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed <input type="radio"/> 2=Some effort against gravity, limb cannot get to or maintain (if cued) 90 degrees <input type="radio"/> 3=No effort against gravity, limb falls <input type="radio"/> 4=No movement <input type="radio"/> Amputation, joint fusion
11	<p style="text-align: center;">Explain if amputation or joint fusion (Motor Arm Right):</p>	

Appendix 7 (cont.)

National Institutes of Health Stroke Scale (NIHSS)

12	<p style="text-align: center;">(8a) Motor Leg Left</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma score "4".</p>	<input type="radio"/> 0=No drift, leg holds 30 degrees position for full 5 seconds <input type="radio"/> 1=Drift, leg falls by the end of the 5 second period but does not hit bed <input type="radio"/> 2=Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity <input type="radio"/> 3=No effort against gravity; leg falls to bed immediately <input type="radio"/> 4>No movement <input type="radio"/> Amputation, joint fusion
13	Explain if amputation/ joint fusion (Motor Leg Left):	
14	<p style="text-align: center;">(8b) Motor Leg Right</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma score "4".</p>	<input type="radio"/> 0=No drift, leg holds 30 degrees position for full 5 seconds <input type="radio"/> 1=Drift, leg falls by the end of the 5 second period but does not hit bed <input type="radio"/> 2=Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity <input type="radio"/> 3=No effort against gravity; leg falls to bed immediately <input type="radio"/> 4>No movement <input type="radio"/> Amputation, joint fusion
15	Explain if amputation/ joint fusion (Motor Leg Right):	
16	<p style="text-align: center;">(7) Limb Ataxia</p> <p>This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, insure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position. Coma score "0".</p>	<input type="radio"/> 0 = Absent <input type="radio"/> 1 = Present in one limb <input type="radio"/> 2 = Present in two limbs <input type="radio"/> UN = amputation or joint fusion
17	Explain if amputation or joint fusion (Limb Ataxia):	

Appendix 7 (cont.)

National Institutes of Health Stroke Scale (NIHSS)

18	<p style="text-align: center;">(8) Sensory</p> <p>Sensation or grimace to pin prick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas [arms (not hands), legs, trunk, face] as needed to accurately check for hemisensory loss. A score of 2, "severe or total," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will therefore probably score 1 or 0. The patient with brain stem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic score 2. Patients in coma (item 1a-3) are arbitrarily given a 2 on this item. Coma Score "2".</p>	<input type="radio"/> 0=Normal; no sensory loss <input type="radio"/> 1=Mild to moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick but patient is aware he/she is being touched <input type="radio"/> 2=Severe to total sensory loss; patient is not aware of being touched in the face, arm & leg
19	<p style="text-align: center;">(9) Best Language</p> <p>A great deal of information about comprehension will be obtained during the preceding sections of the examination. The patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet, and to read from the attached list of sentences. Comprehension is judged from responses here as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in coma (question 1a-3) will arbitrarily score 3 on this item. The examiner must choose a score in the patient with stupor or limited cooperation but a score of 3 should be used only if the patient is mute and follows no one step commands. Coma Score "3".</p>	<input type="radio"/> 0>No aphasia, normal <input type="radio"/> 1=Mild to moderate aphasia; some obvious loss of fluency or facility of comprehension without significant limitation on ideas expressed on form of expression <input type="radio"/> 2=Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener; listener carries burden of communication <input type="radio"/> 3=Mute, global aphasia; no usable speech or auditory comprehension
20	<p style="text-align: center;">(10) Dysarthria</p> <p>If patient is thought to be normal an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barrier to producing speech, may the item be not scored, and the examiner must clearly write an explanation. Do not tell the patient why he/she is being tested. Coma Score "2".</p>	<input type="radio"/> 0=Normal <input type="radio"/> 1=Mild to moderate; patient slurs at least some words and at worst, can be understood with some difficulty <input type="radio"/> 2=Severe; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/ anarthric <input type="radio"/> Intubated or other physical barrier.
21	Explain if intubated or other physical barrier (Dysarthria):	
22	<p style="text-align: center;">(11) Extinction and Inattention (Neglect)</p> <p>Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosognosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable. Coma Score "2".</p>	<input type="radio"/> 0>No abnormality <input type="radio"/> 1=Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities <input type="radio"/> 2=Profound hemi-inattention or hemi-inattention to more than one modality; does not recognize own hand or orients to only one side of space
23	Not applicable	
24	<p>Name of assessor:</p> <p>The assessor must be a study team member who has completed NIHSS certification.</p>	

Appendix 8

Modified Rankin Scale (mRS)

1	Modified Rankin Scale:	<p><input type="radio"/> (0) No symptoms at all</p> <p><input type="radio"/> (1) No significant disability despite symptoms; able to carry out all usual duties and activities</p> <p><input type="radio"/> (2) Slight disability; unable to carry out all previous activities but able to look after own affairs without assistance</p> <p><input type="radio"/> (3) Moderate disability requiring some help, but able to walk without assistance</p> <p><input type="radio"/> (4) Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance</p> <p><input type="radio"/> (5) Severe disability; bedridden, incontinent, and requiring constant nursing care and attention</p>
2	For response 2 above, specify the previous activities that can no longer be carried out:	
3	Name of assessor: The assessor must be a study team member who has completed mRS certification.	

Appendix 9 Glasgow Outcome Scale (GOS)

Note: The scale presented here is based on the original article by Jennett and Bond. It has become common practice in clinical trial administration, however, to use a modified version that places the scores in reverse order (i.e., "good recovery" = 1, "moderate disability" = 2, etc.).

Score	Description
1	DEATH
2	PERSISTENT VEGETATIVE STATE Patient exhibits no <i>obvious cortical function</i> .
3	SEVERE DISABILITY (Conscious but disabled). Patient depends upon others for daily support due to mental or physical disability or both.
4	MODERATE DISABILITY (Disabled but independent). Patient is independent as far as daily life is concerned. The disabilities found include varying degrees of dysphasia, hemiparesis, or ataxia, as well as intellectual and memory deficits and personality changes.
5	GOOD RECOVERY Resumption of normal activities even though there may be minor neurological or psychological deficits.

TOTAL (1-5): _____

References

Jennett B, Bond M. "Assessment of outcome after severe brain damage." *Lancet* 1975 Mar 1;1(7905):480-4

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Appendix 10

Barthel Index (BI)

THE BARTHEL INDEX

Patient Name: _____
 Rater Name: _____
 Date: _____

Activity	Score
FEEDING 0 = unable 5 = needs help cutting, spreading butter, etc., or requires modified diet 10 = independent	_____
BATHING 0 = dependent 5 = independent (or in shower)	_____
GROOMING 0 = needs to help with personal care 5 = independent face/hair/teeth/shaving (implements provided)	_____
DRESSING 0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	_____
BOWELS 0 = incontinent (or needs to be given enemas) 5 = occasional accident 10 = continent	_____
BLADDER 0 = incontinent, or catheterized and unable to manage alone 5 = occasional accident 10 = continent	_____
TOILET USE 0 = dependent 5 = needs some help, but can do something alone 10 = independent (on and off, dressing, wiping)	_____
TRANSFERS (BED TO CHAIR AND BACK) 0 = unable, no sitting balance 5 = major help (one or two people, physical), can sit 10 = minor help (verbal or physical) 15 = independent	_____
MOBILITY (ON LEVEL SURFACES) 0 = immobile or < 50 yards 5 = wheelchair independent, including corners, > 50 yards 10 = walks with help of one person (verbal or physical) > 50 yards 15 = independent (but may use any aid; for example, stick) > 50 yards	_____
STAIRS 0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	_____
	TOTAL (0-100): _____

Appendix 10 (cont.) **Barthel Index (BI)**

The Barthel ADL Index: Guidelines

1. The index should be used as a record of what a patient does, not as a record of what a patient could do.
2. The main aim is to establish degree of independence from any help, physical or verbal, however minor and for whatever reason.
3. The need for supervision renders the patient not independent.
4. A patient's performance should be established using the best available evidence. Asking the patient, friends/relatives and nurses are the usual sources, but direct observation and common sense are also important. However direct testing is not needed.
5. Usually the patient's performance over the preceding 24-48 hours is important, but occasionally longer periods will be relevant.
6. Middle categories imply that the patient supplies over 50 per cent of the effort.
7. Use of aids to be independent is allowed.

Appendix 11 Cognitive and Behavioral Assessments

A. Controlled Oral Word Association Test

Letter L			
Instruct the study participant as follows: <i>"I am going to say a letter and I want you to name, as fast as you can, as many words as you can think of which start with this letter. The letter is L. You will have one minute. Ready? Begin."</i>			
Record the study participant's responses in the following table.			
	Acceptable		Acceptable
1.	<input type="checkbox"/>	21.	<input type="checkbox"/>
2.	<input type="checkbox"/>	22.	<input type="checkbox"/>
3.	<input type="checkbox"/>	23.	<input type="checkbox"/>
4.	<input type="checkbox"/>	24.	<input type="checkbox"/>
5.	<input type="checkbox"/>	25.	<input type="checkbox"/>
6.	<input type="checkbox"/>	26.	<input type="checkbox"/>
7.	<input type="checkbox"/>	27.	<input type="checkbox"/>
8.	<input type="checkbox"/>	28.	<input type="checkbox"/>
9.	<input type="checkbox"/>	29.	<input type="checkbox"/>
10.	<input type="checkbox"/>	30.	<input type="checkbox"/>
11.	<input type="checkbox"/>	31.	<input type="checkbox"/>
12.	<input type="checkbox"/>	32.	<input type="checkbox"/>
13.	<input type="checkbox"/>	33.	<input type="checkbox"/>
14.	<input type="checkbox"/>	34.	<input type="checkbox"/>
15.	<input type="checkbox"/>	35.	<input type="checkbox"/>
16.	<input type="checkbox"/>	36.	<input type="checkbox"/>
17.	<input type="checkbox"/>	37.	<input type="checkbox"/>
18.	<input type="checkbox"/>	38.	<input type="checkbox"/>
19.	<input type="checkbox"/>	39.	<input type="checkbox"/>
20.	<input type="checkbox"/>	40.	<input type="checkbox"/>
Total words generated: <input type="text"/>			
Total acceptable words: <input type="text"/>			

Appendix 11 (cont.) Cognitive and Behavioral Assessments

B . Hopkins Verbal Learning Test-Revised (HVLT-R)

Form 1

Semantic Categories: Four-Legged Animals, Precious Stones, Human Dwellings

Name _____ Sex _____ Age _____ years _____ months

Examiner _____ Date / /

Word List	Learning Trials				Delayed Recall Trial (20-25 min.)
	Trial 1	Trial 2	Trial 3	Trial 4	
LION					
EMERALD					
HORSE					
TENT					
SAPPHIRE					
HOTEL					
CAVE					
OPAL					
TIGER					
PEARL					
COW					
HUT					

Total correct
responses =

Completion Time _____ Start Time _____
Trial 3 _____ Trial 4 _____

→ 3

Appendix 11 (cont.) Cognitive and Behavioral Assessments

Delayed Recognition Trial Instructions

The Delayed Recognition (Forced Choice) trial is administered immediately after the Delayed Recall trial. Say the following:

Now I am going to read a longer list of words to you. Some of them are words from the original list, and some are not. After I read each word, I'd like you to say "Yes" if it was on the original list, or "No" if it was not.

Read the words of the Delayed Recognition trial list in numerical order. Allow the individual as much time as needed to respond. You may use the prompt, "Was horse on the list? Yes or no?" The individual must give you a response for every word. If the individual is not sure, ask for a guess.

Delayed Recognition Trial (Forced Choice)							
1. HORSE	Y N	7. house	Y N	13. HUT	Y N	19. TENT	Y N
2. ruby	Y N	8. OPAL	Y N	14. EMERALD	Y N	20. mountain	Y N
3. CAVE	Y N	9. TIGER	Y N	15. SAPPHIRE	Y N	21. cat	Y N
4. balloon	Y N	10. boat	Y N	16. dog	Y N	22. HOTEL	Y N
5. coffee	Y N	11. scarf	Y N	17. apartment	Y N	23. COW	Y N
6. LION	Y N	12. PEARL	Y N	18. penny	Y N	24. diamond	Y N

Total number of true-positive responses ("hits"): _____ /12 (no shading)

Semantically-related false-positive errors: _____ /6 (light shading)

Semantically-unrelated false-positive errors: _____ /6 (darker shading)

Total number of false-positive errors: _____ /12

	Raw score	T score
Total Recall (sum of total correct responses for Trials 1, 2, & 3)		
Delayed Recall (Trial 4)		
Retention (%) [(Trial 4 ÷ Higher score of Trials 2 and 3) x 100]		
Recognition Discrimination Index (Total no. of true-positives) – (Total no. of false-positives)		

Normative table (Appendix A): _____

4

Appendix 11 (cont.) Cognitive and Behavioral Assessments

C. Digit Symbol Coding from the WAIS III

DIGIT SYMBOL SUBSTITUTION (DSS) TASK

DIGIT SYMBOL	1	2	3	4	5	6	7	8	9
	—	上	□	└	□	○	∧	×	=

6	2	5	1	9	2	8	3	7	4	6	5	9	4	8	3	7	2	6	1	5	4	6	3	7
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Appendix 11 (cont.) Cognitive and Behavioral Assessments

D. Forward and Backward Digit Span Test

FORWARD AND BACKWARD DIGIT SPAN TEST

DIGIT SPAN (FORWARD)*

Ask patient, "Repeat each group of numbers in the same order that I say them." Read numbers at rate of one each second. If patient passes the first trial, advance to the first trial of the next item. If patient fails the first trial, administer the second trial of that item. Stop when patient fails both trials of any item. Score is best performance's maximum number of digits correct.

DIGIT FORWARD	WRITE RESPONSE	CORRECT	
8-4-7		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-7-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No
9-3-4-1		<input type="checkbox"/> Yes	<input type="checkbox"/> No
8-3-9-6		<input type="checkbox"/> Yes	<input type="checkbox"/> No
5-1-6-2-9		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-6-9-2-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No
6-4-3-5-2-7		<input type="checkbox"/> Yes	<input type="checkbox"/> No
9-1-8-4-2-7		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-1-6-9-2-8-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No
2-8-1-4-9-7-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No

DIGIT FORWARD SCORE (3-7)

DIGIT SPAN (BACKWARD)*

Say to patient, "Now I am going to say some more numbers, but this time when I stop, I want you to say them backwards. For example, if I said 7-1-9, what would you say?" Read numbers at rate of one each second. If patient passes the first trial, advance to the first trial of the next item. If patient fails the first trial, administer the second trial of that item. Stop when patient fails both trials of any item. Score is best performance's maximum number of digits correct.

DIGIT FORWARD	WRITE RESPONSE	CORRECT	
5-8		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-1		<input type="checkbox"/> Yes	<input type="checkbox"/> No
6-2-9		<input type="checkbox"/> Yes	<input type="checkbox"/> No
4-9-3		<input type="checkbox"/> Yes	<input type="checkbox"/> No
4-9-6-8		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-8-1-4		<input type="checkbox"/> Yes	<input type="checkbox"/> No
1-5-2-8-6		<input type="checkbox"/> Yes	<input type="checkbox"/> No
6-2-9-7-2		<input type="checkbox"/> Yes	<input type="checkbox"/> No
5-3-9-4-1-8		<input type="checkbox"/> Yes	<input type="checkbox"/> No
7-1-5-2-8-6		<input type="checkbox"/> Yes	<input type="checkbox"/> No

DIGIT BACKWARD SCORE (2-6)

*Wechsler, D. (1987). Wechsler Memory Scale-Revised Manual. San Antonio, TX: Psychological Corporation.

Appendix 11 (cont.) Cognitive and Behavioral Assessments

E. Benton Judgment of Line Orientation

Benton Judgment Of Line Orientation – Form V					
Name _____	No. _____	Date _____	/	/	/
Age _____	Gender _____	Education _____	Handedness _____	Examiner _____	

Instructions: Record each response choice. Circle all errors.

PRACTICE ITEMS

A _____ 1-6	B _____ 4-8	C _____ 4-10	D _____ 7-8	E _____ 2-4
A' _____ 1 6	B' _____ 4 8	C' _____ 4 10	D' _____ 7 8	E' _____ 2 4

TEST ITEMS

1. _____ 5-10 HH	16. _____ 7-8 MM
2. _____ 2-11 MM	17. _____ 3-5 HH
3. _____ 1-2 LL	18. _____ 10-11 MH
4. _____ 1-7 HH	19. _____ 1-4 MM
5. _____ 6-7 HH	20. _____ 3-11 LL
6. _____ 5-6 LL	21. _____ 6-10 LL
7. _____ 4-5 HH	22. _____ 2-9 LL
8. _____ 1-3 MM	23. _____ 3-8 HH
9. _____ 5-11 MM	24. _____ 9-11 HH
10. _____ 1-10 HH	25. _____ 3-4 LM
11. _____ 1-7 MM	26. _____ 8-9 LL
12. _____ 2-6 HH	27. _____ 8-11 HH
13. _____ 7-9 MM	28. _____ 7-10 LL
14. _____ 2-5 HL	29. _____ 3-10 HL
15. _____ 1-9 LL	30. _____ 5-8 HM

Correct

IMPRESSION: _____
OBSERVATIONS: _____
LESION SUMMARY: _____

Appendix 11 (cont.) Cognitive and Behavioral Assessments

F. Animal Naming Test

ANIMAL NAMING

Introduction: "I'd like to ask a question to check your memory."

Instruction: "Tell me the names of as many animals as you can think of, as quickly as possible."

Procedure: Time for 60 seconds and record all responses.
If the person stops before 60 seconds, say "Any more animals?"
If the person says nothing for 15 seconds, say "A dog is an animal.
"Can you tell me more animals?"

1. _____	12. _____
2. _____	13. _____
3. _____	14. _____
4. _____	15. _____
5. _____	16. _____
6. _____	17. _____
7. _____	18. _____
8. _____	19. _____
9. _____	20. _____
10. _____	21. _____
11. _____	22. _____

Scoring: Count the total number of animals (NOT including repetitions or non-animal words): _____

Next step: If the score is less than 14, further testing should be done.

Sager MD, MA; Hermann PhD, BP; LaRue PhD, A; Woodard PhD, JL, Screening for Dementia in Community-based Memory Clinics. Wisconsin Medical Journal 2006;105(7):25-29

Appendix 11 (cont.) Cognitive and Behavioral Assessments

Directions for Scoring Animal Naming Screen

Instructions: "Tell me the names of as many animals as you can think of, as quickly as possible."

If the person says nothing for 15 seconds, say "A dog is an animal. Can you tell me more animals?" If the person stops before 60 seconds, say "Any more animals?"

Scoring: Count all animals, including birds, fish, reptiles, insects, humans, extinct animals, etc. Credit can be given for general category terms (e.g., dog) and for specific instances (e.g., terriers) when both are given. Credit only one item when people name the same animal at different developmental stages (e.g., sheep, lamb).

Note: These minimalist initial instructions date back to an early research study on verbal fluency in aging and dementia by Wilma Rosen (1980) in which subjects were instructed to "give the names of as many animals as you can think of" for 60 seconds. These very brief instructions have been subsequently used in several normative studies (e.g., Tombaugh et al., 1999 and Mayo's MOANS studies such as Lucas et al., 1998) and in some other recent studies of the efficacy of verbal fluency as a diagnostic tool for dementia (e.g., Canning et al., 2004).

Appendix 11 (cont.) Cognitive and Behavioral Assessments

G. Boston Naming Test (BNT) 15-Item Short Form

The following error codes are used to categorize incorrect responses. The error code is to be entered in the designated column below.

ph	Nonword phonemically based paraphasia
ph/v	Real word phonemically based paraphasia (A phonemic paraphasia is scored if more than half of the target word phonology is preserved).
v	Verbal paraphasia, semantically related to the target word
v/u	Unrelated verbal paraphasia
n	Neologism (less than 50% overlap with the phonology of the target word)
mw	Multi-word paraphasic/paragrammatic error
otu	Other off-target utterance (not considered paraphasia)
c	Circumlocution utterance (not considered paraphasia)
p	Perseveration
perc	Perceptual

SHORT FORM

Item	Response	Correct Response	Latency Seconds	Stimulus Cue	Phonemic Cue	Error Code(s)	Multiple Choice
1.	<u>house</u> (home) (a kind of building)						
2.	<u>comb</u> (used for fixing hair)						
3.	<u>toothbrush</u> (used in the mouth)						
4.	<u>octopus</u> (an ocean animal)						
5.	<u>bench</u> (used for sitting)						
6.	<u>volcano</u> (a kind of mountain)						
7.	<u>canoe</u> (used in the water)						
8.	<u>beaver</u> (an animal)						

(2)

Appendix 11 (cont.) Cognitive and Behavioral Assessments

Item	Response	Correct Response	Latency Seconds	Stimulus Cue	Phonemic Cue	Error Code(s)	Multiple Choice
9.	<u>cactus</u> (saguaro) (something that grows)		—	—	—	—	—
10.	<u>hammock</u> (you lie on it)		—	—	—	—	—
11.	<u>stethoscope</u> (used by doctors and nurses)		—	—	—	—	—
12.	<u>unicorn</u> (mythical animal)		—	—	—	—	—
13.	<u>tripod</u> (photographers or surveyors use it)		—	—	—	—	—
14.	<u>sphinx</u> (it's found in Egypt)		—	—	—	—	—
15.	<u>palette</u> (artists use it)		—	—	—	—	—

Appendix 11 (cont.) Cognitive and Behavioral Assessments

Summary of Scores

1. Number of spontaneously given correct responses _____

2. Number of stimulus cues given _____

3. Number of correct responses following a stimulus cue _____

4. Number of phonemic cues _____

5. Number of correct responses following the phonemic cue _____

6. Number of multiple choices given _____

7. Number of correct choices _____

Total Number Correct (1 + 3) _____

Total Score:

(Add credit for items preceding the first item passed. Disregard if you started with item 1.)

Tally of paraphasia types

1. Phonological (sum of ph and ph/v) _____

2. Verbal (sum of v and v/u) _____

3. Neologistic _____

4. Multi-word _____

5. Perceptual _____

Appendix 12

10 Meter Walk Test

Description: The 10 meter walk test (10mWT) is a measure of walking speed.

Equipment: Utilize a quiet hallway or open space at least 14 meters long, digital stopwatch, clipboard, tape measure, and tape to mark standardized course layout, and 2 chairs.

Bracing and Assistive Device: The subject should be wearing comfortable flat shoes or shoes with a heel less than 1/2 inch. Have the subject perform this test with their most often used assistive device and orthotics. Note the specific type of assistive device and orthotics utilized on the source document.

Physical Assistance Provided During Testing:

Physical assistance may be provided as needed to maintain safety and stability. If additional assistance is needed, a non-blinded member of the investigation team can provide the additional assistance. No assistance can be provided for paretic limb advancement. A gait belt may be used during the test. The amount of assistance provided will be recorded with assignment of a Functional Ambulation Category (FAC). See FAC for description of procedure.

