DUal thrombolytic therapy with Mutant pro-urokinase (m-pro-urokinase, HisproUK) and low dose Alteplase for ischemic Stroke

Research protocol for a multicenter randomized controlled phase II trial.

PROTOCOL TITLE: 'Dual thrombolytic therapy with mutant pro-urokinase (m-pro-urokinase) and low dose alteplase for ischemic stroke.'

Protocol ID	DUMAS-1.1
Short title	DUMAS
Dutch MEC number	MEC-2019-0001
EudraCT number	2018-004448-42
Version	1.2
Date	Dec 20 2018 (last amendment March 31, 2020)
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Version number	Change	Date
1.1	Initial version	27-02-2019
1.2	- Changed independent expert to Bart. C. Jacobs - Addition of inclusion criteria for treatment with intravenous thrombolysis beyond 4.5 hours after symptom onset or last seen well. - Change in sample size: an extra patient will be included for each patient included with a discharge diagnosis other than ischemic stroke - Change of secondary clinical outcome: modified Rankin Scale (mRS) at 90 days is changed to mRS at 30 days - Change of safety outcome: death from any cause including intracranial hemorrhage within 90 days is changed to within 30 days	26-03-2020

	- Update in study procedures: Sequences with gadolinium are added to the MRI at 24-48 hours - Addition of NCCT and CT-perfusion as follow-up option in the event of contra-indications for MRI after randomization - Update of the DSMB members and interim analysis - Update in the labeling of IMP - Update in study organization and study committees - An appendix with imaging requirements is added	

PROTOCOL SIGNATURE SHEET

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)
AE	Adverse Event
AR	Adverse Reaction
CA	Competent Authority
CCMO	Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek
CI	Confidence interval
CT	Computed tomography
CTA	Computed tomography angiography
CTP	Computed tomography perfusion
CV	Curriculum Vitae
DSMB	Data Safety Monitoring Board
EU	European Union
EudraCT	European drug regulatory affairs Clinical Trials
GCP	Good Clinical Practice
GDPR	General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)
HisproUK	Mutant pro-urokinase
m-pro-urokinase	Mutant pro-urokinase
IB	Investigator's Brochure
IC	Informed Consent
ICH	Intracranial hemorrhage
IV	Intravenous
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
METC	Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)
MI	Myocardial infarction
MRI	Magnetic resonance imaging
mRS	Modified Rankin Scale
NIHSS	National Institute of Health Stroke Scale

NCCT	Non contrast computed tomography
OR	Odds ratio
PROBE	Prospective randomized open blinded end-point
(S)AE	(Serious) Adverse Event
SPC	Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst
Sponsor	The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator; in this case: Erasmus MC University Medical Center.
Subsidising Party	A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party. In this case: TSI
SUSAR	Suspected Unexpected Serious Adverse Reaction
UAVG	Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG
WMO	Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen

SUMMARY

Rationale: Recombinant tissue plasminogen activator alteplase is the only FDA-approved thrombolytic agent for thrombolytic treatment of ischemic stroke patients. Its effectiveness is limited and the occurrence of intra- and extracerebral hemorrhage is a major limitation. Dual thrombolytic therapy with low dose alteplase pre-treatment followed by a mutant pro-urokinase (m-pro-urokinase, HisproUK), which does not lyse hemostatic fibrin, has a significant potential to be safer and more efficacious than the FDA-approved regimen of standard dose alteplase alone.

Objective: To test the safety and preliminary efficacy of a dual acute thrombolytic treatment consisting of a small intravenous (IV) bolus of alteplase followed by IV infusion of m-pro-urokinase against usual treatment with IV alteplase in patients presenting with ischemic stroke.

Study design: This is a multicenter, phase II, randomized clinical trial with open-label treatment, adaptive design for dose optimization and blinded outcome assessment, comparing low dose IV alteplase + two different dosages of IV m-pro-urokinase with usual thrombolytic treatment of alteplase alone.

Study population: We will enroll 200 patients with a discharge diagnosis of ischemic stroke, intracranial hemorrhage ruled out with non-contrast CT, who can be treated within 4.5 hours from symptom onset, who meet the criteria for standard treatment for IV alteplase, and who are not considered eligible for endovascular thrombectomy.

Intervention and usual care: Bolus of IV alteplase (5 mg) followed by continuous IV infusion of the study medication: m-pro-urokinase 40 mg/hr during 60 minutes (initial dose) or standard treatment with alteplase alone. Depending on results of interim analyses, the alternate dose of m-pro-urokinase may be revised to a lower dose (30 mg/hr during 60 minutes) or a higher dose (50mg/hr during 60 minutes). Usual care consists of a bolus of IV alteplase followed by continuous infusion of alteplase in a total dose of 0.9 mg/kg with a maximum of 90 mg.