Test Administration:

Course Layout: The walking Course is established indoors on a level, non-carpeted surface measuring a length of 14 meters. The course is marked with tape at 0 meters, 2 meters, 12 meters, and 14 meters. 2 chairs can be placed near the 0 meter and 14 meter mark.



3. Instruct the subject in the goal of the 10 meter walk test while the subject is seated:
“You are going to walk a distance of about 40 feet. We will repeat this distance four times. The first two times will be completed at your comfortable pace, the final two times you will walk as quickly and safely as you can. Do you have any questions?”
4. Demonstrate where you want the subject to walk.
5. Walk the subject to the start line (0 meters). Provide the subject with the following instructions:
“You will walk at a comfortable pace to the chair.” (DO NOT refer to the tape on the floor.) The start command is **“Ready and Go”**.
6. When you and the subject are ready, say **“Ready and Go”**. If the subject starts too early, start again.

Appendix 12 (cont.)

10 Meter Walk Test

7. Instructions for Recording Time:

- START** the stopwatch when the subject's first foot crosses the plane of the 2 meter line
- STOP** the stopwatch when the subject's first foot crosses the plane of the 12 meter line.
- Have the subject continue walking until he/she reaches the chair after the 14 meter line.
- Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance

“Comfortable” Pace Trial 1 line on the 10 Meter Walk Test Source Document.

- The subject can rest, if needed, in the chair near the 14 meter line.

8. When the subject is ready, have them repeat the SAME PROCEDURE except they will start from the 14 meter line and end at 0 meter line.

- START** the stopwatch when the subject's first foot crosses the plane of the 12 meter line
- STOP** the stopwatch when the subject's first foot crosses the plane of the 2 meter line.
- Have the subject continue walking until he/she reaches the chair after the 0 meter line.
- Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance on the “Comfortable” Pace Trial 2 line on the 10 Meter Walk Test Source Document.
- The subject can rest, if needed, in the chair near the 0 meter line.

9. Subject Instructions for the last 2 trials:

“You are going to walk the same distances for two additional trials as quickly and as safely as you can. Do you have any questions?”

10. When the subject is ready, walk the subject to the start line (0 meters). Provide the subject with the following instructions:

“You will walk as quickly and as safely as you can to the chair.” (Use an appropriate descriptor for chair/location, but DO NOT refer to the tape on the floor.)
The start command is “Ready and Go”.

11. When you and the subject are ready, say “Ready and Go”. If the subject starts too early, have them start again.

Appendix 12 (cont.)

10 Meter Walk Test

12. Instructions for Recording Time:

- START** the stopwatch when the subject's first foot crosses the plane of the 2 meter line
- STOP** the stopwatch when the subject's first foot crosses the plane of the 12 meter line.
- Have the subject continue walking until he/she reaches the chair after the 14 meter line.
- Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance on the "As Fast As Possible" Pace Trial 1 line on the 10 Meter Walk Test Source Document.
- The subject can rest, if needed, in the chair near the 14 meter line.

13. When the subject is ready, have them repeat the SAME PROCEDURE except they will start from the 14 meter line and end at 0 meter line.

- START** the stopwatch when the subject's first foot crosses the plane of the 12 meter line
- STOP** the stopwatch when the subject's first foot crosses the plane of the 2 meter line.
- Have the subject continue walking until he/she reaches the chair after the 0 meter line.
- Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance on the "As Fast As Possible" Pace Trial 2 line on the 10 Meter Walk Test Source Document.
- The subject can rest, if needed, in the chair near the 0 meter line.

14. Record the assistive device used and the Functional Ambulation Category (FAC) on the 10 Meter Walk Test Source Document.

REFERENCES:

Duncan PW, Sullivan KJ, Behrman AL, et al. Protocol for the Locomotor Experience Applied Post-stroke (LEAPS) trial: A randomized controlled trial. *BMC Neurology* 2007;7(1):39.

Perry J, Garrett M, Gronley JK, et al. Classification of walking handicap in the stroke population. *Stroke* 1995, 26(6):982-89.

Richards CL, Malouin F, Wood-Dauphinee S, et al. Gait velocity as an outcome measure of locomotor recovery after stroke. In: Craik RA, Oatis C, eds. *Gait Analysis: Theory and Applications*. St Louis, Mo: Mosby; 1995:355-64.

Appendix 13

Preferred Terms for Intracranial Hemorrhage

An intracranial hemorrhage (ICH) is considered symptomatic if it is not seen on computed tomography (CT) or magnetic resonance imaging (MRI) scan at baseline and any neurologic decline is attributed to it by the local investigator. Below is the list of preferred terms (PTs) using the MedDRA term to describe the ICH event:

- Basal ganglia hemorrhage
- Brain stem hematoma
- Brain stem hemorrhage
- Brain stem microhemorrhage
- Cerebellar hematoma
- Cerebellar hemorrhage
- Cerebellar microhemorrhage
- Cerebral arteriovenous malformation hemorrhagic
- Cerebral hematoma
- Cerebral hemorrhage
- Cerebral microhemorrhage
- *Epidural hemorrhage*
- Extradural hematoma
- Hemorrhage intracranial
- Hemorrhagic cerebral infarction
- Hemorrhagic stroke
- Hemorrhagic transformation stroke
- Intracranial hematoma
- Intraventricular hemorrhage
- Meningorrhagia
- Pituitary hemorrhage
- Putamen hemorrhage
- Subarachnoid hemorrhage
- Subdural hematoma
- Subdural hemorrhage
- Thalamus hemorrhage
- Traumatic intracranial hemorrhage

Appendix 14
World Health Organization (WHO) Toxicity Grading Scale for
Determining the Severity of Adverse Events

HEMATOLOGY				
ITEM	Grade 1 Toxicity	Grade 2 Toxicity	Grade 3 Toxicity	Grade 4 Toxicity
Hemoglobin	9.5 - 10.5 gm/Dl	8.0 - 9.4 gm/Dl	6.5 - 7.9 gm/Dl	< 6.5 gm/Dl
Absolute Neutrophil Count	1000-1500/mm ³	750-999/mm ³	500-749/mm ³	<500/mm ³
Platelets	75000-99000/mm ³	50000-74999/mm ³	20000-49000/mm ³	<20000/mm ³
Prothrombin Time (PT)	1.01 - 1.25 x ULN	1.26-1.5 x ULN	1.51 -3.0 x ULN	>3 x ULN
Activated Partial Thromboplastin (APPT)	1.01 -1.66 x ULN	1.67 - 2.33 x ULN	2.34 - 3 x ULN	> 3 x ULN
Fibrinogen	0.75 - 0.99 x LLN	0.50 - 0.74 x LLN	0.25 - 0.49 x LLN	< 0.25 x LLN
Fibrin Split Product	20-40 mcg/ml	41-50 mcg/ml	51-60 mcg/ml	> 60 mcg/ml
Methemoglobin	5 - 9.9 %	10.0 - 14.9 %	15.0 - 19.9%	> 20 %
LIVER ENZYMES				
AST (SGOT)	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
ALT (SGPT)	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
GGT	1.25 -2.5 x ULN	1.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Alkaline Phosphatase	1.25 - 2. 5 x ULN	1.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN
CHEMISTRIES				
Hyponatremia	130-135 mEq/L	123-129 mEq/L	116-122 mEq/L	< 116 or mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	158-165 mEq/L	> 165 mEq/L or mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	2.0 - 2.4 mEq/L or intensive replacement Rx required or hospitalization required.	<2.0 mEq/L or paresis or ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	6.6 - 7.0 mEq/l	> 7.0 mEq/L or life-threatening arrhythmia
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or mental status changes or coma

Appendix 14 (cont.)
World Health Organization (WHO) Toxicity Grading Scale for
Determining the Severity of Adverse Events

CHEMISTRIES (continued)				
Hyperglycemia (note if fasting)	116 - 160 mg/dL	161 - 250 mg/dL	251 - 500 mg/dL	> 500 mg/dL, or ketacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	6.9 - 6.1 mg/dL	< 6.1 mg/dL or life threatening arrhythmia or tetany
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	12.6 - 13.5 mg/dL	> 13.5 mg/dL life-threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/L	1.1 - 0.9 mEq/L	0.8 - 0.6 mEq/L	< 0.6 mEq/L or life- threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 - 1.9 mg/dL or replacement Rx required	1.0 - 1.4 mg/dL intensive Rx or hospitalization required	< 1.0 mg/dL or life- threatening arrhythmia
Hyperbilirubinemia	1.1 - 1.5 x ULN	1.6 - 2.5 x ULN	2.6 - 5 x ULN	> 5 x ULN
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Creatinine	1.1 x 1.5 x ULN	1.6 - 3.0 x ULN	3.1 - 6 x ULN	> 6 x ULN or required dialysis
URINALYSIS				
Proteinuria	1+ or < 0.3% or < 3g/L or 200 mg - 1 gm loss/day	2-3+ or 0.3 - 1.0% or 3-10 g/L 1-2 gm loss/day	4+ or > 1.0% or > 10 g/L 2-3.5 gm loss/day	nephrotic syndrome or > 3.5 gm loss/day
Hematuria	microscopic only	gross, no clots	gross+ clots	obstructive or required transfusion
CARDIAC DYSFUNCTION				
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; No Rx required	requires treatment
Hypertension	transient inc. > 20 mm, no Rx	recurrent, chronic, > 20 mm, Rx required	requires acute Rx; No hospitalization	requires hospitalization
Hypotension	transient orthostatic hypotension, No Rx	symptoms correctable with oral fluids Rx	requires IV fluids; no hospitalization required	requires hospitalization
Pericarditis	minimal effusion	mild/moderate asymptomatic effusion, no Rx	symptomatic effusion; pain, EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; > 3 units transfused

Appendix 14 (cont.)
World Health Organization (WHO) Toxicity Grading Scale for
Determining the Severity of Adverse Events

RESPIRATORY				
Cough	transient- no Rx	treatment associated cough local Rx	uncontrolled	
Bronchospasm, Acute	transient; no Rx < 80% - 70% FEV ₁ (or peak flow)	requires Rx normalizes with bronchodilator; FEV ₁ 50% - 70% (or peak flow retractions)	no normalization with bronchodilator; FEV ₁ 25% - 50% (or peak flow or intubated)	cyanosis: FEV ₁ < 25% (or peak flow) or intubated
GASTROINTESTINAL				
Stomatitis	mild discomfort; no limits on activity	some limits on eating/drinking	eating/talking very limited	requires IV fluids
Nausea	mild discomfort; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	severe discomfort; no significant intake; activities limited	minimal fluid intake
Vomiting	transient emesis	occasional/moderate vomiting	orthostatic hypotension or IV fluids required	hypotensive shock or hospitalization required for IV fluid therapy
Constipation	mild	moderate	severe	distensions w/vomiting
Diarrhea	transient 3-4 loose stools/day	5-7 loose stools/day	orthostatic hypotension or > 7 loose stools/day or required IV fluids	hypotensive shock or hospitalization for IV fluid therapy required
NEURO & NEUROMUSCULAR				
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Mood	mild anxiety or depression	moderate anxiety or depression and therapy required	severe anxiety or depression or mania; needs assistance	acute psychosis; incapacitated, requires hospitalization
Neuro Control (ADL = activities of daily living)	mild difficulty concentrating; no Rx; mild confusion/agitation; ADL unaffected	moderate confusion/agitation; some limitation of ADL; minimal Rx	severe confusion/agitation needs assistance for ADL; therapy required	toxic psychosis; hospitalization
Muscle Strength	subjective weakness no objective symptoms/signs	mild objective signs/symptoms no decrease in function	objective weakness function limited	paralysis

Appendix 14 (cont.)
World Health Organization (WHO) Toxicity Grading Scale for
Determining the Severity of Adverse Events

OTHER PARAMETERS				
Fever: oral, \geq 12 hours	37.7 – 38.5 C or 100.0 – 101.5 F	38.6 – 39.5 C or 101.6 – 102.9 F	39.6 – 40.5 C or 103 – 105 F	$>$ 40 C or $>$ 105 F
Headache	mild, no Rx therapy	transient, moderate; Rx required	severe; responds to initial narcotic therapy	intractable; required repeated narcotic therapy
Fatigue	no decrease in ADL	normal activity decreased 25- 50%	normal activity decreased $>$ 50%; can't work	unable to care for self
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Local Reaction	tenderness or erythema	induration $<$ 10 cm or phlebitis or inflammation	induration $>$ 10 cm or ulceration	necrosis
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation, moist desquamation, or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery

PROTOCOL

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: Not applicable

IND NUMBER: 3811

TEST PRODUCT: Alteplase (RO5532960)

LEAD PRINCIPAL INVESTIGATOR: [REDACTED], M.D.

MEDICAL MONITOR: [REDACTED], M.D.

SPONSOR: Genentech, Inc.

DATE FINAL: Version 1: 27 November 2013

DATE AMENDED: Version 2: 22 August 2014
Version 3: See electronic date stamp below

PROTOCOL APPROVAL

Approver's Name

[REDACTED]

Title

[REDACTED]

Date and Time (UTC)

08-Jan-2015 22:46:09

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PROTOCOL AMENDMENT, VERSION 3: RATIONALE

Protocol ML29093 has been amended to clarify the instructions for restarting study treatment in the case of symptoms of intracranial hemorrhage (ICH). Text was revised to clarify that, if ICH is not seen on imaging, study drug infusion should immediately be restarted in order to complete the infusion within 4 hours after the last known well time at the same rate as the previous infusion until the remainder of study drug is infused. It was previously stated that study drug infusion should immediately be restarted up to 5.5 hours after the last known well time.

Additional changes to the protocol are as follows:

- The exclusion criterion regarding allergic reactions to study drug and/or aspirin was expanded to include nonsteroidal anti-inflammatory drugs (NSAIDs) as well.

Additional minor changes have been made to improve clarity and consistency, and to adhere to protocol template standards. Substantive new information appears in *italics*. This amendment represents cumulative changes to the original protocol.

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PROTOCOL AMENDMENT, VERSION 3: SUMMARY OF CHANGES

PROTOCOL SYNOPSIS

The protocol synopsis has been updated to reflect the changes to the protocol, where applicable.

SECTION 4.1.2: Exclusion Criteria

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

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

SECTION 4.2: METHOD OF TREATMENT ASSIGNMENT AND BLINDING

Study Unblinding

~~If the investigator wishes to know the identity of the study drug for any other reason, he or she must contact the Medical Monitor. Treatment codes should not be broken except under circumstances deemed necessary by the site investigator. ...~~

SECTION 4.5.1.1: Informed Consent Forms and Screening Log

...The investigator will maintain a screening log of all patients *who are identified to the stroke team/physician as* arriving in the Emergency Department within 2.5 hours of onset of ischemic stroke who have an initial NIHSS score of ≤ 5 . The investigator will record details of all patients not enrolled despite early arrival and record reasons for non-enrollment.

SECTION 4.7.2: Study Treatment Discontinuation

The study intervention should be discontinued if the patient displays symptoms concerning sICH during the infusion of the study drug. Prompt imaging should be done to assess for ICH. If ICH is seen, study drug infusion should not be resumed and the treatment assignment may be unblinded if blood products (to reverse the biological activity of IV alteplase) are indicated. If ICH is not seen, study drug infusion should immediately be restarted ~~up to 5.5~~ in order to complete the infusion within 4 hours after the last known well time at the same rate as the previous infusion until the remainder of study drug is infused.

SECTION 5.2: SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording AEs, including SAEs and non-serious adverse events of special interest (AESIs), *performing protocol-specified safety laboratory assessments, measurement of performing protocol-specified vital signs, and conducting other protocol-specified tests that are deemed critical to the safety evaluation of the study.*

SECTION 5.3.3.3: Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between patient evaluation timepoints. Such events should only be recorded once on the Adverse Event

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eCRF. The initial severity (*intensity or grade*) of the event should be recorded *at the time the event is first reported*. If a persistent AE becomes more severe, ~~and the most extreme severity should also be updated to reflect the most extreme severity any time the event worsens~~. If the event becomes serious, recorded on the Adverse Event eCRF ~~should be updated to reflect this~~. If the event becomes serious, it should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning that the event became serious; see Section 5.4.1 for reporting instructions). The Adverse Event eCRF should be updated by changing the event from "non-serious" to "serious," providing the date that the event became serious, and completing all data fields related to SAEs.

A recurrent AE is one that resolves between patient evaluation timepoints and subsequently recurs. Each recurrence of an AE should be recorded *as a separately event* on the Adverse Event eCRF.

SECTION 5.3.3.4: Abnormal Laboratory Values

Observations of the same clinically significant laboratory abnormality from visit to visit should ~~not only be repeatedly recorded once~~ on the Adverse Event eCRF ~~unless the etiology changes~~. The initial severity of the event should be recorded, and the severity or seriousness should be updated any time the event worsens (see Section 5.3.3 for details on recording persistent AEs).

SECTION 5.3.3.5: Abnormal Vital Sign Values

Observations of the same clinically significant vital sign abnormality from visit to visit should ~~not only be repeatedly recorded once~~ on the Adverse Event eCRF ~~unless the etiology changes~~. The initial severity of the event should be recorded, and the severity or seriousness should be updated any time the event worsens (see Section 5.3.3 for details on recording persistent AEs).

SECTION 5.3.3.10: Adverse Events Associated with an Overdose or Error in Drug Administration

...If the associated AE fulfills seriousness criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.1).

SECTION 5.4: IMMEDIATE REPORTING REQUIREMENTS FROM INVESTIGATOR TO SPONSOR

...The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- SAEs (see Section 5.4.2 for further details)
- Non-serious AESIs (see Section 5.4.2 for further details)
- Pregnancies (see Section 5.4.3 for further details)

SECTION 5.4.2: Reporting Requirements for Serious Adverse Events and Non-Serious Adverse Events of Special Interest

After initiation of study drug, SAEs and non-serious AESIs will be reported until 90 days after the last dose of study drug. For reports of SAEs and non-serious AESIs, investigators should record all case details that can be gathered immediately (i.e., within 24 hours after learning of the event) on the Adverse Event eCRF and submit the report via the electronic data capture (EDC) system. A report will be generated and sent to the Sponsor's Safety Risk Management department by the EDC system.

In the event that the EDC system is unavailable, a paper the Serious Adverse Event/Adverse Event of Special Interest Reporting Form and fax cover sheet provided to investigators should be completed and faxed submitted to Safety Risk Management the Sponsor or its designee immediately (i.e., no more than 24 hours after learning of the event), either by faxing or by scanning and emailing the form using the fax numbers or email address provided below to investigators (and below). Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

Sites:

Fax No.: (650) 225-4682

Alternate Fax No.: (650) 225-5288

Instructions for reporting post-study AEs are provided in Section 5.6.

SECTION 5.4.3.1: Pregnancies in Female Patients

In the event that the EDC system is unavailable, a the Clinical Trial Pregnancy Reporting Form and fax cover sheet provided to investigators should be completed and submitted faxed to Safety Risk Management the Sponsor or its designee immediately (i.e., no more than 24 hours after learning of the pregnancy), either by faxing or scanning and emailing the form using the fax number or email address provided in Section 5.4.1 to investigators. Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

SECTION 5.5.1: Investigator Follow-Up

During the study period, resolution of AEs (with dates) should be documented on the Adverse Event eCRF and in the patient's medical record to facilitate source data verification. If, after follow up, return to baseline status or stabilization cannot be established, an explanation should be recorded on the Adverse Event eCRF.

SECTION 5.6: POST-STUDY ADVERSE EVENTS

The investigator is not required to actively monitor patients for AEs after the end of the AE reporting period (defined as 90 days after administration of study drug for SAEs and non-serious AESIs, and 30 days for non-serious AEs). However, the Sponsor should be notified if the investigator becomes aware of any SAE, death, development of cancer, or

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a congenital anomaly/birth defect in a subsequently conceived offspring of a female patient exposed to study drug *within 90 days after administration of study drug*.

The investigator should report these events directly to ~~Safety Risk Management~~ the Sponsor via telephone at 1-888-835-2555.

SECTION 8.2: INFORMED CONSENT

The Sponsor's sample Informed Consent Form (and ancillary sample Informed Consent Forms such as a Child's *Informed Assent Form* or ~~Caregiver's~~ *Home Nursing* Informed Consent Form, if applicable) will be provided to each site. ...

SECTION 9.5: PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

This section was added per protocol template standards.

APPENDIX 1: Schedule of Assessments

The Schedule of Assessments has been revised to reflect updates to protocol template language.

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PROTOCOL AMENDMENT ACCEPTANCE FORM

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: Not applicable

IND NUMBER: 3811

TEST PRODUCT: Alteplase (RO5532960)

LEAD PRINCIPAL INVESTIGATOR: [REDACTED], M.D.

MEDICAL MONITOR: [REDACTED], M.D.

SPONSOR: Genentech, Inc.

I agree to conduct the study in accordance with the current protocol.

Principal Investigator's Name (print)

Principal Investigator's Signature

Date

Please return a copy of this form as instructed by your local study monitor and retain a copy for your study files.

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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

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IND NUMBER: 3811

TEST PRODUCT: Alteplase (RO5532960)

PHASE: IIIb

INDICATION: Acute ischemic stroke

SPONSOR: Genentech, Inc.

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.

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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 \geq 18 years (no upper age limit)
- Mild ischemic stroke defined as the most recent pre-treatment NIHSS score of \leq 5 and determined to be 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 \leq 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),

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- 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 ≥ 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-compressible 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 $< 100,000/\text{mm}^3$ (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 inhibitor (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, 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.

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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 (\pm 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 \geq 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 attributed 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. \geq 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

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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 $1/0.95^2$ 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.

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Definition
AE	adverse event
AESI	adverse event of special interest
AIS	acute ischemic stroke
aPTT	activated partial thromboplastin time
BI	Barthel Index
BNT	Boston Naming Test
CES-D	Center for Epidemiologic Studies, Depression
CRO	contract research organization
CT	computed tomography
DW	diffusion-weighted
EC	Ethics Committee
eCRF	electronic Case Report Form
EDC	electronic data capture
EEA	European Economic Area
EQ-5D	European Quality of Life questionnaire
EU	European Union
FDA	U.S. Food and Drug Administration
FLAIR	fluid-attenuated inversion recovery
GOS	Glasgow Outcome Scale
GRE	gradient echo
HIPAA	Health Insurance Portability and Accountability Act
HVLT-R	Hopkins Verbal Learning Test-Revised
ICH	intracranial hemorrhage
ID	identification
iDCC	independent Data Coordinating Center
iDMC	independent Data Monitoring Committee
IMP	investigational medicinal product
IND	Investigational New Drug (application)
INR	international normalized ratio
IRB	Institutional Review Board
IST-3	third International Stroke Trial
ITT	intent-to-treat
IU	international units
IV	intravenous
IWRS	Interactive Web Response System

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Abbreviation	Definition
LHH	likelihood of being helped vs. harmed
LPLV	last patient's last visit
MCA	middle cerebral artery
MedDRA	Medical Dictionary for Regulatory Activities
MRI	magnetic resonance imaging
mRS	modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
NNH	number needed to harm
NNT	number needed to treat
PRO	patient-reported outcome
RISS	rapidly improving stroke symptoms
rPH	remote parenchymal hematoma
SAE	serious adverse event
SAP	Statistical Analysis Plan
sICH	symptomatic intracranial hemorrhage
SIS-16	Stroke Impact Scale-16
STIAMP	Suspected Transmission of an Infectious Agent via a Medicinal Product
SWFI	sterile water for injection
SW	susceptibility-weighted
t-PA	tissue plasminogen activator
U.S.	United States
USPI	U.S. Package Insert
WAIS III	Wechsler Adult Intelligence Scale III
WHO	World Health Organization

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1. BACKGROUND

1.1 BACKGROUND ON ACUTE ISCHEMIC STROKE

Stroke is the fourth leading cause of death and a leading cause of disability in the United States (U.S.). Nearly 800,000 strokes occur in the U.S. annually; of these, approximately 85% are ischemic in nature (Rosamond et al. 2007). Treatment options are limited, as the only approved therapy for acute ischemic stroke (AIS) is alteplase, which is to be used in eligible patients and started within 3 hours from the onset of stroke symptoms. Stroke with “only minor or rapidly improving stroke symptoms” is considered a relative exclusion by national clinical guidelines, as these patients were largely excluded from definitive trials to date (Jauch et al. 2013). Consistent with this recommendation, only 15% of alteplase recipients have mild strokes (Smith et al. 2011).

More than half of all ischemic stroke patients have mild strokes at initial presentation in population-based studies (Dhamoon et al. 2009; Reeves et al. 2013). Yet, a substantial proportion (31%–52%) of ischemic stroke patients who arrive within 2– 3 hours of symptom onset are not treated with intravenous (IV) alteplase primarily due to mild symptoms at the time of the treatment decision (Smith et al. 2011; De Los Rios la Rosa et al. 2012). In a national registry, among 93,517 cases arriving to Emergency Departments within 2 hours, 29,200 (31%) were not treated solely due to mild or improving symptoms, and 80.1% of untreated cases had a National Institutes of Health Stroke Scale (NIHSS) score ≤ 5 (Smith et al. 2011).

While treatment of mild stroke is generally uncommon, leading stroke centers vary widely in their approach. For example, the proportion of mild stroke patients (NIHSS ≤ 3) treated with reperfusion therapies at National Institutes of Health/National Institute of Neurological Disorders and Stroke (NINDS) Specialized Programs of Translational Research in Acute Stroke (SPOTRIAS) centers ranged from 2% – 16% in 2008 – 2009 (Willey et al. 2012). This variability in approach is also evident upon discussion with former leaders of the pivotal NINDS trials; some physicians treat all eligible stroke patients regardless of severity, and others treat only a minority of patients (████████ personal communication). There is unquestionable community equipoise regarding the acute treatment of the mild stroke population.

1.2 BACKGROUND ON ALTEPLASE

1.2.1 Fibrinolysis and Tissue Plasminogen Activator

Physiologic dissolution of a thrombus depends primarily on the proteolytic action of plasmin formed from plasminogen at the site of a clot. The mechanism for this activation in vivo has not been definitively established. Plasminogen, the zymogen precursor of plasmin, may be activated by substances found in the plasma milieu, in tissue (tissue plasminogen activator [t-PA]), in urine (urokinase), and in bacteria (streptokinase). The generated plasmin may be inhibited by plasma proteins such as $\alpha 2$ -antiplasmin and $\alpha 2$ -macroglobulin (Robbins 1982).

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Kinetic analyses suggest that plasminogen activation in the presence of fibrin occurs after the binding of plasminogen and t-PA at the clot site. t-PA has a high affinity for fibrin, thereby efficiently activating the fibrin-bound plasminogen and converting it to plasmin. Plasmin, which when bound to fibrin is protected from rapid inactivation by α_2 -antiplasmin, then proceeds to digest the fibrin clot.

See the Activase® (alteplase) U.S. Package Insert (USPI) for additional details on nonclinical and clinical studies.

1.3 STUDY RATIONALE AND BENEFIT-RISK ASSESSMENT

Alteplase is the only approved therapeutic agent for the treatment of AIS and is widely accepted as the standard of care for eligible patients (Edlow et al. 2013; Jauch et al. 2013). The currently approved alteplase 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 (Jauch et al. 2013). 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 on who constitutes this patient population, and the absence of definitive evidence of alteplase efficacy in this setting (Levine et al. 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” throughout the protocol.

To date, all completed major randomized acute stroke trials have excluded patients with mild strokes to varying degrees. In the two NINDS trials, such patients were excluded according to the protocol (largely to prevent patients with transient ischemic attacks from being enrolled if they were having rapidly improving symptoms), but did not include an explicit definition of mild stroke except for pure sensory stroke, isolated ataxia, isolated dysarthria, and isolated facial weakness (NINDS rt-PA Study Group 1995). The operational definition for stroke symptoms too mild to treat was left to the judgment of the randomizing physician (Levine et al. 2013). Interestingly, a post hoc analysis of the 624 patients in the NINDS trial revealed that there were 58 patients (9%) enrolled with an NIHSS score ≤ 5 , none of whom had isolated motor symptoms, isolated facial droop, isolated ataxia, dysarthria, isolated sensory, and three or fewer of each of additional isolated deficits (Khatri et al. 2010). Perhaps more significantly, 2971 patients were excluded primarily due to “rapidly improving” or “minor symptoms.” However, a significant unmet medical need exists for patients with mild stroke. Available outcome data for patients with mild stroke who do not receive alteplase consistently demonstrate a high rate of suboptimal outcomes. Specifically, in two different prospective databases

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of patients with mild deficits (NIHSS score ≤ 5) and no baseline disability, approximately one-third (29%–32%) were significantly disabled (as measured by modified Rankin Scale [mRS] score 2–6) at 90 days (Fischer et al. 2010; Khatri et al. 2012; [REDACTED], personal communication).

Existing, albeit limited, data suggest that the potential benefit-risk ratio for alteplase in mild stroke should be favorable. Gonzales et al. demonstrated better functional outcomes among mild strokes treated with IV alteplase compared to those not treated in a large, single-center, retrospective cohort; this was shown with two different thresholds of stroke severity (NIHSS ≤ 7 and NIHSS ≤ 3) (Gonzales et al. 2006). Additionally, a recent post hoc analysis of the subset of the third International Stroke Trial (IST-3) patients with mild strokes (pre-treatment NIHSS ≤ 5 who met all other NINDS rt-PA study eligibility criteria) showed a trend toward benefit for alteplase ([REDACTED], personal communication). For PRISMS, the primary efficacy outcome assumption (mRS 0–1) is a 9% absolute treatment effect of alteplase relative to placebo. This assumption is based on several retrospective analyses of similar patient populations (Köhrmann et al. 2009; Fischer et al. 2010; Hassan et al. 2010b; Khatri et al. 2012) as well as the aforementioned IST-3 trial subgroup analysis ([REDACTED], personal communication).

The most prevalently reported and life-threatening adverse event (AE) associated with alteplase treatment for acute ischemic stroke is symptomatic intracranial hemorrhage (sICH). Post hoc analyses, case series, and retrospective studies in patients with mild stroke suggest an absolute sICH rate of 0–2% with alteplase treatment (NINDS rt-PA Study Group 2005; Baumann 2006; Gonzales et al. 2006; Köhrmann et al. 2009; Steffenhagen et al. 2009; Hassan et al. 2010a; Hassan et al. 2010b; Huisa et al. 2012). These data are consistent with analyses of IV alteplase trials of more severe patients suggesting that stroke severity, as measured by the NIHSS score, is a strong predictor of sICH risk (Khatri et al. 2007). No data exist to estimate the sICH rate for patients with mild stroke who did not receive t-PA; therefore, for this study, we are using a 0% sICH rate for the placebo arm, which is likely a conservative estimate. An absolute sICH difference of 2% at 36 hours (primary safety endpoint) has been assumed for the alteplase arm vs. the placebo control arm in the proposed study. For this study, sICH has been defined as any neurologic decline attributed to intracranial hemorrhage (ICH) seen on imaging by the local investigator within 36 hours of study drug administration, consistent with the NINDS trial definition for sICH (NINDS rt-PA Study Group 1995).

Assuming the proposed study demonstrates both a 9% absolute treatment effect rate, the number of patients needed to treat (NNT) to show benefit (i.e., mRS score of 0–1) for one patient is 11. Assuming a 2% absolute difference in sICH rate in patients treated with alteplase, the corresponding value for the number needed to harm (NNH), specifically for sICH, is conservatively estimated to be 50. The actual number NNH, or the number of patients who would have worse outcomes at 90 days with alteplase compared to placebo treatment, is likely to be much higher than is reflected by the sICH

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rate alone, as many patients who experience sICH have severe baseline infarcts and are destined for poor outcomes (Saver 2007).

For comparison, the absolute treatment effect for mRS score of 0–1 at 90 days was 13% for the NINDS-sponsored pivotal study for alteplase. This equates to an NNT of 7.9. In the same study, the absolute difference in sICH was 5.8% at 24±12 hours post-treatment, or an NNH of 17.2 (NINDS rt-PA Study Group 1995).

Table 1 Comparison of PRISMS and NINDS Studies

Study	Absolute Treatment Effect (%)	Absolute Difference in sICH (%)	NNT	NNH	LHH ^a
Proposed study in patients with mild stroke and RISS	9	2	11	50	0.22
NINDS-sponsored pivotal study for alteplase	13	5.8 ^b	7.9	17.2	0.46

LHH=likelihood of being helped vs. harmed; NINDS=National Institute of Neurological Disorders and Stroke; NNH=number needed to harm; NNT=number needed to treat; RISS=rapidly improving stroke symptoms; sICH=symptomatic intracranial hemorrhage.

^a LHH is the likelihood of being helped versus harmed and is the ratio between the NNT and the NNH. The LHH has been proposed as an expression to capture both the NNT and the NNH into a single value. An LHH below 1.0 indicates the expected benefits outweigh the possible harm. The lower the LHH, the more favorable the benefit-risk ratio is for the treatment in the patient population of interest (Demaerschalk 2007).

^b In the NINDS study, patients who received alteplase and developed sICH within 36 hours had a mortality rate of 45% (NINDS rt-PA Study Group 2005).

2. OBJECTIVES

2.1 PRIMARY OBJECTIVE

The primary objective of this study is to determine the efficacy of IV alteplase for treatment of AIS in patients with mild stroke (also known as “minor neurologic deficit” and “rapidly improving stroke symptoms”), defined as an NIHSS score ≤ 5 and not clearly disabling, within 3 hours of last known well time as measured by the proportion of patients with mRS score of 0–1 at Day 90.

2.2 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:

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- sICH within 36 hours (primary safety assessment)
- Any ICH within 36 hours
- Mortality within 90 days
- Stroke-related and neurological deaths within 90 days
- Incidence, severity, and spectrum of all AEs and serious adverse events (SAEs)

2.3 EXPLORATORY OBJECTIVES

The exploratory objectives for this study include subgroup analyses of the primary efficacy outcome variable as defined by age (<65 vs. \geq 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 (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 test, form V; HVLT-R trial 4 and recognition; semantic fluency [Animal Naming test]; 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

3. STUDY DESIGN

3.1 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

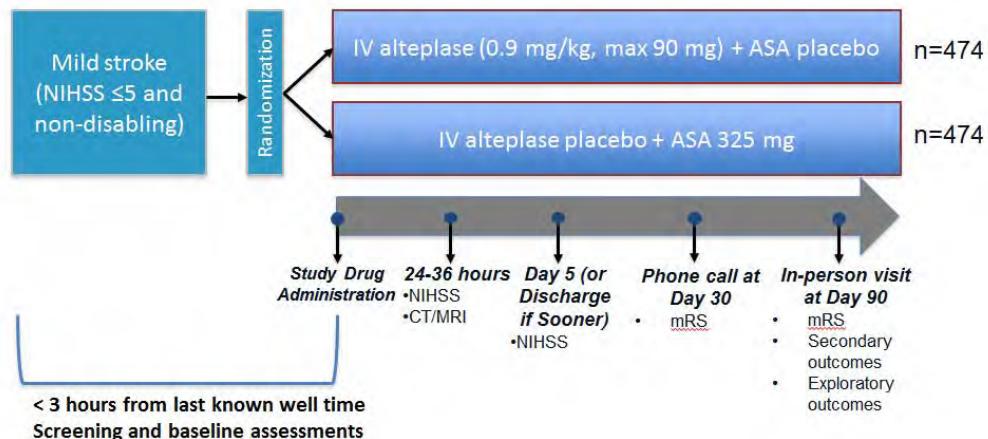
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2. One dose of IV alteplase placebo and one dose of oral aspirin 325 mg (Figure 1).

Every effort should be made to ensure both study drugs are initiated within 3 hours of last known well time. If institutional policies on oral medications make it challenging to administer the oral aspirin/aspirin placebo within 3 hours of last known well time, the oral study drug may be given up to 24 hours after the last known well time. IV alteplase/IV alteplase placebo MAY NOT be initiated beyond 3 hours of last known well time under any circumstances.

A full schedule of assessments is provided in [Appendix 1](#).

Figure 1 Abbreviated Study Schema



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

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 stratified by site 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 informed consent and eligibility confirmation. Further details on step forward randomization can be found in Section 4.2, Method of Treatment Assignment and Blinding.

An independent Data Monitoring Committee (iDMC) will provide ongoing review of potential safety concerns and review efficacy and safety outcomes to inform a futility interim analysis after 50% of patients have achieved the primary endpoint (following completion of the Day 90 visit). The composition of the iDMC and analysis will be documented in detail in a separate iDMC charter.

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3.2 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.

3.3 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 (\pm 14 days) after the last patient is enrolled into the study.

3.3.1 Number of Patients

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

3.4 RATIONALE FOR STUDY DESIGN

This study is designed to assess whether alteplase administered within 3 hours of last known well time is superior to standard of care (i.e., aspirin) in patients with mild stroke. Patients will be randomized 1:1 to receive either IV alteplase plus aspirin placebo or IV alteplase placebo plus aspirin 325 mg; hence, this is an active-comparator trial. Other than the study intervention, patients will receive standard of care for AIS at the enrolling institutions. The primary endpoint using the mRS assessment of global disability at 90 days is standard for current AIS trials.

The study design of PRISMS has the following characteristics:

- **Consistent with the currently approved indication in the alteplase USPI:** Treatment with alteplase in PRISMS will occur in otherwise eligible AIS patients within 3 hours from the last known well time.
- **Conduct of a large, multicenter study:** The Sponsor plans to enroll 948 patients across approximately 75 sites in North America. (For comparison, the two NINDS trials enrolled a total of 624 patients.)
- **Multiple endpoints involving different events:** In addition to the primary efficacy endpoint of mRS at Day 90, which measures disability, secondary endpoints at Day 90 will include an ordinal mRS assessment and global favorable recovery, as calculated using the Global Outcome Measure derived from the mRS, NIHSS, BI, and GOS.

3.4.1 Rationale for Alteplase Dosage

The dose selected for alteplase (0.9 mg/kg, maximum 90 mg) is the approved dose for eligible patients with AIS in North America and is considered the standard of care by the stroke community ([Jauch et al. 2013](#)).

3.4.2 Rationale for Patient Population

The patients for this trial will be recruited from all AIS patients presenting to Emergency Departments in whom treatment can be initiated within 3 hours of last known well time at approximately 75 clinical sites within North America. As described in Section 1.3, eligible patients with mild stroke will be enrolled in this study. Currently, the alteplase USPI warns against the use of alteplase in patients with “minor neurologic deficit and rapidly improving stroke symptoms” due to lack of data. Retrospective and observational data suggest potential benefit of alteplase in these patients, with a favorable benefit-risk profile.

The definition of mild stroke for PRISMS is an NIHSS score ≤ 5 and not clearly disabling. This includes patients whose symptoms are persistently mild as well as those with RISS.

An NIHSS score ≤ 5 was chosen as one criterion in the definition of mild stroke for PRISMS for the following reasons:

1. An NIHSS score ≤ 5 is commonly used as an exclusion criterion for many current AIS studies, suggesting that these patients are underrepresented in current stroke research and are treated differently by the stroke community, as shown in [Table 2](#).
2. The largest retrospective analysis of mild stroke from the Get With the Guidelines® registry included 29,200 stroke patients who did not receive alteplase due to mild or improving symptoms. This analysis found that 80.1% of patients with mild or rapidly improving stroke who did not receive alteplase (and had a recorded NIHSS score) had an initial NIHSS score ≤ 5 ([Smith et al. 2011](#)).

Based on these findings, an NIHSS score ≤ 5 was determined to be the most appropriate cut-off to define the patient population with mild stroke.

Table 2 NIHSS Exclusion Criteria of Recently Completed and Ongoing Acute Ischemic Stroke Trials

Recently Completed AIS Trials	Exclusion	Ongoing AIS Trials	Exclusion
Combined Approach to Lysis Utilizing Eptifibatide and rt-PA in Acute Ischemic Stroke (CLEAR Stroke) Trial	NIHSS≤5	ARTSS	NIHSS≤9
Intravenous Thrombolysis Plus Hypothermia for Acute Treatment of Ischemic Stroke	NIHSS≤6	SHINE	NIHSS<3
Albumin in Acute Ischemic Stroke Trial (ALIAS) Part One	NIHSS≤5	The Intravascular Cooling in the Treatment of Stroke 2/3 (ICTuS 2/3) Trial (ICTuS2/3)	NIHSS≤6
Study of the Combination Therapy of rt-PA and Eptifibatide to Treat Acute Ischemic Stroke (CLEAR-ER)	NIHSS≤5	Safety/Feasibility of Autologous Mononuclear Bone Marrow Cells in Stroke Patients	NIHSS≤5
Albumin in Acute Ischemic Stroke Trial (ALIAS) Part Two	NIHSS≤5	SWIFT PRIME	NIHSS≤7
Mechanical Retrieval and Recanalization of Stroke Clots Using Embolectomy (MR RESCUE)	NIHSS≤5	PENUMBRA THERAPY	NIHSS≤7
NeuroThera Efficacy and Safety Trial-3 (NEST3)	NIHSS≤6	PENUMBRA 3D	NIHSS≤7
		CLOTBUST-ER	NIHSS≤9

AIS=acute ischemic stroke; NIHSS=National Institutes of Health Stroke Scale.

Source: www.clinicaltrials.gov. Accessed August 2013. Note that the NIHSS criteria for the trials listed are mentioned in the inclusion criteria, whereas the entries in the above table were adapted to list the corresponding exclusion criteria.

It is notable that the alteplase USPI (2011) distinguishes patients with minor neurological deficit from those with RISS. However, PRISMS combines these two patient groups into one operationally defined patient population (mild stroke): all eligible patients with an NIHSS score≤5 at the time of randomization without clearly disabling symptoms, irrespective of previous NIHSS scores. Data demonstrating low alteplase use in patients with NIHSS scores≤5 do not distinguish between whether the patient's deficit was persistently mild or improved to this status (Smith et al. 2011). Furthermore, no data exist that suggest patients who improved to a mild status at the time of treatment with alteplase have different outcomes from patients whose neurologic deficit is persistently mild. To date, available data suggest that alteplase therapy would potentially be beneficial in both patients with RISS who improve to an NIHSS score≤5 and patients with persistently mild deficits with an NIHSS score≤5.

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Conversely, patients who improve to an NIHSS score > 5 at the time of treatment decision will be excluded from PRISMS as they are not considered to have RISS. Current clinical practice suggests that these patients would still have a moderate or severe stroke at the time of treatment decision and should receive alteplase, if eligible, rather than be randomized to a study. Such patients should not be defined as rapidly improving, given that they have not improved to an NIHSS score low enough to merit withholding therapy. This definition of RISS is consistent with a recent consensus statement by leading stroke and emergency medicine experts (Levine et al. 2013).

PRISMS will also exclude patients who are assessed at the time of pre-treatment to be clearly disabled. These clearly disabling deficits at presentation may include, but are not limited to, homonymous hemianopsia, severe limb weakness, severe aphasia, hemineglect, or cortical blindness. Patients with clearly disabling deficits will be excluded from this study irrespective of the NIHSS score at time of randomization, as current clinical practice and ethical responsibility suggest that these patients should be evaluated to receive alteplase rather than be enrolled in a randomized study where they may receive alteplase placebo.

3.4.3 Rationale for Control Group

The overall rationale for this trial is to determine whether IV alteplase improves outcomes in patients with mild stroke. The control group (i.e., mild stroke patients who receive standard of care) has been included so that outcomes in the active treatment group can be compared to those who receive standard of care. The control group will receive IV alteplase placebo and 325 mg of oral aspirin. The rationale for the control group receiving aspirin is that it would be unethical to withhold a dose of aspirin from patients who would not be receiving active study drug. The standard of care for patients suffering from AIS who are ineligible for IV alteplase therapy is oral aspirin administered within the first 24 hours after the onset of their stroke symptoms.

3.5 OUTCOME MEASURES

3.5.1 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 are measured at Day 90 and listed below:

- Ordinal mRS
- Global favorable recovery (Global Outcome Measure derived from mRS 0 – 1, NIHSS 0 – 1, BI \geq 95, and GOS = 1)

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3.5.2 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 attributed 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

Symptomatic Intracranial Hemorrhage

In this study, an ICH is considered symptomatic if it is not seen on computed tomography (CT) or magnetic resonance imaging (MRI) scan at baseline and any neurologic decline is attributed to it by the local investigator. To detect intracranial hemorrhage, neuroimaging (CT or MRI) scan is required at 22 to 36 hours. Additional neuroimaging should be performed at any time based on the investigator's discretion that clinical findings suggested hemorrhage. Details on MRI types are described in Section 4.5.1.6.

Intracranial hemorrhage events will include classifications of intracerebral hemorrhage (hemorrhagic infarct type 1 or type 2, parenchymal hematoma type 1 or type 2, or remote intraparenchymal hemorrhage type 1 or type 2). Descriptions of each type of intracerebral hemorrhage are shown in the table below. Additionally, subarachnoid hemorrhage, subdural hemorrhage, or epidural hemorrhage should be noted.

Table 3 Intracerebral Hemorrhage Definitions

Type	Description
HI type 1	Small petechiae along margin of infarct
HI type 2	More confluent petechiae within the infarcted area but without space-occupying effect
PH type 1	Hematoma in $\leq 30\%$ of the infarcted area with some slight space-occupying effect
PH type 2	Dense hematoma $> 30\%$ total of the infarcted area with substantial space-occupying effect or any hemorrhagic area outside the infarcted area
rPH type 1	Small or medium sized blood clots located remote from the actual infarct; a mild space-occupying effect could be present. Remote primary intracerebral hemorrhage
rPH type 2	Large confluent dense blood clots in an area remote from the actual infarct; substantial space-occupying effect might be present.