Primary and secondary outcomes: The primary outcome is any post-intervention intracranial hemorrhage on MRI according to the Heidelberg Bleeding Classification within 24-48 hours of study drug administration. Secondary outcomes include stroke severity measured with the National Institutes of Health Stroke Scale (NIHSS) at 24 hours and 5-7 days, score on the modified Rankin Scale (mRS) assessed at 30 days, dichotomized mRS, infarct volume measured with MRI at 24-48 hours, change (pre-treatment vs. post-treatment) in abnormal perfusion volume and secondary blood biomarkers of thrombolysis at 24 hours (including d-dimers and fibrinogen level). Safety endpoints include symptomatic intracranial hemorrhage, death and major extracranial hemorrhage.

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

M-pro-urokinase has an improved safety profile and similar effectiveness as alteplase in ex- and in-vivo experimental studies as well as in a clinical study in myocardial infarction. The informed consent procedure takes on average one hour, both in proxies and in stroke patients themselves. For every 15 minutes of delay of IV thrombolytic treatment, the likelihood of a good functional outcome is reduced by 1% (absolute risk difference). We will therefore defer consent and ask for written informed consent as early as deemed appropriate according to the treating physician.

Trial registration: http://www.trialregister.nl, NTR7634

1. INTRODUCTION AND RATIONALE

Currently, recombinant tissue plasminogen activator alteplase is the only FDA-approved thrombolytic agent for thrombolytic treatment of ischemic stroke. Its effectiveness is limited and it carries a risk of symptomatic intracerebral hemorrhage (ICH) of 6-7%.[1-3] The drug is given intravenously in a dose of 0.9 mg/kg, with 10% bolus followed by a continuous infusion over 60 minutes of the remaining 90%. Its use is limited to patients presenting within 4.5 hours after symptom onset and patients with unknown time of onset with a mismatch between diffusion-weighted imaging and fluid-attenuated inversion recovery (FLAIR).[4] Recently, it has been shown that alteplase is also beneficial for patients presenting between 4.5 hours to 12 hours from symptom onset or last seen well if they have still viable ischemic brain tissue which can be identified with advanced imaging.[5]

Additional treatment with endovascular thrombectomy is effective in patients with an occlusion, located in the intracranial carotid, or the horizontal segment of the middle cerebral artery (M1 and proximal M2). This type of occlusion is present in at most 30% of ischemic stroke patients presenting at the emergency department. [8]

Patients without an intracranial occlusion can only be treated with a thrombolytic agent. This thrombolytic treatment with alteplase in patients with ischemic stroke leads on average to improved reperfusion in about 30% of patients and increases the likelihood of good clinical outcome in 1 of every 10 treated patients.[1] Apart from its limited efficacy, the occurrence of intra-and extracranial hemorrhage is a major limitation in the treatment with alteplase. In the Cochrane analysis, thrombolytic treatments consistently increased the risk of symptomatic intracranial hemorrhage fourfold, from 1.7% to 7.5% (OR 3.75, 95%CI 3.11 to 4.51, P < 0.00001), with no statistically significant heterogeneity (p=0.36). [1]

Several classifications of intracranial hemorrhage are in use, i.e. NINDS classification, ECASS II classification, and the recent Heidelberg Bleeding Classification. An overview of these classifications is provided in Table 1 (Section 15.1). An intracranial hemorrhage can either be classified symptomatic or asymptomatic. In most studies, symptomatic intracranial hemorrhage is defined as an increase in neurological deficit of 4 points or more on the NIH stroke scale, or death, with hemorrhage confirmed by neuroimaging, with a distinction being made between hemorrhagic infarction, intracerebral hemorrhage, subarachnoid and intraventricular hemorrhage.[9 10] This implies that several hemorrhages may cause more subtle deterioration and still be classified as asymptomatic. In many instances, intracranial hemorrhage or hemorrhagic infarction does not lead to overt clinical deterioration and the hemorrhage is classified as asymptomatic. The incidence of asymptomatic intracranial hemorrhage or any intracranial hemorrhage is often not reported. The classification of hemorrhage on CT leaves considerable room for interpretation and interobserver variability.