HI=hemorrhagic infarct; PH=parenchymal hematoma; rPH=remote parenchymal hematoma.

Source: [Wahlgren et al. 2007](#).

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3.5.3 Exploratory Outcome Measures

The exploratory outcome measures for this study are as follows:

- NIHSS at Day 90
- Instrumental activities of daily living, as measured by the BI at Day 90
- GOS at Day 90
- 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 test, form V; HVLT-R trial 4 and recognition; semantic fluency [Animal Naming test]; Boston Naming Test [BNT 15-item short form]) at Day 90
- Ambulatory performance (as measured by walking speed) at Day 90
- Depression (CES-D score) at Day 90
- Quality of life (EQ-5D questionnaire) at Day 90
- SIS-16 at Day 90

4. MATERIALS AND METHODS

4.1 PATIENTS

This study will include adult patients with AIS.

4.1.1 Inclusion Criteria

Patients must meet the following criteria for study entry:

- Age \geq 18 years (no upper age limit)
- Mild ischemic stroke defined as the most recent pre-treatment NIHSS score of ≤ 5 and determined to be 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.

4.1.2 Exclusion Criteria

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

- CT or 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 ≥ 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-compressible 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 (aPTT) outside of the normal range as specified by the center's local laboratory
 - Blood glucose <50 mg/dL
 - International normalized ratio (INR) >1.7 (Note: This does not need to be verified prior to randomization if there is no clinical suspicion of abnormality.)
 - Platelet count of $<100,000/\text{mm}^3$ (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 inhibitor (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, aspirin, *or* nonsteroidal anti-inflammatory drugs (NSAIDs)

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- 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

4.2 METHOD OF TREATMENT ASSIGNMENT AND BLINDING

Step Forward Randomization

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 process 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. This procedure will be implemented using an Interactive Web Response System (IWRS) where study sites enter identification (ID) information of a patient AFTER he/she is treated (within 8 hours of initiation of the patient's study treatment). The IWRS will then assign drug kit IDs for the next patient that becomes eligible at that site. The pharmacist will prepare "Use Next" labels and place them on the appropriate study drug kits based on the drug kit IDs via IWRS. When the next potentially eligible patient is identified, the investigator (or designee) will notify the pharmacist (or designee) to begin pre-mixing study drug from the alteplase/alteplase-placebo drug kit. Pre-mixing may occur prior to signing informed consent but should be performed after key eligibility criteria (e.g., NIHSS≤5 and not clearly disabling, study drug initiation will occur within 3 hours of time last known well, CT/MRI negative for ICH) to minimize drug wastage. Study drug bolus will be administered after confirming that all eligibility criteria have been met and informed consent signed, and this will indicate enrollment in the trial. This approach will ensure minimal delays to treatment. Following study drug administration (within 8 hours), the study site should enter the patient's ID, including date of birth, patient initials, screening ID, and confirm drug kit IDs from the kits with which the patient is treated. Once this information is entered into IWRS, it will result in two actions:

1. IWRS will assign a patient ID to the currently treated patient, which will be correlated to the drug kit ID previously labeled as "Use Next," via the randomization visit record.
2. IWRS will assign a new pair of drug kit IDs for the next patient that becomes eligible at that site to be labeled as "Use Next."

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In the case when pre-mixed IV drug is not used (e.g., patient is determined to be ineligible for study or declines informed consent prior to drug administration), sites will enter drug kit IDs into IWRS and indicate that the kits were not used. IWRS will then assign a new set of drug kit IDs for the pharmacist to label with “Use Next” labels. The pharmacist will properly dispose of the pre-mixed IV drug and the matching aspirin/aspirin-placebo kit in accordance with institutional guidelines.

Prior to the first patient enrolled at a site, a site will register with IWRS as part of site activation, which will assign drug kit IDs ahead of time for the first potential patient. Once the first patient is enrolled at a site, the procedure described above will commence.

Study Unblinding

If a clinical situation arises in which the investigator thinks it is necessary to unblind in the interest of patient safety, the Medical Monitor must be notified immediately to discuss the intended unblinding. Unblinding should occur only in settings where identification of the study treatment is critical for treatment decisions. IWRS will be used for the unblinding process. All unblinded patients will remain in the study and require all follow-up visits.

Treatment codes should not be broken except under circumstances deemed necessary by the site investigator. The investigator should document and provide an explanation for any premature unblinding (e.g., unblinding due to sICH or severe systemic bleeding that requires blood products if IV alteplase was administered, or unblinding due to clinical worsening during the IV alteplase time window that would lead to IV alteplase administration if not already given).

For regulatory reporting purposes, and if required by local health authorities, the Sponsor will break the treatment code for all serious, unexpected suspected adverse reactions (see Section 5.7) that are considered by the investigator or Sponsor to be related to study drug.

4.3 STUDY TREATMENT

4.3.1 Formulation, Packaging, and Handling

4.3.1.1 Alteplase

Alteplase is a single polypeptide containing 527 amino acids; it has three carbohydrate side chains and is predominantly one-chain t-PA. It is a recombinant protein produced in a continuous mammalian tissue culture system and is purified by a series of chromatographic steps. The manufacturing process includes fermentation with use of the antibiotic gentamicin; however, the presence of the antibiotic is not detectable in the final product.

Alteplase is a sterile, white to pale yellow, lyophilized powder for IV administration after reconstitution with sterile water for injection (SWFI), USP, without preservatives. Phosphoric acid and/or sodium hydroxide may be used prior to lyophilization for

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pH adjustment. Biologic potency is determined by an in vitro clot lysis assay and is expressed in international units (IU) as tested against the World Health Organization (WHO) standard.

Each 50-mg vial of Activase contains 50 mg of alteplase, 1.7 g of L-arginine, 5 mg of polysorbate 80, and 0.5 g of phosphoric acid.

Each kit will contain 2 vials of 50-mg of alteplase labeled for “clinical trial use only.”

Vials of 50-mg alteplase must be stored at 2°C–30°C and protected from light.

4.3.1.2 Alteplase Placebo

Alteplase placebo is the same formulation as the active without the alteplase protein. Each 50-mg vial contains 1.7 g of L-arginine, 5 mg of polysorbate 80, and 0.5 g of phosphoric acid.

Each kit will contain 2 vials of 50 mg of alteplase placebo labeled for “clinical trial use only.”

Alteplase placebo vials should be stored at 2°C–30°C and protected from light.

4.3.1.3 Aspirin

Aspirin (325 mg) will be packaged for the study. Each kit will contain one bottle, which has 100 tablets labeled for “clinical trial use only.” Only one tablet will be administered to the patient, and the remainder of the bottle will be kept at the site for drug accountability.

Aspirin tablets should be stored at 25°C with excursions permitted between 15°C–30°C.

4.3.1.4 Aspirin Placebo

Aspirin placebo will be identical in appearance to active aspirin to maintain treatment blinding. Each bottle of aspirin placebo contains 100 tablets labeled for “clinical trial use only.” Only one tablet will be administered to the patient, and the remainder of the bottle will be kept at the site for drug accountability.

Placebo tablets should be stored in the same manner as aspirin tablets: 25°C with excursions permitted between 15°C–30°C.

4.3.2 Dosage, Administration, and Compliance

4.3.2.1 Alteplase

For AIS, the recommended dose is 0.9 mg/kg infused over 60 minutes with 10% of the total dose administered as an initial IV bolus over 1 minute. The total dose should not exceed 90 mg.

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Because alteplase and alteplase placebo contain no antibacterial preservatives, 50-mg vials should be reconstituted immediately before use by adding 50 mL of SWFI, USP, to the vial; this yields a concentration of 1 mg/mL. Using aseptic technique, direct the flow of SWFI directly into the lyophilized cake of study drug. It is recommended that an 18-gauge needle be used for the reconstitution of the 50-mg vial. Once reconstituted, gently swirl the vial. **Do not shake.** Slight foaming upon reconstitution is not unusual; allowing the vial to stand undisturbed for several minutes is usually sufficient to allow dissipation of any large bubbles. Before further dilution or administration, the product should be visually inspected for particulate matter and discoloration. If a precipitate is seen, the product should not be used.

After reconstitution, alteplase should be administered at 1 mg/mL. As an alternative, the reconstituted solution may be diluted further immediately before administration in an equal volume of 0.9% sodium chloride injection, USP, or 5% dextrose injection, USP, to yield a concentration of 0.5 mg/mL. The osmolality of this solution is approximately 215 mOsm/kg. Polyvinyl chloride bags, glass bottles, or polypropylene syringes are acceptable. Do not use filter needles for infusion. Alteplase is stable for up to 8 hours in these solutions at room temperature. Exposure to light has no effect on the stability of these solutions. Excessive agitation during dilution should be avoided; mixing should be accomplished with gentle swirling and/or slow inversion.

No other medication should be added to infusion solutions containing alteplase. Any unused infusion solution should be discarded.

See [Appendix 2](#) (Alteplase Administration) and [Appendix 3](#) (Reconstitution of 50-mg Vials) for further details.

4.3.3 Investigational Medicinal Product Accountability

All investigational medicinal products (IMPs) required for completion of this study will be provided by the Sponsor where required by local health authority regulations. The investigational site will acknowledge receipt of IMPs using the IWRS to confirm the shipment condition and content. Any damaged shipments will be replaced.

IMPs will either be disposed of at the study site according to the study site's institutional standard operating procedure or returned to the Sponsor with the appropriate documentation. The site's method of IMP destruction must be agreed to by the Sponsor. The site must obtain written authorization from the Sponsor before any IMP is destroyed, and IMP destruction must be documented on the appropriate form.

Accurate records of all IMPs received, dispensed, returned, and disposed of by the study site should be recorded on the Drug Inventory Log.

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4.3.4 Investigational Medicinal Product

4.3.4.1 Alteplase and Placebo

Test Product

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.

4.3.4.2 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.

4.3.5 Post-Trial Access to Alteplase

The Sponsor does not intend to provide alteplase, any investigational or non-investigational products (specified in this protocol), or any other study interventions to patients after the conclusion of the study or to patients who are withdrawn earlier.

4.4 CONCOMITANT THERAPY

4.4.1 Permitted Therapy

Concomitant therapy includes any medication (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by a patient from 7 days prior to screening to the study completion/discontinuation visit. All concomitant medications should be reported to the investigator and recorded on the Concomitant Medications electronic Case Report Form (eCRF).

4.4.2 Prohibited Therapy

Available endovascular therapies have not demonstrated clinical efficacy in definitive trials to date and therefore are not permitted.

As indicated in the exclusion criteria, use of the following therapies is prohibited prior to the study:

- Treatment with unfractionated heparin within the last 48 hours and aPTT outside of the normal range as specified by the center's local laboratory

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- Treatment with a direct thrombin inhibitor or factor Xa inhibitor (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
- Treatment with warfarin and INR >1.7 (Note: INR does not need to be verified prior to randomization if there is no clinical suspicion of abnormality.)

The above lists of medications are not necessarily comprehensive. The investigator should contact the Medical Monitor if questions arise regarding medications not listed above. For further reference about drug interaction guidance from the U.S. Food and Drug Administration (FDA), as well as a list of common cytochrome P450 interactions, the following resources are available online:

<http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM292362.pdf>

<http://medicine.iupui.edu/clinpharm/ddis/table.aspx>

4.5 STUDY ASSESSMENTS

Please see [Appendix 1](#) for the schedule of assessments performed during the study.

4.5.1 Description of Study Assessments

4.5.1.1 Informed Consent Forms and Screening Log

Written informed consent for participation in the study must be obtained before treatment. All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before treatment. The investigator will maintain a screening log of all patients *who are identified to the stroke team/physician as arriving in the Emergency Department within 2.5 hours of onset of ischemic stroke who have an initial NIHSS score of ≤5*. The investigator will record details of all patients not enrolled despite early arrival and record reasons for non-enrollment.

4.5.1.2 Medical History and Demographic Data

Medical history includes clinically significant diseases, such as stroke history and smoking history, use of alcohol and drugs of abuse, and all medications (e.g., prescription drugs, over-the-counter drugs, herbal or homeopathic remedies, nutritional supplements) used by the patient within 7 days prior to the screening visit, and history of allergy to any food or medication.

Demographic data will include date of birth, sex, and self-reported race/ethnicity.

4.5.1.3 Neurological Examinations

A partial neurological examination is to be performed for each patient. The NIHSS will be a component of this neurological examination and will be performed only by practitioners who are certified to perform an NIHSS assessment. Changes from

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baseline should be recorded in patient notes. Additional or supplemental neurological exams will also be performed, including assessment of mental status, cranial nerves, motor function, and coordination.

4.5.1.4 Vital Signs

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

4.5.1.5 Laboratory Assessments

Specimens for the following laboratory tests will be sent to the study site's local laboratory for analysis:

- Complete blood count without differential
- Serum glucose
- Coagulation (INR, aPTT)
- Pregnancy test (serum or urine) for women of childbearing potential including those who have had a tubal ligation

The following laboratory test values will be reported in the eCRFs:

- Complete blood count without differential
- Serum glucose
- Coagulation values (INR, aPTT)
- Pregnancy test for women of childbearing potential including those who have had a tubal ligation

For sampling procedures, storage conditions, and shipment instructions, see the site's local laboratory manual.

4.5.1.6 Other Disease-Specific Assessments

Neuroimaging may consist of a non-contrast CT or MRI of the brain. If an MRI is used in the acute setting (to determine study eligibility), it must include the following at a minimum: (1) DW MRI, (2) either susceptibility-weighted (SW) imaging or gradient echo (GRE) sequences, and (3) FLAIR sequences.

Non-contrast CT or MRI will be performed at baseline to ensure that the patient does not have evidence of acute 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. An NIHSS should be performed at the time this CT scan or MRI is performed, but prior to reviewing imaging results to avoid biasing the assessment. If an MRI is performed, in addition to the above requirements for the baseline visit MRI, it must also include T1 and T2 sequences.

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Additional CT scans or MRIs should be performed based on the investigator's discretion, or if any clinical findings suggest ICH (such as new headache, nausea, vomiting, significant deterioration of neurological status, or rapid increase of blood pressure). If an ICH is identified, the NIHSS score should be reassessed at the time and recorded on the Adverse Event eCRF.

CT scans or MRIs performed at baseline, between 22 to 36 hours, and additional imaging performed due to clinical suspicion of deterioration up until 36 hours after study drug administration, will be evaluated by a central team of radiologists blinded to the clinical attributes of the case. These images will need to be sent to the central radiologists by the local site.

4.5.2 Patient-Reported Outcomes

Patient-reported outcomes (PRO) data will be elicited from the patients in this study to more fully characterize the clinical profile of alteplase in this patient population. The PRO questionnaires will be distributed by the investigator staff and completed in their entirety by the patient or their surrogate.

This study will assess the impact of alteplase treatment on health-related quality of life, physical function, daily functioning, and symptoms of depression.

Health-related quality of life will be assessed using the EQ-5D ([Appendix 4](#)). 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.

Physical functioning will be evaluated using the SIS-16 ([Appendix 5](#)), which is a validated, stroke-specific, quality of life measure to assess the impact of stroke on a patient's health and life.

The CES-D ([Appendix 6](#)) will be used to evaluate the proportion of patients with depressive symptoms (defined as a score of ≥ 16 at 90 days).

The EQ-5D, SIS-16, and CES-D will be provided in Spanish for those patients requiring translations.

AE reports will not be derived from PRO data by the Sponsor. However, any PRO responses suggestive of a possible AE identified during site review of the PRO data should be reported as outlined in Section [5.3.3.11](#).

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4.6 TIMING OF STUDY ASSESSMENTS

4.6.1 Screening/Baseline (Visit 1)

The following standard of care assessments performed prior to obtaining informed consent will be used in the screening process. Once consent is obtained, the results of the standard of care assessments may be used for the study.

- Medical history
- Demographics
- Neurological examination
- Vital signs
- Complete blood count without differential
- Coagulation status
- Pregnancy test (serum or urine)
- Serum blood glucose
- Non-contrast CT scan or MRI, including DW MRI, either SW or GRE, and FLAIR images
- NIHSS score (Note: This must be performed by an NIHSS-certified practitioner; see [Appendix 7](#))
- Concomitant medications

All screening evaluations with the exception of certain laboratory results (e.g., coagulation and complete blood count) must be completed and reviewed to confirm that patients have met all eligibility criteria prior to treatment. Serum glucose is required to confirm eligibility prior to enrollment; however, if the investigator feels there is clinical suspicion that would be related to an exclusion laboratory test that would preclude the patient from eligibility, the results of those laboratory tests must be obtained prior to treatment.

The investigator will maintain a screening log to record details of all patients consented to confirm eligibility or record reasons for screening failure, as applicable.

4.6.2 Visit 2 (22 to 36 Hours after Treatment Initiation)

A non-contrast cranial CT or MRI (including DW MRI, either SW or GRE, FLAIR, and T1 and T2 sequences) will be performed at 22 to 36 hours after treatment with study drug has been initiated (bolus). Either a CT scan or MRI may be performed, as consistent with the institutional standard of care; the main criterion is to perform radiographic imaging of the brain by a modality sensitive to the presence of blood. An NIHSS will also be performed during this visit, but prior to review of the imaging results (or by an

investigator blinded to the results). AEs¹ and concomitant medications will also be assessed at this visit.

4.6.3 Visit 3 (Day 5 or Discharge from Hospital, if Sooner than Day 5)

On Day 5 (or discharge from hospital, if sooner than Day 5), NIHSS score, stroke etiology, AEs, and concomitant medications will be collected. Discharge location will be collected at the time of hospital discharge.

4.6.4 Visit 4 (Day 30 [30 ± 7 Days])

A phone call to the patient or caregiver will be made at Day 30 and an mRS will be assessed (see [Appendix 8](#)). AEs, concomitant medications, and patient survival will be assessed during this phone visit. Pregnancy will also be assessed for female patients of childbearing potential.

4.6.5 Visit 5 (Day 90 [± 14 Days])/Study Completion)

Primary and secondary efficacy endpoints, as well as all exploratory endpoints, will be assessed at Day 90 with the patient. The following assessments will be performed:

- NIHSS score ([Appendix 7](#))
- Patient survival
- mRS ([Appendix 8](#))
- GOS ([Appendix 9](#))
- BI ([Appendix 10](#))
- EQ-5D ([Appendix 4](#))
- SIS-16 ([Appendix 5](#))
- CES-D ([Appendix 6](#))
- Cognitive assessments ([Appendix 11](#))
 - 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 test, form V
 - HVLT-R trial 4 and recognition
 - Animal Naming test

¹ AE/SAE collection should be started as soon as study drug administration (bolus) is initiated.

- BNT 15-item short form

The above cognitive assessments will be provided in Spanish for those patients requiring translations with the exception of the BNT 15-item short form.

- Walking speed ([Appendix 12](#))
- Concomitant medications
- SAEs and certain AEs (See Section [5.3.1](#))

4.6.6 Early Discontinuation

Concomitant medication, AEs, and patient survival will be assessed via a phone call, if possible.

4.7 PATIENT, TREATMENT, STUDY, AND SITE DISCONTINUATION

4.7.1 Patient Discontinuation

Patients have the right to voluntarily withdraw from the study at any time and for any reason. In addition, the investigator has the right to withdraw a patient from the study at any time. Reasons for withdrawal from the study may include, but are not limited to, the following:

- Patient withdrawal of consent at any time
- Any medical condition that the investigator or Sponsor determines may jeopardize the patient's safety if he or she continues in the study
- Investigator or Sponsor determines it is in the best interest of the patient
- Patient non-compliance, specifically defined as unwillingness to participate in a 3-month assessment of neurological status and function

Every effort should be made to obtain information on patients who withdraw from the study. The primary reason for withdrawal from the study should be documented on the appropriate eCRF. However, patients will not be followed for any reason after consent has been withdrawn. Patients who withdraw from the study will not be replaced.

4.7.2 Study Treatment Discontinuation

The study intervention should be discontinued if the patient displays symptoms concerning sICH during the infusion of the study drug. Prompt imaging should be done to assess for ICH. If ICH is seen, study drug infusion should not be resumed and the treatment assignment may be unblinded if blood products (to reverse the biological activity of IV alteplase) are indicated. If ICH is not seen, study drug infusion should immediately be restarted *in order to complete the infusion within 4 hours after the last known well time at the same rate as the previous infusion until the remainder of study drug is infused.*

Patients must discontinue study treatment if they experience any of the following:

- Anaphylactoid reaction (including angioedema)
- Serious bleeding (not controllable by local pressure) at puncture site or serious bleeding in a critical location (e.g., intracranial, gastrointestinal, retroperitoneal, pericardial)

The primary reason for study treatment discontinuation should be documented on the appropriate eCRF. Patients who discontinue study treatment prematurely will not be replaced. Patients who discontinue study treatment prematurely will continue in the study and be followed through Day 90, based on the intent-to-treat (ITT) principle.

4.7.3 Study and Site Discontinuation

The Sponsor has the right to terminate this study at any time. The Sponsor will notify the investigator if the Sponsor decides to discontinue the study. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of AEs in this or other studies indicates a potential health hazard to patients.
- Patient enrollment is unsatisfactory
- A pre-specified boundary at the interim analysis was crossed

The Sponsor has the right to close a site at any time. Reasons for closing a site may include, but are not limited to, the following:

- Excessively slow recruitment
- Poor protocol adherence
- Inaccurate or incomplete data recording
- Non-compliance with the International Conference on Harmonisation guideline for Good Clinical Practice
- No study activity (i.e., all patients have completed study assessments and all obligations have been fulfilled)

5. ASSESSMENT OF SAFETY

5.1 SAFETY PLAN

The safety profile of alteplase across indications is generally well established. The principal adverse reactions to alteplase are bleeding and allergic reactions.

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5.1.1 Risk of Bleeding

Bleeding is the most frequent adverse reaction associated with alteplase in all approved indications, including AIS. This may be either superficial from punctures or damaged blood vessels or internal bleeding at any site or body cavity. Bleeding may result in life-threatening situations, permanent disability, or death.

- The incidence of ICH, especially symptomatic ICH, in patients with AIS is higher in alteplase-treated patients than placebo-treated patients in published studies (for detailed information, see the alteplase USPI).

Management of Bleeding

Patients will be excluded for the presence of conditions related to risks of bleeding (as outlined in Section 4.1.2, Exclusion Criteria). A non-contrast cranial CT or MRI (including either SW or GRE to detect ICH) should be performed at baseline to ensure that the patient does not have evidence of ICH prior to study drug administration.

Fibrin, which is part of the hemostatic plug formed at needle puncture sites, may be lysed during alteplase therapy. Therefore, alteplase therapy requires careful attention to potential bleeding sites, e.g., catheter-insertion sites and arterial-puncture sites. Arterial and venous punctures should be minimized. Non-compressible arterial, as well as internal jugular and subclavian venous punctures, should be avoided to minimize bleeding from non-compressible sites. In the event of serious bleeding (not controllable by local pressure) or occurring in a critical location (intracranial, gastrointestinal, retroperitoneal, or pericardial), study drug should be discontinued immediately.

Supportive care and medical management of AEs are at the discretion of the investigator.

5.1.2 Risk of Allergic Reactions

Allergic-type reactions, e.g., anaphylactoid reaction, laryngeal edema, orolingual angioedema, rash, and urticaria, have been reported. When such reactions occur, they usually respond to conventional therapy.

There have been postmarketing reports of orolingual angioedema associated with the use of alteplase. Most were patients treated for AIS. Many of these patients received concomitant angiotensin-converting enzyme inhibitors, and most cases resolved with prompt treatment.

Management of Allergic Reaction

If an anaphylactic reaction occurs, the infusion of study drug should be discontinued immediately and appropriate therapy should be promptly instituted.

5.2 SAFETY PARAMETERS AND DEFINITIONS

Safety assessments will consist of monitoring and recording AEs, including SAEs and non-serious adverse events of special interest (AESIs), *performing protocol-specified safety laboratory assessments, performing protocol-specified vital signs, and conducting*

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other protocol-specified tests that are deemed critical to the safety evaluation of the study.

Certain types of events require immediate reporting to the Sponsor, as outlined in Sections [5.2.2](#) and [5.2.3](#).

5.2.1 Adverse Events

According to the International Conference on Harmonisation guideline for Good Clinical Practice, an AE is any untoward medical occurrence in a clinical investigation patient administered a pharmaceutical product, regardless of causal attribution. An AE can therefore be any of the following:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product
- Any new disease or exacerbation of an existing disease (a worsening in the character, frequency, or severity of a known condition), except as described in Section [5.3.3.9](#)
- Recurrence of an intermittent medical condition (e.g., headache) not present at baseline
- Any deterioration in a laboratory value or other clinical test (e.g., electrocardiogram, X-ray) that is associated with symptoms or leads to a change in study treatment or concomitant treatment or discontinuation from study drug

5.2.2 Serious Adverse Events (Immediately Reportable to the Sponsor)

An SAE is any AE that meets any of the following criteria:

- Is fatal (i.e., the AE actually causes or leads to death)
- Is life-threatening (i.e., the AE, in the view of the investigator, places the patient at immediate risk of death)

This does not include any AE that, had it occurred in a more severe form or was allowed to continue, might have caused death.

- Requires or prolongs inpatient hospitalization (see Section [5.3.3.9](#))
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the patient's ability to conduct normal life functions)
- Is a congenital anomaly/birth defect in a neonate/infant born to a mother exposed to study drug
- Is a significant medical event in the investigator's judgment (e.g., may jeopardize the patient or may require medical/surgical intervention to prevent one of the outcomes listed above)

The terms "severe" and "serious" are not synonymous. Severity refers to the intensity of an adverse event (rated as mild, moderate, or severe); the event itself may be of

relatively minor medical significance (such as severe headache). “Serious” is a regulatory definition and is based on patient or event outcome or action criteria usually associated with events that pose a threat to a patient’s life or vital functions. Seriousness (not severity) serves as the guide for defining regulatory reporting obligations.

Severity and seriousness need to be independently assessed for each AE recorded on the eCRF.

SAEs 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](#) for reporting instructions).

5.2.3 Non-Serious Adverse Events of Special Interest (Immediately Reportable to the Sponsor)

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 [5.4.1](#) 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](#))
- Stroke recurrence: report if the AE resulted in a substantial disruption of a person’s ability to conduct normal life functions (i.e., the AE resulted in a significant, persistent, or permanent change; impairment; or damage or disruption in the patient’s body function/structure, physical activities, and/or quality of life).
- In addition, Suspected Transmission of an Infectious Agent via a Medicinal Product (STIAMP) by the study drug in a Genentech-sponsored trial qualifies as an AESI and requires immediate reporting to the Sponsor.

5.3 METHODS AND TIMING FOR CAPTURING AND ASSESSING SAFETY PARAMETERS

The investigator is responsible for ensuring that all AEs (see Section [5.2.1](#) for definition) are recorded on the Adverse Event eCRF and reported to the Sponsor in accordance with instructions provided in this section and in Sections [5.4–5.6](#).

For each AE recorded on the Adverse Event eCRF, the investigator will make an assessment of seriousness (see Section [5.2.2](#) for seriousness criteria), severity (see Section [5.3.2](#)), and causality (see Section [5.3.3](#)).

5.3.1 Adverse Event Reporting Period

Investigators will seek information on AEs at each patient contact. All AEs, whether reported by the patient or noted by study personnel, will be recorded in the patient’s medical record and on the Adverse Event eCRF.

After initiation of study drug, all AEs (including serious and non-serious AEs), regardless of relationship to study drug, will be reported until 30 days after administration of study drug.

After 30 days until 90 days after administration of study drug, the following AEs should be recorded on the AE CRF:

- All SAEs
- Non-serious ICHs
- Non-serious AESIs
- All AEs that resulted in withdrawal from the study

All SAEs and non-serious AESIs require immediate reporting to the Sponsor (see Section 5.4).

After 90 days, the investigator is not required to actively monitor patients for AEs; however, the Sponsor should be notified if the investigator becomes aware of any post-study SAEs (see Section 5.6).

5.3.2 Assessment of Severity of Adverse Events

The WHO toxicity grading scale (see [Appendix 14](#)) will be used for assessing adverse event severity. Table 4 will be used for assessing severity for adverse events that are not specifically listed in the WHO toxicity grading scale.

Table 4 Adverse Event Severity Grading Scale

Grade	Severity
1	Mild; transient or mild discomfort (<48 hours); no medical intervention or therapy required
2	Moderate; mild to moderate limitation in activity; some assistance may be needed; no or minimal medical intervention or therapy required
3	Severe; marked limitation in activity; some assistance usually required; medical intervention or therapy required; hospitalization possible
4	Life-threatening; extreme limitation in activity; significant assistance required; significant medical intervention or therapy required, hospitalization or hospice care probable

Notes: Developed by the Division of Microbiology and Infectious Diseases.

Regardless of severity, some events may also meet seriousness criteria. Refer to definition of a serious adverse event (see Section 5.2.2).

5.3.3 Assessment of Causality of Adverse Events

Investigators should use their knowledge of the patient, the circumstances surrounding the event, and an evaluation of any potential alternative causes to determine whether or not an AE is considered to be related to the study drug, indicating "yes" or "no" accordingly. The following guidance should be taken into consideration (see also [Table 5](#)):

- Temporal relationship of event onset to the initiation of study drug
- Course of the event, considering especially the effects of dose reduction, discontinuation of study drug, or reintroduction of study drug (as applicable)
- Known association of the event with the study drug or with similar treatments
- Known association of the event with the disease under study
- Presence of risk factors in the patient or use of concomitant medications known to increase the occurrence of the event
- Presence of non-treatment-related factors that are known to be associated with the occurrence of the event

Table 5 Causal Attribution Guidance

Is the adverse event suspected to be caused by the study drug on the basis of facts, evidence, science-based rationales, and clinical judgment?	
YES	There is a plausible temporal relationship between the onset of the adverse event and administration of the study drug, and the adverse event cannot be readily explained by the patient's clinical state, intercurrent illness, or concomitant therapies; and/or the adverse event follows a known pattern of response to the study drug; and/or the adverse event abates or resolves upon discontinuation of the study drug or dose reduction and, if applicable, reappears upon re-challenge.
NO	Adverse events will be considered related, unless they fulfill the criteria as specified below. Evidence exists that the adverse event has an etiology other than the study drug (e.g., preexisting medical condition, underlying disease, intercurrent illness, or concomitant medication); and/or the adverse event has no plausible temporal relationship to administration of the study drug (e.g., cancer diagnosed 2 days after first dose of study drug).

5.3.3.1 **Diagnosis versus Signs and Symptoms**

A diagnosis (if known) should be recorded on the Adverse Event eCRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be medically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded on the Adverse Event eCRF. If a diagnosis is subsequently established, all previously reported AEs based on signs and symptoms should be nullified and replaced by one AE report based on the single diagnosis, with a starting date that corresponds to the starting date of the first symptom of the eventual diagnosis.

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5.3.3.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause, with the exception of severe or serious secondary events. However, medically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the Adverse Event eCRF. For example:

- If vomiting results in mild dehydration with no additional treatment in a healthy adult, only vomiting should be reported on the eCRF.
- If vomiting results in severe dehydration, both events should be reported separately on the eCRF.
- If a severe gastrointestinal hemorrhage leads to renal failure, both events should be reported separately on the eCRF.
- If dizziness leads to a fall and consequent fracture, all three events should be reported separately on the eCRF.
- If neutropenia is accompanied by an infection, both events should be reported separately on the eCRF.

All AEs should be recorded separately on the Adverse Event eCRF if it is unclear as to whether the events are associated.

5.3.3.3 Persistent or Recurrent Adverse Events

A persistent AE is one that extends continuously, without resolution, between patient evaluation timepoints. Such events should only be recorded once on the Adverse Event eCRF. The initial severity (*intensity or grade*) of the event should be recorded *at the time the event is first reported*. *If a persistent AE becomes more severe, the most extreme severity should also be recorded on the Adverse Event eCRF. If the event becomes serious, it should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning that the event became serious; see Section 5.4.1 for reporting instructions). The Adverse Event eCRF should be updated by changing the event from "non-serious" to "serious," providing the date that the event became serious, and completing all data fields related to SAEs.*

A recurrent AE is one that resolves between patient evaluation timepoints and subsequently recurs. Each recurrence of an AE should be recorded *as a separate event* on the Adverse Event eCRF.

5.3.3.4 Abnormal Laboratory Values

Not every laboratory abnormality qualifies as an AE. A laboratory test result must be reported as an AE if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)

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- Results in a medical intervention (e.g., potassium supplementation for hypokalemia) or a change in concomitant therapy
- Is clinically significant in the investigator's judgment

It is the investigator's responsibility to review all laboratory findings. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an AE.

If a clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 times the upper limit of normal associated with cholestasis), only the diagnosis (i.e., cholestasis) should be recorded on the Adverse Event eCRF.

If a clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded on the Adverse Event eCRF, along with a descriptor indicating if the test result is above or below the normal range (e.g., "elevated potassium" as opposed to "abnormal potassium"). If the laboratory abnormality can be characterized by a precise clinical term per standard definitions, the clinical term should be recorded as the AE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia."

Observations of the same clinically significant laboratory abnormality from visit to visit should *only* be recorded *once* on the Adverse Event eCRF (*see Section 5.3.3.3 for details on recording persistent AEs*).

5.3.3.5 Abnormal Vital Sign Values

Not every vital sign abnormality qualifies as an AE. A vital sign result must be reported as an AE if it meets any of the following criteria:

- Is accompanied by clinical symptoms
- Results in a change in study treatment (e.g., dosage modification, treatment interruption, or treatment discontinuation)
- Results in a medical intervention or a change in concomitant therapy
- Is clinically significant in the investigator's judgment

It is the investigator's responsibility to review all vital sign findings. Medical and scientific judgment should be exercised in deciding whether an isolated vital sign abnormality should be classified as an AE.

If a clinically significant vital sign abnormality is a sign of a disease or syndrome (e.g., high blood pressure), only the diagnosis (i.e., hypertension) should be recorded on the Adverse Event eCRF.

Observations of the same clinically significant vital sign abnormality from visit to visit should *only* be recorded *once* on the Adverse Event eCRF (see Section 5.3.3.3 for details on recording persistent AEs).

5.3.3.6 Deaths

All deaths that occur during the protocol-specified AE reporting period (Section 5.3.1) regardless of relationship to study drug, must be recorded on the Adverse Event eCRF and immediately reported to the Sponsor (see Section 5.2.2). This includes death attributed to progression of AIS.

Death should be considered an outcome and not a distinct event. The event or condition that caused or contributed to the fatal outcome should be recorded as the single medical concept on the Adverse Event eCRF. Generally, only one such event should be reported. The term "sudden death" should only be used for the occurrence of an abrupt and unexpected death due to presumed cardiac causes in a patient with or without preexisting heart disease, within 1 hour of the onset of acute symptoms or, in the case of an unwitnessed death, within 24 hours after the patient was last seen alive and stable. If the cause of death is unknown and cannot be ascertained at the time of reporting, "unexplained death" should be recorded on the Adverse Event eCRF. If the cause of death later becomes available (e.g., after autopsy), "unexplained death" should be replaced by the established cause of death.

If the death is attributed to progression of AIS, "acute ischemic stroke progression" should be recorded on the Adverse Event eCRF.

5.3.3.7 Preexisting Medical Conditions

A preexisting medical condition is one that is present at the screening visit for this study. Such conditions should be recorded on the General Medical History and Baseline Conditions eCRF.

A preexisting medical condition should be recorded as an AE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on the Adverse Event eCRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., more frequent headaches").

5.3.3.8 Lack of Efficacy or Worsening of Acute Ischemic Stroke

Medical occurrences or symptoms of deterioration that are considered part of the disease course or disease process should be recorded as an AE if judged by the investigator to have unexpectedly worsened in severity or frequency or unexpectedly changed in nature at any time during the study. If there is uncertainty, it should be reported as an AE. When recording unexpected worsening of AIS on the Adverse Event eCRF, it is important to convey the concept that the condition has changed by including applicable descriptors of the event.

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5.3.3.9 Hospitalization or Prolonged Hospitalization

Any AE that results in hospitalization (i.e., in-patient admission to a hospital) or prolonged hospitalization should be documented and reported as an SAE (per the definition of an SAE in Section 5.2.2), except as outlined below.

The following hospitalization scenarios are not considered to be AEs:

- Hospitalization for respite care
- Hospitalization for a preexisting condition, provided that all of the following criteria are met:
 - The hospitalization was planned prior to the study or was scheduled during the study when elective surgery became necessary because of the expected normal progression of the disease.
 - The patient has not suffered an AE.

5.3.3.10 Adverse Events Associated with an Overdose or Error in Drug Administration

An overdose is the accidental or intentional use of a drug in an amount higher than the dose being studied. An overdose or incorrect administration of study treatment is not itself an AE, but it may result in an AE. All AEs associated with an overdose or incorrect administration of study drug should be recorded on the Adverse Event eCRF. If the associated AE fulfills *seriousness* criteria, the event should be reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.1).

5.3.3.11 Patient-Reported Outcome Data

AE reports will not be derived from PRO data by the Sponsor, and safety analyses will not be performed using PRO data. However, if any PRO responses suggestive of a possible AE are identified during site review of the PRO data, the investigator will determine whether the criteria for an AE have been met and, if so, will report the event on the Adverse Event eCRF.

5.4 IMMEDIATE REPORTING REQUIREMENTS FROM INVESTIGATOR TO SPONSOR

Certain events require immediate reporting to allow the Sponsor to take appropriate measures to address potential new risks in a clinical trial. The investigator must report such events to the Sponsor immediately; under no circumstances should reporting take place more than 24 hours after the investigator learns of the event. The following is a list of events that the investigator must report to the Sponsor within 24 hours after learning of the event, regardless of relationship to study drug:

- SAEs (see Section 5.4.2 for further details)
- Non-serious AESIs (see Section 5.4.2 for further details)
- Pregnancies (see Section 5.4.3 for further details)

The investigator must report new significant follow-up information for these events to the Sponsor immediately (i.e., no more than 24 hours after becoming aware of the information). New significant information includes the following:

- New signs or symptoms or a change in the diagnosis
- Significant new diagnostic test results
- Change in causality based on new information
- Change in the event's outcome, including recovery
- Additional narrative information on the clinical course of the event

Investigators must also comply with local requirements for reporting SAEs to the local health authority and Institutional Review Board/Ethics Committee (IRB/EC).

5.4.1 Emergency Medical Contacts

Medical Monitor Contact Information

PPD Medical Monitor contact information:

Medical Monitor: [REDACTED], M.D.

Telephone Nos.: [REDACTED]

Alternate Medical Monitor contact information for all sites:

Medical Monitor: [REDACTED], M.D.

Telephone No.: [REDACTED]

5.4.2 Reporting Requirements for Serious Adverse Events and Non-Serious Adverse Events of Special Interest

After initiation of study drug, SAEs and non-serious AESIs will be reported until 90 days after the last dose of study drug. Investigators should record all case details that can be gathered immediately (i.e., within 24 hours after learning of the event) on the Adverse Event eCRF and submit the report via the electronic data capture (EDC) system. A report will be generated and sent to Safety Risk Management by the EDC system.

In the event that the EDC system is unavailable, the Serious Adverse Event/Adverse Event of Special Interest Reporting Form provided to investigators should be completed and submitted to the Sponsor or its designee immediately (i.e., no more than 24 hours after learning of the event), either by faxing or by scanning and emailing the form using the fax number or email address provided to investigators (and below). Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

Sites:

Fax No.: (650) 225-4682

Alternate Fax No.: (650) 225-5288

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Instructions for reporting post-study AEs are provided in Section 5.6.

5.4.3 Reporting Requirements for Pregnancies

5.4.3.1 Pregnancies in Female Patients

Female patients of childbearing potential will be instructed to immediately inform the investigator if they become pregnant within 30 (± 7) days after the last dose of study drug. A Pregnancy Report eCRF should be completed by the investigator immediately (i.e., no more than 24 hours after learning of the pregnancy) and submitted via the EDC system. A pregnancy report will automatically be generated and sent to Safety Risk Management. Pregnancy should not be recorded on the Adverse Event eCRF. The investigator should counsel the patient, discussing the risks of the pregnancy and the possible effects on the fetus. Monitoring of the patient should continue until conclusion of the pregnancy. Any SAEs associated with the pregnancy (e.g., an event in the fetus, an event in the mother during or after the pregnancy, or a congenital anomaly/birth defect in the child) should be reported on the Adverse Event eCRF.

In the event that the EDC system is unavailable, *the Clinical Trial Pregnancy Reporting Form provided to investigators* should be completed and *submitted to the Sponsor or its designee immediately* (i.e., no more than 24 hours after learning of the pregnancy), *either by faxing or scanning and emailing the form using the fax number or email address provided to investigators*. Once the EDC system is available, all information will need to be entered and submitted via the EDC system.

5.4.3.2 Congenital Anomalies/Birth Defects and Abortions

Any congenital anomaly/birth defect in a child born to a female patient exposed to study drug should be classified as an SAE, recorded on the Adverse Event eCRF, and reported to the Sponsor immediately (i.e., no more than 24 hours after learning of the event; see Section 5.4.1). Any spontaneous abortion should be reported in the same fashion (as the Sponsor considers spontaneous abortions to be medically significant events).

5.5 FOLLOW-UP OF PATIENTS AFTER ADVERSE EVENTS

5.5.1 Investigator Follow-Up

The investigator should follow each AE until the event has resolved to baseline grade or better and is assessed as stable by the investigator, the patient is lost to follow-up, or the patient withdraws consent. Every effort should be made to follow all SAEs considered to be related to study drug or trial-related procedures until a final outcome can be reported.

During the study period, resolution of AEs (with dates) should be documented on the Adverse Event eCRF and in the patient's medical record to facilitate source data verification.

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All pregnancies reported during the study should be followed until pregnancy outcome. If the EDC system is not available at the time of pregnancy outcome, follow reporting instructions provided in Section 5.4.3.

5.5.2 Sponsor Follow-Up

For SAEs, AESIs, and pregnancies, the Sponsor or a designee may follow up by telephone, fax, electronic mail, and/or a monitoring visit to obtain additional case details and outcome information (e.g., from hospital discharge summaries, consultant reports, autopsy reports) in order to perform an independent medical assessment of the reported case.

5.6 POST-STUDY ADVERSE EVENTS

At the time of study completion or study discontinuation, the investigator should instruct each patient to report to the investigator any subsequent AEs that the patient's personal physician believes could be related to prior study drug treatment or study procedures.

The investigator is not required to actively monitor patients for AEs after the end of the AE reporting period (defined as 90 days after administration of study drug *for SAEs and non-serious AESIs, and 30 days for non-serious AEs*). However, the Sponsor should be notified if the investigator becomes aware of any SAE, death, development of cancer, or a congenital anomaly/birth defect in a subsequently conceived offspring of a female patient exposed to study drug *within 90 days after administration of study drug*.

The investigator should report these events directly to *the Sponsor* via telephone at 1-888-835-2555.

5.7 EXPEDITED REPORTING TO HEALTH AUTHORITIES, INVESTIGATORS, INSTITUTIONAL REVIEW BOARDS, AND ETHICS COMMITTEES

The Sponsor will promptly evaluate all SAEs and non-serious AESI against cumulative product experience to identify and expeditiously communicate possible new safety findings to investigators, IRBs, ECs, and applicable health authorities based on applicable legislation.

To determine reporting requirements for single AE cases, the Sponsor will assess the expectedness of these events using the following reference document:

- Alteplase USPI

The Sponsor will compare the severity of each event and the cumulative event frequency reported for the study with the severity and frequency reported in the applicable reference document.

Reporting requirements will also be based on the investigator's assessment of causality and seriousness, with allowance for upgrading by the Sponsor as needed.

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6. STATISTICAL METHODS

This study will test whether treatment of patients with mild stroke with IV alteplase will lead to a higher proportion (9% absolute difference) of patients with a favorable outcome (mRS of 0 or 1) at 90 days.

6.1 DETERMINATION OF SAMPLE SIZE

A sample size of 856 is required in order to achieve 80% power in 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 for this 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 size 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.

6.2 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 ITT principle. As a sensitivity analysis, results from the univariate Pearson's chi-square test will also be presented.

As a supportive, secondary analysis, an ordinal (or "shift") analysis of the distribution of the mRS will be analyzed via proportional odds model if the proportionality assumption holds, and by the Mann-Whitney superiority measure if the proportionality assumption does not hold. The proportionality assumption will be assessed using the score test as well as clinical judgment on the similarities of the different odds ratios. As a secondary efficacy outcome, the global outcome score at 90 days will be analyzed using the global odds ratio test based on the generalized estimating equations analysis (Wald-type χ^2)

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test) with unstructured covariance-correlation matrix. These results will be treated as supportive evidence (or lack thereof) of the treatment effect rather than conclusive evidence. The significance of each test is determined at the two-sided alpha level of 0.05. The specific statistical model for analyzing each of these outcome measures will be detailed in the Statistical Analysis Plan (SAP).

6.3 SAFETY ANALYSIS

Incidence and severity of AEs will be described for all treated patients by treatment group as received and by the Medical Dictionary for Regulatory Activities (MedDRA) classification.

Safety outcomes include incidence of sICH within 36 hours (primary safety assessment), any ICH within 36 hours, overall mortality, and stroke-related and neurological deaths within 90 days of study drug initiation. The cumulative incidences of each outcome will be compared via Fisher's exact test. Mortality will be analyzed by Log-Rank test and the Kaplan-Meier estimates will be plotted over the observation period of 90 days. No alpha adjustment for multiple testing on the safety endpoints is foreseen because those analyses will be exploratory.

6.4 EXPLORATORY ANALYSES

As exploratory analyses, heterogeneity 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 (<65 vs. ≥65)
- Pre-treatment NIHSS score (0–2 vs. 3–5)
- Last known well time to treatment (0–2 vs. >2–3 hours)
- Stroke subgroup (RISS vs. non-RISS)

Additional heterogeneity analyses will be considered and detailed in the SAP. Forest plots will be constructed to illustrate subgroup analyses. In addition, the two-way interactions of IV alteplase treatment effect with age, NIHSS score, and stroke subgroups (RISS vs. non-RISS) on the primary outcome will be explored through multivariable logistic regression. Additional exploratory analyses will be conducted to explore treatment effects on quality of life endpoints and other exploratory endpoints.

6.5 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.

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

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An iDMC will be organized for review of the interim analysis results. The composition of the iDMC and analysis in detail will be documented in a separate iDMC charter.

6.6 MISSING DATA HANDLING

Under the ITT principle, all patients who are randomized will be included in the analysis. The multiple-imputation method will be used for handling missing data, including the last observation (e.g., the Day 30 mRS) among the variables used in imputation. As supportive analyses, missing primary outcome data will be imputed by 1) using last observation carried forward and 2) assuming missing outcomes to be unfavorable.

7. DATA COLLECTION AND MANAGEMENT

7.1 DATA QUALITY ASSURANCE

The Sponsor will supply eCRF specifications for this study. A contract research organization (CRO) will be responsible for data management of this study, including quality checking of the data. Data entered manually will be collected via EDC using eCRFs. Sites will be responsible for data entry into the EDC system. In the event of discrepant data, the CRO will request data clarification from the sites, which the sites will resolve electronically in the EDC system.

The CRO will produce a Data Quality Plan that describes the quality checking to be performed on the data with the input from the Sponsor. For this study, local laboratories will be used and data will be captured in the EDC system and will then be transferred to the Sponsor. This will include the eCRF data using the Sponsor's standard procedures and processes to transfer data.

The Sponsor will perform oversight of the data management of this study, including approval of the CRO's data management plans and specifications. Data will be transferred electronically from the CRO to the Sponsor, and the Sponsor's standard procedures will be used to handle and process the electronic transfer of these data.

eCRFs and correction documentation will be maintained in the EDC system's audit trail. The EDC vendor has back-ups for data stored, and records retention for the study data will be consistent with the CRO's standard procedures.

Data from paper PRO questionnaires will be entered into the EDC system by site staff.

7.2 ELECTRONIC CASE REPORT FORMS

eCRFs are to be completed using a Sponsor-designated EDC system. Sites will receive training and have eCRF completion guidelines for the eCRF completion. eCRFs will be submitted electronically to the Sponsor and should be handled in accordance with instructions from the Sponsor.

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All eCRFs should be completed by designated trained site staff. eCRFs should be reviewed and electronically signed and dated by the investigator or a designee.

At the end of the study, the investigator will receive patient data for his or her site in a readable format on a compact disc that must be kept with the study records.

Acknowledgement of receipt of the compact disc is required.

7.3 SOURCE DATA DOCUMENTATION

Study monitors will perform ongoing source data verification to confirm that critical protocol data (e.g., source data) entered into the eCRFs by authorized site personnel are accurate, complete, and verifiable from source documents. Source documents (paper or electronic) are those in which patient data are recorded and documented for the first time. They include, but are not limited to, hospital records, clinical and office charts, laboratory notes, memoranda, patient-reported outcomes, evaluation checklists, pharmacy dispensing records, recorded data from automated instruments, copies of transcriptions that are certified after verification as being accurate and complete, microfiche, photographic negatives, microfilm or magnetic media, X-rays, patient files, and records kept at pharmacies, laboratories, and medico-technical departments involved in a clinical trial.

Before study initiation, the types of source documents that are to be generated will be clearly defined in the Study Trial Monitoring Plan. This includes any protocol data to be entered directly into the eCRFs (i.e., no prior written or electronic record of the data) and considered source data.

Source documents that are required to verify the validity and completeness of data entered into the eCRFs must not be obliterated or destroyed and must be retained per the policy for retention of records described in Section [7.5](#).

To facilitate source data verification, the investigators and institutions must provide the Sponsor direct access to applicable source documents and reports for trial-related monitoring, Sponsor audits, and IRB/EC review. The investigational site must also allow inspection by applicable health authorities.

7.4 USE OF COMPUTERIZED SYSTEMS

When clinical observations are entered directly into an investigational site's computerized medical record system (i.e., in lieu of original hardcopy records), the electronic record can serve as the source document if the system has been validated in accordance with health authority requirements pertaining to computerized systems used in clinical research. An acceptable computerized data collection system allows preservation of the original entry of data. If original data are modified, the system should maintain a viewable audit trail that shows the original data as well as the reason for the change, name of the person making the change, and date of the change.

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7.5 RETENTION OF RECORDS

Records and documents pertaining to the conduct of this study and the distribution of IMP, including eCRFs, PRO data (if applicable), Informed Consent Forms, laboratory test results, and medication inventory records, must be retained by the Principal Investigator for at least 15 years after completion or discontinuation of the study, or for the length of time required by relevant national or local health authorities, whichever is longer. After that period of time, the documents may be destroyed, subject to local regulations.

No records may be disposed of without the written approval of the Sponsor. Written notification should be provided to the Sponsor prior to transferring any records to another party or moving them to another location.

8. ETHICAL CONSIDERATIONS

8.1 COMPLIANCE WITH LAWS AND REGULATIONS

This study will be conducted in full conformance with the International Conference on Harmonisation E6 guideline for Good Clinical Practice and the principles of the Declaration of Helsinki, or the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study will comply with the requirements of the International Conference on Harmonisation E2A guideline (Clinical Safety Data Management: Definitions and Standards for Expedited Reporting). Studies conducted in the United States or under a U.S. Investigational New Drug (IND) application will comply with FDA regulations and applicable local, state, and federal laws. Studies conducted in the European Union (EU)/European Economic Area (EEA) will comply with the EU Clinical Trial Directive (2001/20/EC).

8.2 INFORMED CONSENT

The Sponsor's sample Informed Consent Form (and ancillary sample Informed Consent Forms such as a Child's *Informed Assent Form* or *Home Nursing* Informed Consent Form, if applicable) will be provided to each site. If applicable, it will be provided in a certified translation of the local language. The Sponsor or its designee must review and approve any proposed deviations from the Sponsor's sample Informed Consent Forms or any alternate consent forms proposed by the site (collectively, the "Consent Forms") before IRB/EC submission. The final IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes according to local requirements.

The Consent Forms should be revised whenever there are changes to study procedures or when new information becomes available that may affect the willingness of the patient to participate. The final revised IRB/EC-approved Consent Forms must be provided to the Sponsor for health authority submission purposes.

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Patients must be re-consented to the most current version of the Consent Forms (or to a significant new information/findings addendum in accordance with applicable laws and IRB/EC policy) during their participation in the study. For any updated or revised Consent Forms, the case history or clinical records for each patient shall document the informed consent process and that written informed consent was obtained using the updated/revised Consent Forms for continued participation in the study.

A copy of each signed Consent Form must be provided to the patient or the patient's legally authorized representative. All signed and dated Consent Forms must remain in each patient's study file or in the site file and must be available for verification by study monitors at any time.

For sites in the U.S., each Consent Form may also include patient authorization to allow use and disclosure of personal health information in compliance with the U.S. Health Insurance Portability and Accountability Act of 1996 (HIPAA). If the site utilizes a separate Authorization Form for patient authorization for use and disclosure of personal health information under the HIPAA regulations, the review, approval, and other processes outlined above apply except that IRB review and approval may not be required per study site policies.

8.3 INSTITUTIONAL REVIEW BOARD OR ETHICS COMMITTEE

This protocol, the Informed Consent Forms, any information to be given to the patient, and relevant supporting information must be submitted to the IRB/EC by the Principal Investigator and reviewed and approved by the IRB/EC before the study is initiated. In addition, any patient recruitment materials must be approved by the IRB/EC.

The Principal Investigator is responsible for providing written summaries of the status of the study to the IRB/EC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/EC. Investigators are also responsible for promptly informing the IRB/EC of any protocol amendments (Section 9.6).

In addition to the requirements for reporting all AEs to the Sponsor, investigators must comply with requirements for reporting SAEs to the local health authority and IRB/EC. Investigators may receive written IND safety reports or other safety-related communications from the Sponsor. Investigators are responsible for ensuring that such reports are reviewed and processed in accordance with health authority requirements and the policies and procedures established by their IRB/EC, and archived in the site's study file.

8.4 CONFIDENTIALITY

The Sponsor maintains confidentiality standards by coding each patient enrolled in the study through assignment of a unique patient ID number. This means that patient names are not included in data sets that are transmitted to any Sponsor location.

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Patient medical information obtained by this study is confidential and may only be disclosed to third parties as permitted by the Informed Consent Form (or separate authorization for use and disclosure of personal health information) signed by the patient, unless permitted or required by law.

Medical information may be given to a patient's personal physician or other appropriate medical personnel responsible for the patient's welfare, for treatment purposes.

Data generated by this study must be available for inspection upon request by representatives of the FDA and other national and local health authorities, Sponsor monitors, representatives, and collaborators, and the IRB/EC for each study site, as appropriate.

8.5 FINANCIAL DISCLOSURE

Investigators will provide the Sponsor with sufficient, accurate financial information in accordance with local regulations to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate health authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study (i.e., LPLV).

9. STUDY DOCUMENTATION, MONITORING, AND ADMINISTRATION

9.1 STUDY DOCUMENTATION

The investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented, including but not limited to the protocol, protocol amendments, Informed Consent Forms, and documentation of IRB/EC and governmental approval. In addition, at the end of the study, the investigator will receive the patient data, which includes an audit trail containing a complete record of all changes to data.

9.2 PROTOCOL VIOLATIONS

The investigator should document and explain any protocol violations. The investigator should promptly report any violations that might impact patient safety and data integrity to the Sponsor and to the IRB/EC in accordance with established IRB/EC policies and procedures.

9.3 SITE INSPECTIONS

Site visits will be conducted by the Sponsor or an authorized representative for inspection of study data, patients' medical records, and eCRFs. The investigator will permit national and local health authorities, Sponsor monitors, representatives, and collaborators, and the IRBs/ECs to inspect facilities and records relevant to this study.

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9.4 ADMINISTRATIVE STRUCTURE

Genentech, Inc., is the Sponsor and will have oversight of study management, including project management, oversight of data management, and statistical programming. A CRO will assist with study management, including project management, medical monitoring (as needed), clinical monitoring, data monitoring, and data management. Patients will be enrolled at approximately 75 sites in North America.

The CRO will manage site activation, study drug supply, patient enrollment and tracking, and clinical monitoring. Genentech will handle study drug distribution.

A Steering Committee will provide scientific oversight for this study. The Steering Committee will be composed of Genentech representatives and external advisors. The Steering Committee will perform functions such as:

- Provide recommendations regarding changes in the study conduct
- Review results of the final analyses for primary and secondary endpoints
- Participate in the drafting and publishing of final study results

An iDMC will be responsible for monitoring the overall efficacy and safety of the patients in the trial after treatment with alteplase. The iDMC will be composed of external advisors. The iDMC will perform functions such as:

- Review all accumulated safety data at interim analysis
- Review efficacy data analysis performed for interim analysis for consideration of potential discontinuation of the trial for futility
- Make recommendation to the Sponsor as to if it is appropriate to discontinue the study after assessing the interim efficacy and safety data in aggregate
- Determine if the study should continue after review of interim analysis results

An iDCC will be responsible for the preparation and review of unmasked data.

A central IRB will be utilized when possible to ensure the timely submission and approval of site regulatory documents for sites not required to use a local IRB. The CRO will be responsible for management of the central IRB.

An EDC system will be used to capture data for the study.

An IWRS will be utilized for randomization, patient tracking, and drug inventory management for the study.

9.5 PUBLICATION OF DATA AND PROTECTION OF TRADE SECRETS

Regardless of the outcome of a trial, the Sponsor is dedicated to openly providing information on the trial to healthcare professionals and to the public, both at scientific congresses and in peer-reviewed journals. The Sponsor will comply with all

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requirements for publication of study results. For more information, refer to the Roche Global Policy on Sharing of Clinical Trials Data at the following Web site:

<http://www.rochetrials.com/pdf/RocheGlobalDataSharingPolicy.pdf>

The results of this study may be published or presented at scientific congresses. For all clinical trials in patients involving an IMP for which a marketing authorization application has been filed or approved in any country, the Sponsor aims to submit a journal manuscript reporting primary clinical trial results within 6 months after the availability of the respective clinical study report. In addition, for all clinical trials in patients involving an IMP for which a marketing authorization application has been filed or approved in any country, the Sponsor aims to publish results from analyses of additional endpoints and exploratory data that are clinically meaningful and statistically sound.

The investigator must agree to submit all manuscripts or abstracts to the Sponsor prior to submission for publication or presentation. This allows the Sponsor to protect proprietary information and to provide comments based on information from other studies that may not yet be available to the investigator.

In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter trials only in their entirety and not as individual center data. In this case, a coordinating investigator will be designated by mutual agreement.

Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors authorship requirements. Any formal publication of the study in which contribution of Sponsor personnel exceeded that of conventional monitoring will be considered as a joint publication by the investigator and the appropriate Sponsor personnel.

Any inventions and resulting patents, improvements, and/or know-how originating from the use of data from this study will become and remain the exclusive and unburdened property of the Sponsor, except where agreed otherwise.

9.6 PROTOCOL AMENDMENTS

Any protocol amendments will be prepared by the Sponsor. Protocol amendments will be submitted to the IRB/EC and to regulatory authorities in accordance with local regulatory requirements.

Approval must be obtained from the IRB/EC and regulatory authorities (as locally required) before implementation of any changes, except for changes necessary to eliminate an immediate hazard to patients or changes that involve logistical or administrative aspects only (e.g., change in Medical Monitor or contact information).

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Appendix 1

Schedule of Assessments

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 (± 14) Days/Study Completion (Visit 5)	Early Discontinuation (Phone call, if possible)
Informed consent	X						
Review of eligibility	X						
Medical history and baseline conditions ^a	X						
Demographics	X						
Neurological 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					

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Appendix 1 (cont.) Schedule of Assessments

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 (± 14) Days/Study Completion (Visit 5)	Early Discontinuation (Phone call, if possible)
Discharge location				X			X
Patient survival					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 assessments ^l						X	
Walking 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; SW MRI=susceptibility-weighted MRI; WAIS III=Wechsler Adult Intelligence Scale III.

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Appendix 1 (cont.) Schedule of Assessments

- ^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.
- ^c Includes pulse and systolic/diastolic blood pressure (while patient is in a supine position). *Record abnormalities on the Adverse Event eCRF.*
- ^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 walking 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.

Appendix 2

Alteplase Administration

Administration of bolus



Step 1:

Inspect solution

After reconstitution to 1 mg/mL, inspect solution for particulate matter and discoloration prior to administration

Step 2:

Discard excess

To ensure proper dosing, discard excess by removing from vial any quantity of drug in excess of that specified for patient treatment. When drawing off excess solution, be sure to insert the needle into the peripheral area of the vial top, away from the puncture site caused by the transfer device

Step 3:

Prepare bolus

Withdraw 10% of the 0.9-mg/kg dose in one of the following ways:

- Remove from vial using a syringe and needle,
- Remove from port (second injection site) on infusion line after infusion set is primed, or
- Program infusion pump to deliver bolus at infusion initiation

Step 4:

Administer bolus

Administer initial IV bolus over 1 minute

Administration of remainder of dose

Infuse the remaining 90% of 0.9-mg/kg dose over 60 minutes.

50-mg vials—Administer using either a polyvinyl chloride bag or glass vial and infusion set.

No medication should be added to the infusion solutions that contain alteplase.

For specifics regarding dosing and administration, please see the [Activase full Prescribing Information](#).

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Appendix 3 Reconstitution of 50-mg Vials

A single 50-mg vial of Activase may be used to treat patients weighing up to 122 lbs or 55.5 kilograms. Reconstitute Activase immediately before administration, using aseptic technique at all times—including thorough hand washing and the use of gloves.



Step 1:

Assemble the 50-mg vial of Activase, the vial of Sterile Water for Injection (SWFI), USP (included with the Activase package), a 50-mL syringe, a large-bore needle (eg, 18-gauge), and alcohol wipes. Also, prepare a polyvinyl chloride bag or glass vial along with an infusion set.

Step 2:

Remove the protective cap from the top of the Activase vial and the vial of SWFI. Swab the top of each vial with an alcohol wipe to reduce the risk of contamination.

Step 3:

Withdraw 50-mL of SWFI, without preservatives. Diluent is included. DO NOT USE Bacteriostatic Water for Injection, USP.

Step 4:

Inject the 50-mL into the 50-mg Activase vial using a large-bore needle and syringe, directing the stream into the lyophilized cake. DO NOT USE IF VACUUM IS NOT PRESENT. The syringe should not be primed with air during preparation and should be inserted into the Activase vial stopper. Slight foaming upon reconstitution is normal. Let the solution stand undisturbed for several minutes to allow any large bubbles to dissipate.

Step 5:

Mix with a gentle swirl or slow inversion. DO NOT SHAKE. Visually inspect the Activase solution for particulate matter and discoloration before administration.

Activase is stable for up to 8 hours in these solutions at room temperature. Do not freeze solutions containing Activase. No other medications should be added to infusions containing Activase. Any unused infusion solution should be discarded.

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Appendix 4

European Quality of Life-5D (EQ-5D) Questionnaire

By placing a checkmark in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about
I have some problems in walking about
I am confined to bed

Self-Care

I have no problems with self-care
I have some problems washing or dressing myself
I am unable to wash or dress myself

Usual Activities (e.g. work, study, housework, family or leisure activities)

I have no problems with performing my usual activities
I have some problems with performing my usual activities
I am unable to perform my usual activities

Pain/Discomfort

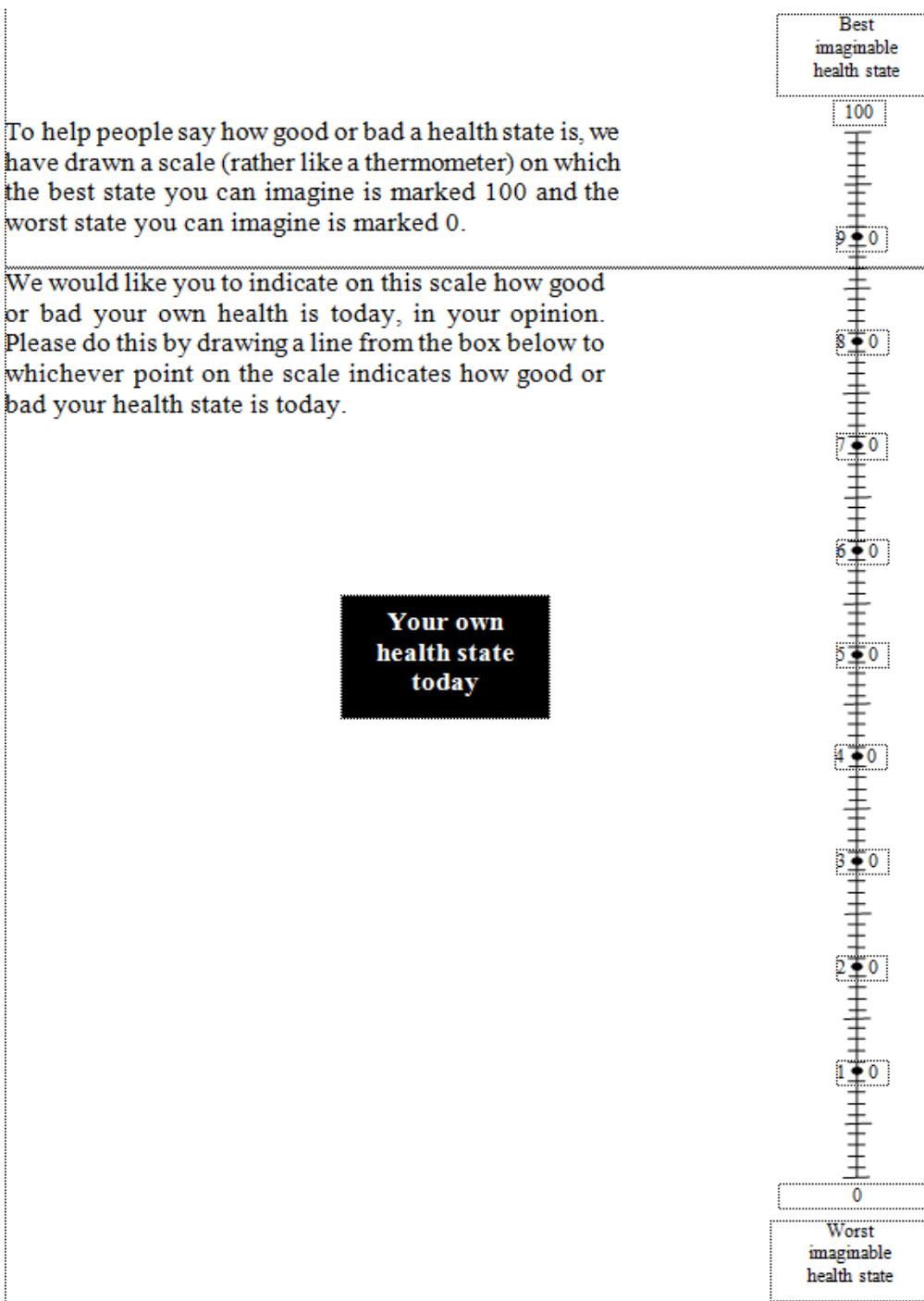
I have no pain or discomfort
I have moderate pain or discomfort
I have extreme pain or discomfort

Anxiety/Depression

I am not anxious or depressed
I am moderately anxious or depressed
I am extremely anxious or depressed

Appendix 4 (cont.)

European Quality of Life-5D (EQ-5D) Questionnaire



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Appendix 5
Stroke Index Scale (SIS-16)

In the past 2 weeks, how difficult was it to...	Not difficult at all	A little difficult	Somewhat difficult	Very difficult	Could not do at all
a. Dress the top part of your body?	5	4	3	2	1
b. Bathe yourself?	5	4	3	2	1
c. Get to the toilet on time?	5	4	3	2	1
d. Control your bladder (not have an accident)?	5	4	3	2	1
e. Control your bowels (not have an accident)?	5	4	3	2	1
f. Stand without losing balance?	5	4	3	2	1
g. Go shopping?	5	4	3	2	1
h. Do heavy household chores (e.g., vacuum, laundry or yard work)?	5	4	3	2	1
i. Stay sitting without losing your balance?	5	4	3	2	1
j. Walk without losing your balance?	5	4	3	2	1
k. Move from a bed to a chair?	5	4	3	2	1
l. Walk fast?	5	4	3	2	1
m. Climb one flight of stairs?	5	4	3	2	1
n. Walk one block?	5	4	3	2	1
o. Get in and out of a car?	5	4	3	2	1
p. Carry heavy objects (e.g. bag of groceries) with your affected hand?	5	4	3	2	1

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Appendix 6
Center for Epidemiologic Studies, Depression (CES-D) Scale,
NIMH

Below is a list of the ways you might have felt or behaved. Please tell me how often you have felt this way during the past week.

Week	During the Past			
	Rarely or none of the time (less than 1 day)			
	Some or a little of the time (1-2 days)	Occasionally or a moderate amount of time (3-4 days)	Most or all of the time (5-7 days)	
1. I was bothered by things that usually don't bother me.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
2. I did not feel like eating; my appetite was poor.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
3. I felt that I could not shake off the blues even with help from my family or friends.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
4. I felt I was just as good as other people.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
5. I had trouble keeping my mind on what I was doing.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
6. I felt depressed.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
7. I felt that everything I did was an effort.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
8. I felt hopeful about the future.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
9. I thought my life had been a failure.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
10. I felt fearful.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
11. My sleep was restless.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
12. I was happy.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
13. I talked less than usual.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
14. I felt lonely.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
15. People were unfriendly.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
16. I enjoyed life.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
17. I had crying spells.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
18. I felt sad.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
19. I felt that people dislike me.	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
20. I could not get "going."	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

SCORING: zero for answers in the first column, 1 for answers in the second column, 2 for answers in the third column, 3 for answers in the fourth column. The scoring of positive items is reversed. Possible range of scores is zero to 60, with the higher scores indicating the presence of more symptomatology.

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Appendix 7

National Institutes of Health Stroke Scale (NIHSS)

<p>Administer stroke scale items in the order listed. Record performance in each category after each subscale exam. Do not go back and change scores. Follow directions provided for each exam technique. Scores should reflect what the patient does, not what the clinician thinks the patient can do. The clinician should record answers while administering the exam and work quickly. Except where indicated, the patient should not be coached (i.e., repeated requests to patient to make a special effort).</p>		
1	<p>Has the subject had a stroke (either as the index event at randomization or as an outcome event)? If no, form is complete.</p>	<p><input type="radio"/> No <input type="radio"/> Yes</p>
2	(1a) Level of Consciousness	<p><input type="radio"/> 0=Alert; keenly responsive <input type="radio"/> 1=Not alert, but arousable by minor stimulation to obey, answer or respond <input type="radio"/> 2=Not alert, requires repeated stimulation to attend, or is obtunded and requires strong or painful stimulation to make movements (not stereotyped) <input type="radio"/> 3=Responds only with reflex motor or autonomic effects or totally unresponsive, flaccid, areflexic (Complete form using coma scoring)</p>
3	(1b) LOC Questions	<p><input type="radio"/> 0=Answers both questions correctly <input type="radio"/> 1=Answers one question correctly <input type="radio"/> 2=Answers neither question correctly</p>
4	(1c) LOC Commands	<p><input type="radio"/> 0=Performs both tasks correctly <input type="radio"/> 1=Performs one task correctly <input type="radio"/> 2=Performs neither task correctly</p>
5	(2) Best Gaze	<p><input type="radio"/> 0=Normal <input type="radio"/> 1=Partial gaze palsy. This score is given when gaze is abnormal in one or both eyes, but where forced deviation or total gaze paresis are not present <input type="radio"/> 2=Forced deviation, or total gaze paresis not overcome by the oculocephalic maneuver</p>

Appendix 7 (cont.)

National Institutes of Health Stroke Scale (NIHSS)

8	<p style="text-align: center;">(3) Visual</p> <p>Visual fields (upper and lower quadrants) are tested by confrontation, using finger counting or visual threat as appropriate. Patient must be encouraged, but if they look at the side of the moving fingers appropriately, this can be scored as normal. If there is unilateral blindness or enucleation, visual fields in the remaining eye are scored. Score 1 only if a clear-cut asymmetry, including quadrantanopia is found. If patient is blind from any cause, score 3. Double simultaneous stimulation is performed at this point. If there is extinction patient receives a 1 and the results are used to answer question 22. Score as examined, using bilateral threat.</p>	<input type="radio"/> 0=No visual loss <input type="radio"/> 1=Partial hemianopia <input type="radio"/> 2=Complete hemianopia <input type="radio"/> 3=Bilateral hemianopia (blind including cortical blindness)
7	<p style="text-align: center;">(4) Facial Palsy</p> <p>Ask, or use pantomime to encourage the patient to show teeth or raise eyebrows and close eyes. Score symmetry of grimace in response to noxious stimuli in the poorly responsive or non-comprehending patient. If facial trauma/bandages, orotracheal tube, tape or other physical barrier obscures the face, these should be removed to the extent possible. Coma Score "3".</p>	<input type="radio"/> 0=Normal symmetrical movement <input type="radio"/> 1=Minor paralysis (flattened nasolabial fold, asymmetry of smiling) <input type="radio"/> 2=Partial paralysis (total or near total paralysis of lower face) <input type="radio"/> 3=Complete paralysis of one or both sides (absence of facial movement in the upper and lower face)
8	<p style="text-align: center;">(5a) Motor Arm Left</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma Score "4".</p>	<input type="radio"/> 0=No drift, limb holds 90 (or 45) degrees for full 10 seconds <input type="radio"/> 1=Drift, limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed <input type="radio"/> 2=Some effort against gravity, limb cannot get to or maintain (if cued) 90 degrees <input type="radio"/> 3=No effort against gravity, limb falls <input type="radio"/> 4=No movement <input type="radio"/> Amputation, joint fusion
9	<p>Explain if amputation or joint fusion (Motor Arm Left):</p>	
10	<p style="text-align: center;">(5b) Motor Arm Right</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma score "4".</p>	<input type="radio"/> 0=No drift, limb holds 90 (or 45) degrees for full 10 seconds <input type="radio"/> 1=Drift, limb holds 90 (or 45) degrees, but drifts down before full 10 seconds; does not hit bed <input type="radio"/> 2=Some effort against gravity, limb cannot get to or maintain (if cued) 90 degrees <input type="radio"/> 3=No effort against gravity, limb falls <input type="radio"/> 4=No movement <input type="radio"/> Amputation, joint fusion
11	<p>Explain if amputation or joint fusion (Motor Arm Right):</p>	

Appendix 7 (cont.)
National Institutes of Health Stroke Scale (NIHSS)

12	<p>(6a) Motor Leg Left</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma score "4".</p>	<input type="radio"/> 0=No drift, leg holds 30 degrees position for full 5 seconds <input type="radio"/> 1=Drift, leg falls by the end of the 5 second period but does not hit bed <input type="radio"/> 2=Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity <input type="radio"/> 3=No effort against gravity; leg falls to bed immediately <input type="radio"/> 4>No movement <input type="radio"/> Amputation, joint fusion
13	Explain if amputation/ joint fusion (Motor Leg Left):	
14	<p>(6b) Motor Leg Right</p> <p>The limb is placed in the appropriate position: extend the arms (palms down) 90 degrees (if sitting) or 45 degrees (if supine) and the leg 30 degrees (always tested supine). Drift is scored if the arm falls before 10 seconds or the leg before 5 seconds. The aphasic patient is encouraged using urgency in the voice and pantomime but not noxious stimulation. Each limb is tested in turn, beginning with the non-paretic arm. Only in cases of amputation or joint fusion at the shoulder or hip can the examiner indicate no score and an explanation must be provided. Coma score "4".</p>	<input type="radio"/> 0=No drift, leg holds 30 degrees position for full 5 seconds <input type="radio"/> 1=Drift, leg falls by the end of the 5 second period but does not hit bed <input type="radio"/> 2=Some effort against gravity; leg falls to bed by 5 seconds, but has some effort against gravity <input type="radio"/> 3=No effort against gravity; leg falls to bed immediately <input type="radio"/> 4>No movement <input type="radio"/> Amputation, joint fusion
15	Explain if amputation/ joint fusion (Motor Leg Right):	
16	<p>(7) Limb Ataxia</p> <p>This item is aimed at finding evidence of a unilateral cerebellar lesion. Test with eyes open. In case of visual defect, insure testing is done in intact visual field. The finger-nose-finger and heel-shin tests are performed on both sides, and ataxia is scored only if present out of proportion to weakness. Ataxia is absent in the patient who cannot understand or is paralyzed. Only in the case of amputation or joint fusion, the examiner should record the score as untestable (UN), and clearly write the explanation for this choice. In case of blindness, test by having the patient touch nose from extended arm position. Coma score "0".</p>	<input type="radio"/> 0 = Absent <input type="radio"/> 1 = Present in one limb <input type="radio"/> 2 = Present in two limbs <input type="radio"/> UN = amputation or joint fusion
17	Explain if amputation or joint fusion (Limb Ataxia):	

Appendix 7 (cont.)
National Institutes of Health Stroke Scale (NIHSS)

18	<p>(8) Sensory</p> <p>Sensation or grimace to pin prick when tested, or withdrawal from noxious stimulus in the obtunded or aphasic patient. Only sensory loss attributed to stroke is scored as abnormal and the examiner should test as many body areas (arms (not hands), legs, trunk, face) as needed to accurately check for hemisensory loss. A score of 2, "severe or total," should only be given when a severe or total loss of sensation can be clearly demonstrated. Stuporous and aphasic patients will therefore probably score 1 or 0. The patient with brain stem stroke who has bilateral loss of sensation is scored 2. If the patient does not respond and is quadriplegic score 2. Patients in coma (item 1a-3) are arbitrarily given a 2 on this item. Coma Score "2".</p>	<input type="radio"/> 0=Normal; no sensory loss <input type="radio"/> 1=Mild to moderate sensory loss; patient feels pinprick is less sharp or is dull on the affected side; or there is a loss of superficial pain with pinprick but patient is aware he/she is being touched <input type="radio"/> 2=Severe to total sensory loss; patient is not aware of being touched in the face, arm & leg
19	<p>(9) Best Language</p> <p>A great deal of information about comprehension will be obtained during the preceding sections of the examination. The patient is asked to describe what is happening in the attached picture, to name the items on the attached naming sheet, and to read from the attached list of sentences. Comprehension is judged from responses here as well as to all of the commands in the preceding general neurological exam. If visual loss interferes with the tests, ask the patient to identify objects placed in the hand, repeat, and produce speech. The intubated patient should be asked to write. The patient in coma (question 1a-3) will arbitrarily score 3 on this item. The examiner must choose a score in the patient with stupor or limited cooperation but a score of 3 should be used only if the patient is mute and follows no one step commands. Coma Score "3".</p>	<input type="radio"/> 0=No aphasia, normal <input type="radio"/> 1=Mild to moderate aphasia; some obvious loss of fluency or facility of comprehension without significant limitation on ideas expressed on form of expression <input type="radio"/> 2=Severe aphasia; all communication is through fragmentary expression; great need for inference, questioning, and guessing by the listener; listener carries burden of communication <input type="radio"/> 3=Mute, global aphasia; no usable speech or auditory comprehension
20	<p>(10) Dysarthria</p> <p>If patient is thought to be normal an adequate sample of speech must be obtained by asking patient to read or repeat words from the attached list. If the patient has severe aphasia, the clarity of articulation of spontaneous speech can be rated. Only if the patient is intubated or has other physical barrier to producing speech, may the item be not scored, and the examiner must clearly write an explanation. Do not tell the patient why he/she is being tested. Coma Score "2".</p>	<input type="radio"/> 0=Normal <input type="radio"/> 1=Mild to moderate; patient slurs at least some words and at worst, can be understood with some difficulty <input type="radio"/> 2=Severe; patient's speech is so slurred as to be unintelligible in the absence of or out of proportion to any dysphasia, or is mute/ anarthric <input type="radio"/> Intubated or other physical barrier.
21	Explain if intubated or other physical barrier (Dysarthria):	
22	<p>(11) Extinction and Inattention (Neglect)</p> <p>Sufficient information to identify neglect may be obtained during the prior testing. If the patient has a severe visual loss preventing visual double simultaneous stimulation, and the cutaneous stimuli are normal, the score is normal. If the patient has aphasia but does appear to attend to both sides, the score is normal. The presence of visual spatial neglect or anosognosia may also be taken as evidence of abnormality. Since the abnormality is scored only if present, the item is never untestable. Coma Score "2".</p>	<input type="radio"/> 0=No abnormality <input type="radio"/> 1=Visual, tactile, auditory, spatial, or personal inattention or extinction to bilateral simultaneous stimulation in one of the sensory modalities <input type="radio"/> 2=Profound hemi-inattention or hemi-inattention to more than one modality; does not recognize own hand or orients to only one side of space
23	Not applicable	
24	<p>Name of assessor:</p> <p>The assessor must be a study team member who has completed NIHSS certification.</p>	

Appendix 8

Modified Rankin Scale (mRS)

1	Modified Rankin Scale:	<input type="radio"/> (0) No symptoms at all <input type="radio"/> (1) No significant disability despite symptoms; able to carry out all usual duties and activities <input type="radio"/> (2) Slight disability; unable to carry out all previous activities but able to look after own affairs without assistance <input type="radio"/> (3) Moderate disability requiring some help, but able to walk without assistance <input type="radio"/> (4) Moderately severe disability; unable to walk without assistance and unable to attend to own bodily needs without assistance <input type="radio"/> (5) Severe disability; bedridden, incontinent, and requiring constant nursing care and attention
2	For response 2 above, specify the previous activities that can no longer be carried out:	
3	Name of assessor: The assessor must be a study team member who has completed mRS certification.	

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Appendix 9

Glasgow Outcome Scale (GOS)

Note: The scale presented here is based on the original article by Jennett and Bond. It has become common practice in clinical trial administration, however, to use a modified version that places the scores in reverse order (i.e., "good recovery" = 1, "moderate disability" = 2, etc.).

Score	Description
1	DEATH
2	PERSISTENT VEGETATIVE STATE Patient exhibits no <i>obvious cortical</i> function.
3	SEVERE DISABILITY (Conscious but disabled). Patient depends upon others for daily support due to mental or physical disability or both.
4	MODERATE DISABILITY (Disabled but independent). Patient is independent as far as daily life is concerned. The disabilities found include varying degrees of dysphasia, hemiparesis, or ataxia, as well as intellectual and memory deficits and personality changes.
5	GOOD RECOVERY Resumption of normal activities even though there may be minor neurological or psychological deficits.

TOTAL (1-5): _____

References

Jennett B, Bond M. "Assessment of outcome after severe brain damage." *Lancet* 1975 Mar 1;1(7905):480-4

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Appendix 10 Barthel Index (BI)

THE BARTHEL INDEX

Patient Name: _____
Rater Name: _____
Date: _____

Activity	Score
FEEDING 0 = unable 5 = needs help cutting, spreading butter, etc., or requires modified diet 10 = independent	_____
BATHING 0 = dependent 5 = independent (or in shower)	_____
GROOMING 0 = needs to help with personal care 5 = independent face/hair/teeth/shaving (implements provided)	_____
DRESSING 0 = dependent 5 = needs help but can do about half unaided 10 = independent (including buttons, zips, laces, etc.)	_____
BOWELS 0 = incontinent (or needs to be given enemas) 5 = occasional accident 10 = continent	_____
BLADDER 0 = incontinent, or catheterized and unable to manage alone 5 = occasional accident 10 = continent	_____
TOILET USE 0 = dependent 5 = needs some help, but can do something alone 10 = independent (on and off, dressing, wiping)	_____
TRANSFERS (BED TO CHAIR AND BACK) 0 = unable, no sitting balance 5 = major help (one or two people, physical), can sit 10 = minor help (verbal or physical) 15 = independent	_____
MOBILITY (ON LEVEL SURFACES) 0 = immobile or < 50 yards 5 = wheelchair independent, including corners, > 50 yards 10 = walks with help of one person (verbal or physical) > 50 yards 15 = independent (but may use any aid; for example, stick) > 50 yards	_____
STAIRS 0 = unable 5 = needs help (verbal, physical, carrying aid) 10 = independent	_____
	TOTAL (0-100): _____

Appendix 10 (cont.) **Barthel Index (BI)**

The Barthel ADL Index: Guidelines

1. The index should be used as a record of what a patient does, not as a record of what a patient could do.
2. The main aim is to establish degree of independence from any help, physical or verbal, however minor and for whatever reason.
3. The need for supervision renders the patient not independent.
4. A patient's performance should be established using the best available evidence. Asking the patient, friends/relatives and nurses are the usual sources, but direct observation and common sense are also important. However direct testing is not needed.
5. Usually the patient's performance over the preceding 24-48 hours is important, but occasionally longer periods will be relevant.
6. Middle categories imply that the patient supplies over 50 per cent of the effort.
7. Use of aids to be independent is allowed.

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Appendix 11 Cognitive and Behavioral Assessments

A. Controlled Oral Word Association Test

Letter L			
Instruct the study participant as follows:			
<i>"I am going to say a letter and I want you to name, as fast as you can, as many words as you can think of which start with this letter. The letter is L. You will have one minute. Ready? Begin."</i>			
Record the study participant's responses in the following table.			
	Acceptable		Acceptable
1.	<input type="checkbox"/>	21.	<input type="checkbox"/>
2.	<input type="checkbox"/>	22.	<input type="checkbox"/>
3.	<input type="checkbox"/>	23.	<input type="checkbox"/>
4.	<input type="checkbox"/>	24.	<input type="checkbox"/>
5.	<input type="checkbox"/>	25.	<input type="checkbox"/>
6.	<input type="checkbox"/>	26.	<input type="checkbox"/>
7.	<input type="checkbox"/>	27.	<input type="checkbox"/>
8.	<input type="checkbox"/>	28.	<input type="checkbox"/>
9.	<input type="checkbox"/>	29.	<input type="checkbox"/>
10.	<input type="checkbox"/>	30.	<input type="checkbox"/>
11.	<input type="checkbox"/>	31.	<input type="checkbox"/>
12.	<input type="checkbox"/>	32.	<input type="checkbox"/>
13.	<input type="checkbox"/>	33.	<input type="checkbox"/>
14.	<input type="checkbox"/>	34.	<input type="checkbox"/>
15.	<input type="checkbox"/>	35.	<input type="checkbox"/>
16.	<input type="checkbox"/>	36.	<input type="checkbox"/>
17.	<input type="checkbox"/>	37.	<input type="checkbox"/>
18.	<input type="checkbox"/>	38.	<input type="checkbox"/>
19.	<input type="checkbox"/>	39.	<input type="checkbox"/>
20.	<input type="checkbox"/>	40.	<input type="checkbox"/>
Total words generated: <input type="text"/>			
Total acceptable words: <input type="text"/>			

Appendix 11 (cont.)
Cognitive and Behavioral Assessments

B . Hopkins Verbal Learning Test-Revised (HVLT-R)

Form 1

Semantic Categories: Four-Legged Animals, Precious Stones, Human Dwellings

Name _____ Sex _____ Age _____ years _____ months
Examiner _____ Date _____ / _____ / _____

Word List	Learning Trials			Delayed Recall Trial (20-25 min.)
	Trial 1	Trial 2	Trial 3	
LION				
EMERALD				
HORSE				
TENT				
SAPPHIRE				
HOTEL				
CAVE				
OPAL				
TIGER				
PEARL				
COW				
HUT				
Total correct responses =				

Completion Time _____ Start Time _____
Trial 3 _____ Trial 4 _____



3

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Appendix 11 (cont.) Cognitive and Behavioral Assessments

Delayed Recognition Trial Instructions

The Delayed Recognition (Forced Choice) trial is administered immediately after the Delayed Recall trial. Say the following:

Now I am going to read a longer list of words to you. Some of them are words from the original list, and some are not. After I read each word, I'd like you to say "Yes" if it was on the original list, or "No" if it was not.

Read the words of the Delayed Recognition trial list in numerical order. Allow the individual as much time as needed to respond. You may use the prompt, "Was horse on the list? Yes or no?" The individual must give you a response for every word. If the individual is not sure, ask for a guess.

Delayed Recognition Trial (Forced Choice)							
1. HORSE	Y N	7. house	Y N	13. HUT	Y N	19. TENT	Y N
2. ruby	Y N	8. OPAL	Y N	14. EMERALD	Y N	20. mountain	Y N
3. CAVE	Y N	9. TIGER	Y N	15. SAPPHIRE	Y N	21. cat	Y N
4. balloon	Y N	10. boat	Y N	16. dog	Y N	22. HOTEL	Y N
5. coffee	Y N	11. scarf	Y N	17. apartment	Y N	23. COW	Y N
6. LION	Y N	12. PEARL	Y N	18. penny	Y N	24. diamond	Y N

Total number of true-positive responses ("hits"): _____ /12 (no shading)

Semantically-related false-positive errors: _____ /6 (light shading)

Semantically-unrelated false-positive errors: _____ /6 (darker shading)

Total number of false-positive errors: _____ /12

	Raw score	T score
Total Recall (sum of total correct responses for Trials 1, 2, & 3)		
Delayed Recall (Trial 4)		
Retention (%) [(Trial 4 ÷ Higher score of Trials 2 and 3) × 100]		
Recognition Discrimination Index (Total no. of true-positives) – (Total no. of false-positives)		

Normative table (Appendix A): _____

Appendix 11 (cont.)

Cognitive and Behavioral Assessments

C. Digit Symbol Coding from the WAIS III

DIGIT SYMBOL SUBSTITUTION (DSS) TASK

DIGIT	1	2	3	4	5	6	7	8	9
SYMBOL	-	上	□	└	□	○	∧	×	=

1	5	4	2	7	6	3	5	7	2	8	5	4	6	3	7	2	8	1	9	5	8	4	7	3
---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---	---

Appendix 11 (cont.) Cognitive and Behavioral Assessments

D. Forward and Backward Digit Span Test

FORWARD AND BACKWARD DIGIT SPAN TEST

DIGIT SPAN (FORWARD)*

Ask patient, "Repeat each group of numbers in the same order that I say them." Read numbers at rate of one each second. If patient passes the first trial, advance to the first trial of the next item. If patient fails the first trial, administer the second trial of that item. Stop when patient fails both trials of any item. Score is best performance's maximum number of digits correct.

DIGIT FORWARD	WRITE RESPONSE	CORRECT	
8-4-7		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-7-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No
9-3-4-1		<input type="checkbox"/> Yes	<input type="checkbox"/> No
8-3-9-6		<input type="checkbox"/> Yes	<input type="checkbox"/> No
5-1-6-2-9		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-6-9-2-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No
6-4-3-5-2-7		<input type="checkbox"/> Yes	<input type="checkbox"/> No
9-1-8-4-2-7		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-1-6-9-2-8-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No
2-8-1-4-9-7-5		<input type="checkbox"/> Yes	<input type="checkbox"/> No

DIGIT FORWARD SCORE (3-7)

DIGIT SPAN (BACKWARD)*

Say to patient, "Now I am going to say some more numbers, but this time when I stop, I want you to say them backwards. For example, if I said 7-1-9, what would you say?" Read numbers at rate of one each second. If patient passes the first trial, advance to the first trial of the next item. If patient fails the first trial, administer the second trial of that item. Stop when patient fails both trials of any item. Score is best performance's maximum number of digits correct.

DIGIT FORWARD	WRITE RESPONSE	CORRECT	
5-8		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-1		<input type="checkbox"/> Yes	<input type="checkbox"/> No
6-2-9		<input type="checkbox"/> Yes	<input type="checkbox"/> No
4-9-3		<input type="checkbox"/> Yes	<input type="checkbox"/> No
4-9-6-8		<input type="checkbox"/> Yes	<input type="checkbox"/> No
3-8-1-4		<input type="checkbox"/> Yes	<input type="checkbox"/> No
1-5-2-8-6		<input type="checkbox"/> Yes	<input type="checkbox"/> No
6-2-9-7-2		<input type="checkbox"/> Yes	<input type="checkbox"/> No
5-3-9-4-1-8		<input type="checkbox"/> Yes	<input type="checkbox"/> No
7-1-5-2-8-6		<input type="checkbox"/> Yes	<input type="checkbox"/> No

DIGIT BACKWARD SCORE (2-6)

*Wechsler, D. (1987). Wechsler Memory Scale-Revised Manual. San Antonio, TX: Psychological Corporation

Appendix 11 (cont.) Cognitive and Behavioral Assessments

E. Benton Judgment of Line Orientation

Benton Judgment Of Line Orientation – Form V				
Name _____	No. _____	Date _____	/ /	
Age _____	Gender _____	Education _____	Handedness _____	Examiner _____

Instructions: Record each response choice. Circle all errors.

PRACTICE ITEMS

A _____ 1-6	B _____ 4-8	C _____ 4-10	D _____ 7-8	E _____ 2-4
A' _____ 1 6	B' _____ 4 8	C' _____ 4 10	D' _____ 7 8	E' _____ 2 4

TEST ITEMS

1. _____ 5-10 HH	16. _____ 7-8 MM
2. _____ 2-11 MM	17. _____ 3-5 HH
3. _____ 1-2 LL	18. _____ 10-11 MH
4. _____ 1-7 HH	19. _____ 1-4 MM
5. _____ 6-7 HH	20. _____ 3-11 LL
6. _____ 5-6 LL	21. _____ 6-10 LL
7. _____ 4-5 HH	22. _____ 2-9 LL
8. _____ 1-3 MM	23. _____ 3-8 HH
9. _____ 5-11 MM	24. _____ 9-11 HH
10. _____ 1-10 HH	25. _____ 3-4 LM
11. _____ 1-7 MM	26. _____ 8-9 LL
12. _____ 2-6 HH	27. _____ 8-11 HH
13. _____ 7-9 MM	28. _____ 7-10 LL
14. _____ 2-5 HL	29. _____ 3-10 HL
15. _____ 1-9 LL	30. _____ 5-8 HM

Correct

IMPRESSION: _____
OBSERVATIONS: _____
LESION SUMMARY: _____

Appendix 11 (cont.) Cognitive and Behavioral Assessments

F. Animal Naming Test

ANIMAL NAMING

Introduction: "I'd like to ask a question to check your memory."

Instruction: "Tell me the names of as many animals as you can think of, as quickly as possible."

Procedure: Time for 60 seconds and record all responses.
If the person stops before 60 seconds, say "Any more animals?"
If the person says nothing for 15 seconds, say "A dog is an animal.
"Can you tell me more animals?"

1. _____	12. _____
2. _____	13. _____
3. _____	14. _____
4. _____	15. _____
5. _____	16. _____
6. _____	17. _____
7. _____	18. _____
8. _____	19. _____
9. _____	20. _____
10. _____	21. _____
11. _____	22. _____

Scoring: Count the total number of animals (NOT including repetitions or non-animal words): _____

Next step: If the score is less than 14, further testing should be done.

Sager MD, MA; Hermann PhD, BP; LaRue PhD, A; Woodard PhD, JL, Screening for Dementia in Community-based Memory Clinics. Wisconsin Medical Journal 2006;105(7):25-29

Appendix 11 (cont.) **Cognitive and Behavioral Assessments**

Directions for Scoring Animal Naming Screen

Instructions: "Tell me the names of as many animals as you can think of, as quickly as possible."

If the person says nothing for 15 seconds, say "A dog is an animal. Can you tell me more animals?" If the person stops before 60 seconds, say "Any more animals?"

Scoring: Count all animals, including birds, fish, reptiles, insects, humans, extinct animals, etc. Credit can be given for general category terms (e.g., dog) and for specific instances (e.g., terriers) when both are given. Credit only one item when people name the same animal at different developmental stages (e.g., sheep, lamb).

Note: These minimalist initial instructions date back to an early research study on verbal fluency in aging and dementia by Wilma Rosen (1980) in which subjects were instructed to "give the names of as many animals as you can think of" for 60 seconds. These very brief instructions have been subsequently used in several normative studies (e.g., Tombaugh et al., 1999 and Mayo's MOANS studies such as Lucas et al., 1998) and in some other recent studies of the efficacy of verbal fluency as a diagnostic tool for dementia (e.g., Canning et al., 2004).

Appendix 11 (cont.)
Cognitive and Behavioral Assessments

G. Boston Naming Test (BNT) 15-Item Short Form

SHORT FORM						
Item	Response	Correct Response	Latency Seconds	Stimulus Cue	Phonemic Cue	Multiple Choice
1. <u>house</u> (home)	—	—	—	—	—	—
	(a kind of building)					
2. <u>comb</u>	—	—	—	—	—	—
	(used for fixing hair)					
3. <u>toothbrush</u>	—	—	—	—	—	—
	(used in the mouth)					
4. <u>octopus</u>	—	—	—	—	—	—
	(an ocean animal)					
5. <u>bench</u>	—	—	—	—	—	—
	(used for sitting)					
6. <u>volcano</u>	—	—	—	—	—	—
	(a kind of mountain)					
7. <u>canoe</u>	—	—	—	—	—	—
	(used in the water)					
8. <u>beaver</u>	—	—	—	—	—	—
	(an animal)					

Appendix 11 (cont.)

Cognitive and Behavioral Assessments

Item	Response	Correct Response	Latency Seconds	Stimulus Cue	Phonemic Cue	Multiple Choice
9.	<u>cactus</u> (<i>saguaro</i>) (something that grows)		—	—	—	—
10.	<u>hammock</u> (you lie on it)		—	—	—	—
11.	<u>stethoscope</u> (used by doctors and nurses)		—	—	—	—
12.	<u>unicorn</u> (mythical animal)		—	—	—	—
13.	<u>tripod</u> (photographers or surveyors use it)		—	—	—	—
14.	<u>sphinx</u> (it's found in Egypt)		—	—	—	—
15.	<u>palette</u> (artists use it)		—	—	—	—

Appendix 11 (cont.) Cognitive and Behavioral Assessments

Summary of Scores

1. Number of spontaneously given correct responses _____
2. Number of stimulus cues given _____
3. Number of correct responses following a stimulus cue _____
4. Number of phonemic cues _____
5. Number of correct responses following the phonemic cue _____
6. Number of multiple choices given _____
7. Number of correct choices _____

Total Number Correct (1 + 3) _____

Total Score:
(Add credit for items preceding the first item passed. Disregard if you started with item 1.) _____

Appendix 12

10 Meter Walk Test

Description: The 10 meter walk test (10mWT) is a measure of walking speed.

Equipment: Utilize a quiet hallway or open space at least 14 meters long, digital stopwatch, clipboard, tape measure, and tape to mark standardized course layout, and 2 chairs.

Bracing and Assistive Device: The subject should be wearing comfortable flat shoes or shoes with a heel less than 1/2 inch. Have the subject perform this test with their most often used assistive device and orthotics. Note the specific type of assistive device and orthotics utilized on the source document.

Physical Assistance Provided During Testing:

Physical assistance may be provided as needed to maintain safety and stability. If additional assistance is needed, a non-blinded member of the investigation team can provide the additional assistance. No assistance can be provided for paretic limb advancement. A gait belt may be used during the test. The amount of assistance provided will be recorded with assignment of a Functional Ambulation Category (FAC). See FAC for description of procedure.

Test Administration:

Course Layout: The walking Course is established indoors on a level, non-carpeted surface measuring a length of 14 meters. The course is marked with tape at 0 meters, 2 meters, 12 meters, and 14 meters. 2 chairs can be placed near the 0 meter and 14 meter mark.



1. Instruct the subject in the goal of the 10 meter walk test while the subject is seated:
“You are going to walk a distance of about 40 feet. We will repeat this distance four times. The first two times will be completed at your comfortable pace, the final two times you will walk as quickly and safely as you can. Do you have any questions?”
2. Demonstrate where you want the subject to walk.
3. Walk the subject to the start line (0 meters). Provide the subject with the following instructions:
“You will walk at a comfortable pace to the chair.” (DO NOT refer to the tape on the floor.) The start command is **“Ready and Go”**.
4. When you and the subject are ready, say **“Ready and Go”**. If the subject starts too early, start again.

Appendix 12 (cont.)

10 Meter Walk Test

5. Instructions for Recording Time:
 - START** the stopwatch when the subject's first foot crosses the plane of the 2 meter line
 - STOP** the stopwatch when the subject's first foot crosses the plane of the 12 meter line.
 - Have the subject continue walking until he/she reaches the chair after the 14 meter line.
 - Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance
"Comfortable" Pace Trial 1 line on the 10 Meter Walk Test Source Document.
 - The subject can rest, if needed, in the chair near the 14 meter line.
6. When the subject is ready, have them repeat the SAME PROCEDURE except they will start from the 14 meter line and end at 0 meter line.
 - START** the stopwatch when the subject's first foot crosses the plane of the 12 meter line
 - STOP** the stopwatch when the subject's first foot crosses the plane of the 2 meter line.
 - Have the subject continue walking until he/she reaches the chair after the 0 meter line.
 - Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance on the "Comfortable" Pace Trial 2 line on the 10 Meter Walk Test Source Document.
 - The subject can rest, if needed, in the chair near the 0 meter line.
7. Subject Instructions for the last 2 trials:

"You are going to walk the same distances for two additional trials as quickly and as safely as you can. Do you have any questions?"
8. When the subject is ready, walk the subject to the start line (0 meters). Provide the subject with the following instructions:

"You will walk as quickly and as safely as you can to the chair." (Use an appropriate descriptor for chair/location, but DO NOT refer to the tape on the floor.)
The start command is "Ready and Go".
9. When you and the subject are ready, say "Ready and Go". If the subject starts too early, have them start again.

Appendix 12 (cont.) 10 Meter Walk Test

10. Instructions for Recording Time:

- START** the stopwatch when the subject's first foot crosses the plane of the 2 meter line
- STOP** the stopwatch when the subject's first foot crosses the plane of the 12 meter line.
- Have the subject continue walking until he/she reaches the chair after the 14 meter line.
- Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance on the "As Fast As Possible" Pace Trial 1 line on the 10 Meter Walk Test Source Document.
- The subject can rest, if needed, in the chair near the 14 meter line.

11. When the subject is ready, have them repeat the SAME PROCEDURE except they will start from the 14 meter line and end at 0 meter line.

- START** the stopwatch when the subject's first foot crosses the plane of the 12 meter line
- STOP** the stopwatch when the subject's first foot crosses the plane of the 2 meter line.
- Have the subject continue walking until he/she reaches the chair after the 0 meter line.
- Record (in seconds to the hundredths) the time it took for the subject to walk the ten meter distance on the "As Fast As Possible" Pace Trial 2 line on the 10 Meter Walk Test Source Document.
- The subject can rest, if needed, in the chair near the 0 meter line.

12. Record the assistive device used and the Functional Ambulation Category (FAC) on the 10 Meter Walk Test Source Document.

REFERENCES:

Duncan PW, Sullivan KJ, Behrman AL, et al. Protocol for the Locomotor Experience Applied Post-stroke (LEAPS) trial: A randomized controlled trial. *BMC Neurology* 2007;7(1):39.

Perry J, Garrett M, Gronley JK, et al. Classification of walking handicap in the stroke population. *Stroke* 1995, 26(6):982-89.

Richards CL, Malouin F, Wood-Dauphinee S, et al. Gait velocity as an outcome measure of locomotor recovery after stroke. In: Craik RA, Oatis C, eds. *Gait Analysis: Theory and Applications*. St Louis, Mo: Mosby; 1995:355-64.

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Appendix 13 **Preferred Terms for Intracranial Hemorrhage**

An intracranial hemorrhage (ICH) is considered symptomatic if it is not seen on computed tomography (CT) or magnetic resonance imaging (MRI) scan at baseline and any neurologic decline is attributed to it by the local investigator. Below is the list of preferred terms (PTs) using the MedDRA term to describe the ICH event:

- Basal ganglia hemorrhage
- Brain stem hematoma
- Brain stem hemorrhage
- Brain stem microhemorrhage
- Cerebellar hematoma
- Cerebellar hemorrhage
- Cerebellar microhemorrhage
- Cerebral arteriovenous malformation hemorrhagic
- Cerebral hematoma
- Cerebral hemorrhage
- Cerebral microhemorrhage
- Epidural hemorrhage
- Extradural hematoma
- Hemorrhage intracranial
- Hemorrhagic cerebral infarction
- Hemorrhagic stroke
- Hemorrhagic transformation stroke
- Intracranial hematoma
- Intraventricular hemorrhage
- Meningorrhagia
- Pituitary hemorrhage
- Putamen hemorrhage
- Subarachnoid hemorrhage
- Subdural hematoma
- Subdural hemorrhage
- Thalamus hemorrhage
- Traumatic intracranial hemorrhage

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Appendix 14
World Health Organization (WHO) Toxicity Grading Scale for
Determining the Severity of Adverse Events

HEMATOLOGY				
ITEM	Grade 1 Toxicity	Grade 2 Toxicity	Grade 3 Toxicity	Grade 4 Toxicity
Hemoglobin	9.5 - 10.5 gm/Dl	8.0 - 9.4 gm/Dl	6.5 - 7.9 gm/Dl	< 6.5 gm/Dl
Absolute Neutrophil Count	1000-1500/mm ³	750-999/mm ³	500-749/mm ³	<500/mm ³
Platelets	75000-99000/mm ³	50000-74999/mm ³	20000-49000/mm ³	<20000/mm ³
Prothrombin Time (PT)	1.01 - 1.25 x ULN	1.26-1.5 x ULN	1.51 - 3.0 x ULN	>3 x ULN
Activated Partial Thromboplastin (APPT)	1.01 -1.66 x ULN	1.67 - 2.33 x ULN	2.34 - 3 x ULN	> 3 x ULN
Fibrinogen	0.75 - 0.99 x LLN	0.50 - 0.74 x LLN	0.25 - 0.49 x LLN	< 0.25 x LLN
Fibrin Split Product	20-40 mcg/ml	41-50 mcg/ml	51-60 mcg/ml	> 60 mcg/ml
Methemoglobin	5 - 9.9 %	10.0 - 14.9 %	15.0 - 19.9 %	> 20 %
LIVER ENZYMES				
AST (SGOT)	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
ALT (SGPT)	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
GGT	1.25 - 2.5 x ULN	1.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Alkaline Phosphatase	1.25 - 2.5 x ULN	1.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Amylase	1.1 - 1.5 x ULN	1.6 - 2.0 x ULN	2.1 - 5.0 x ULN	> 5.1 x ULN
CHEMISTRIES				
Hyponatremia	130-135 mEq/L	123-129 mEq/L	116-122 mEq/L	< 116 or mental status changes or seizures
Hypernatremia	146-150 mEq/L	151-157 mEq/L	158-165 mEq/L	> 165 mEq/L or mental status changes or seizures
Hypokalemia	3.0 - 3.4 mEq/L	2.5 - 2.9 mEq/L	2.0 - 2.4 mEq/L or intensive replacement Rx required or hospitalization required.	< 2.0 mEq/L or paresis or ileus or life-threatening arrhythmia
Hyperkalemia	5.6 - 6.0 mEq/L	6.1 - 6.5 mEq/L	6.6 - 7.0 mEq/L	> 7.0 mEq/L or life-threatening arrhythmia
Hypoglycemia	55-64 mg/dL	40-54 mg/dL	30-39 mg/dL	<30 mg/dL or mental status changes or coma

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Appendix 14 (cont.)
World Health Organization (WHO) Toxicity Grading Scale for
Determining the Severity of Adverse Events

CHEMISTRIES (continued)				
Hyperglycemia (note if fasting)	116 - 160 mg/dL	161 - 250 mg/dL	251 - 500 mg/dL	> 500 mg/dL or ketoacidosis or seizures
Hypocalcemia (corrected for albumin)	8.4 - 7.8 mg/dL	7.7 - 7.0 mg/dL	6.9 - 6.1 mg/dL	< 6.1 mg/dL or life threatening arrhythmia or tetany
Hypercalcemia (correct for albumin)	10.6 - 11.5 mg/dL	11.6 - 12.5 mg/dL	12.6 - 13.5 mg/dL	> 13.5 mg/dL life-threatening arrhythmia
Hypomagnesemia	1.4 - 1.2 mEq/L	1.1 - 0.9 mEq/L	0.8 - 0.6 mEq/L	< 0.6 mEq/L or life- threatening arrhythmia
Hypophosphatemia	2.0 - 2.4 mg/dL	1.5 - 1.9 mg/dL or replacement Rx required	1.0 - 1.4 mg/dL intensive Rx or hospitalization required	< 1.0 mg/dL or life- threatening arrhythmia
Hyperbilirubinemia	1.1 - 1.5 x ULN	1.6 - 2.5 x ULN	2.6 - 5 x ULN	> 5 x ULN
BUN	1.25 - 2.5 x ULN	2.6 - 5 x ULN	5.1 - 10 x ULN	> 10 x ULN
Creatinine	1.1 x 1.5 x ULN	1.6 - 3.0 x ULN	3.1 - 6 x ULN	> 6 x ULN or required dialysis
URINALYSIS				
Proteinuria	1+ or < 0.3% or < 3g/L or 200 mg - 1 gm loss/day	2-3+ or 0.3 - 1.0% or 3-10 g/L 1-2 gm loss/day	4+ or > 1.0% or > 10 g/L 2-3.5 gm loss/day	nephrotic syndrome or > 3.5 gm loss/day
Hematuria	microscopic only	gross, no clots	gross* clots	obstructive or required transfusion
CARDIAC DYSFUNCTION				
Cardiac Rhythm		asymptomatic, transient signs, no Rx required	recurrent/persistent; No Rx required	requires treatment
Hypertension	transient inc. > 20 mm; no Rx	recurrent, chronic, > 20 mm, Rx required	requires acute Rx; No hospitalization	requires hospitalization
Hypotension	transient orthostatic hypotension, No Rx	symptoms correctable with oral fluids Rx	requires IV fluids; no hospitalization required	requires hospitalization
Pericarditis	minimal effusion	mild/moderate asymptomatic effusion, no Rx	symptomatic effusion; pain; EKG changes	tamponade; pericardiocentesis or surgery required
Hemorrhage, Blood Loss	microscopic/occult	mild, no transfusion	gross blood loss; 1-2 units transfused	massive blood loss; > 3 units transfused

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Appendix 14 (cont.)
World Health Organization (WHO) Toxicity Grading Scale for
Determining the Severity of Adverse Events

RESPIRATORY				
Cough	transient- no Rx	treatment associated cough local Rx	uncontrolled	
Bronchospasm, Acute	transient; no Rx < 80% - 70% FEV ₁ (or peak flow)	requires Rx normalizes with bronchodilator; FEV ₁ 25% - 50% (or peak flow retractions)	no normalization with bronchodilator; FEV ₁ 25% - 50% (or peak flow retractions)	cyanosis; FEV ₁ <25% (or peak flow) or intubated
GASTROINTESTINAL				
Stomatitis	mild discomfort; no limits on activity	some limits on eating/drinking	eating/talking very limited	requires IV fluids
Nausea	mild discomfort; maintains reasonable intake	moderate discomfort; intake decreased significantly; some activity limited	severe discomfort; no significant intake; activities limited	minimal fluid intake
Vomiting	transient emesis	occasional/moderate vomiting	orthostatic hypotension or IV fluids required	hypotensive shock or hospitalization required for IV fluid therapy
Constipation	mild	moderate	severe	distensions w/vomiting
Diarrhea	transient 3-4 loose stools/day	5-7 loose stools/day	orthostatic hypotension or > 7 loose stools/day or required IV fluids	hypotensive shock or hospitalization for IV fluid therapy required
NEURO & NEUROMUSCULAR				
Neuro-Cerebellar	slight incoordination dysdiadochokinesis	intention tremor, dysmetria, slurred speech; nystagmus	locomotor ataxia	incapacitated
Mood	mild anxiety or depression	moderate anxiety or depression and therapy required	severe anxiety or depression or mania; needs assistance	acute psychosis; incapacitated, requires hospitalization
Neuro Control (ADL = activities of daily living)	mild difficulty concentrating; no Rx; mild confusion/agitation; ADL unaffected	moderate confusion/agitation some limitation of ADL; minimal Rx	severe confusion/agitation needs assistance for ADL; therapy required	toxic psychosis; hospitalization
Muscle Strength	subjective weakness no objective symptoms/signs	mild objective signs/symptoms no decrease in function	objective weakness function limited	paralysis

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Appendix 14 (cont.)
World Health Organization (WHO) Toxicity Grading Scale for
Determining the Severity of Adverse Events

OTHER PARAMETERS				
Fever: oral, > 12 hours	37.7 – 38.5 C or 100.0 – 101.5 F	38.6 – 39.5 C or 101.6 – 102.9 F	39.6 – 40.5 C or 103 – 105 F	> 40 C or > 105 F
Headache	mild, no Rx therapy	transient, moderate; Rx required	severe; responds to initial narcotic therapy	intractable; required repeated narcotic therapy
Fatigue	no decrease in ADL	normal activity decreased 25- 50%	normal activity decreased > 50% can't work	unable to care for self
Allergic Reaction	pruritus without rash	localized urticaria	generalized urticaria; angioedema	anaphylaxis
Local Reaction	tenderness or erythema	induration < 10 cm or phlebitis or inflammation	induration > 10 cm or ulceration	necrosis
Mucocutaneous	erythema; pruritus	diffuse, maculo papular rash, dry desquamation	vesiculation, moist desquamation, or ulceration	exfoliative dermatitis, mucous membrane involvement or erythema, multiforme or suspected Stevens-Johnson or necrosis requiring surgery

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