In the NOR-TEST trial of IV tenecteplase versus IV alteplase in 1100 patients with ischemic stroke, the incidence of symptomatic intracranial hemorrhage was only 2% and the incidence of any ICH was 9% in the alteplase group.[11] In the SITS-MOST, a multicenter registry of 6483 patients who were treated with IV alteplase, the incidence of symptomatic hemorrhage was 4.6%, and the incidence of asymptomatic hemorrhage was 17%. In the MR CLEAN trial, the likelihood of any intracerebral hemorrhage (ICH) or hemorrhagic transformation according to the ECASS II classification in patients who had been treated with IV alteplase was 46%. 7% had a symptomatic ICH. Thrombectomy did not influence this rate.[12]
It has been suggested that Asian patients are at increased risk of symptomatic intracranial hemorrhage after treatment with alteplase.[13] However, studies show inconsistent results and have not led to altered recommendations in Dutch or US guidelines regarding dose changes for Asian patients.[14-18]
There is a need for a better and safer thrombolytic therapy, that expands the number of patients that will be treated safely and successfully. Tenecteplase at a dose of 0.25 mg/kg is a promising alternative to alteplase, because of its ease of administration, but until now, superiority or even non-inferiority has not been sufficiently demonstrated. Also, the rate of hemorrhage in patients treated with tenecteplase and alteplase are similar.[11, 19-22]
Preclinical and clinical studies have indicated that dual thrombolytic therapy, mimicking the physiological design of thrombolysis, with low dose alteplase pre-treatment followed by a mutant pro-urokinase (m-pro-urokinase, brand-name: HisproUK) has a significant potential to be safer and more efficacious than the FDA-approved regimen of standard dose alteplase alone (0.9 mg/kg).[23-25] M-pro-urokinase is a mutation of pro-urokinase with less susceptibility to non-specific activation to urokinase. Moreover, m-pro-urokinase by itself does not lyse hemostatic fibrin, only degraded fibrin.[26] When alteplase is cleared from the systemic circulation, m-pro-urokinase will only induce intravascular clot lysis while sparing hemostatic fibrin. Therefore, this therapeutic regimen has the potential to be safer. However, everywhere where alteplase is bound to plasminogen, activation of m-pro-urokinase may occur. These considerations argue for using all intracranial hemorrhages as the primary outcome. They lead to the necessity of having a core lab for consistent and blinded assessment of all follow up scans for hemorrhage classification.

2. OBJECTIVES

To test the safety and preliminary efficacy of a dual acute thrombolytic treatment consisting of a small bolus of intravenous (IV) alteplase followed by IV infusion of mutant pro-urokinase (m-pro-urokinase) against usual treatment with IV alteplase in patients presenting acutely with ischemic stroke.

3. STUDY DESIGN

This is a multicenter, phase II, randomized clinical trial with open-label treatment and blinded outcome assessment (PROBE) study comparing low dose IV alteplase + two different dosages of IV m-pro-urokinase with usual thrombolytic treatment. Sequential interim analyses will be performed allowing adaptation of the IV m-pro-urokinase dose, because the exact optimal dose of IV m-pro-urokinase in patients with ischemic stroke is still unknown. This study will run for 2 years in several hospitals in the Netherlands. An overview of the study and the main procedures that subjects will undergo is provided in Figure 1 (Section 16.1).

4. STUDY POPULATION

4.1 Population (base)

The study population will be drawn from patients with a clinical diagnosis of ischemic stroke at the Emergency Department. Patients meeting the inclusion and exclusion criteria as set below will be entered in the trial.

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- A clinical diagnosis of ischemic stroke;
- A score of at least 1 on the NIH Stroke Scale;
- CT or MRI ruling out intracranial hemorrhage;
- Treatment possible for patients who: 1) can be treated within 4.5 hours from symptom onset or last seen well, or 2) can be treated between 4.5 to 12 hours from symptom onset or last seen well **and** have an infarct core ≤ 25 mL **and** have a penumbra of at least the same size as the infarct core (i.e. total ischemic volume/infarct core mismatch ≥ 2.0),[5] or 3) have an unknown time of onset **and** a mismatch between diffusion-weighted imaging and FLAIR[4];

- Meet the criteria for standard treatment for IV alteplase according to national guidelines[27];
- Age of 18 years or older;
- Written informed consent (deferred).

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Candidate for endovascular thrombectomy (i.e., a proximal intracranial large artery occlusion on CTA or MRA);
- Contra-indication for treatment with IV alteplase according to national guidelines[27]:
    - Arterial blood pressure exceeding 185/110 mmHg and not responding to treatment
    - Blood glucose less than 2.7 or over 22.2 mmol/L
    - Cerebral infarction in the previous 6 weeks with residual neurological deficit or signs of recent infarction on neuro-imaging
    - Head trauma in the previous 4 weeks
    - Major surgery or serious trauma in the previous 2 weeks
    - Gastrointestinal or urinary tract hemorrhage in the previous 2 weeks
    - Previous intracerebral hemorrhage
    - Use of anticoagulant with INR exceeding 1.7 or APTT exceeding 50 seconds
    - Known thrombocyte count less than 90 x 10^9/L. When the treating physician suspects a thrombocyte count below 90x109/L (e.g. suspected hemorrhagic diathesis), the thrombocyte count in the laboratory should be awaited prior to inclusion in DUMAS.
    - Treatment with direct thrombin or factor X inhibitors, unless specific antidotum has been given, i.e. idarucizumab in case of dabigatran use.
- Pre-stroke disability which interferes with the assessment of functional outcome at 30 days, i.e. mRS > 2;
- Known pregnancy or if pregnancy cannot be excluded, i.e. did not have intercourse for > 6 months and no clinical signs of pregnancy, adequate use of any contraceptive method (e.g. intrauterine devices) or sterilization of the subject herself.
- Contra-indication for an MRI scan, i.e.:
    - an MRI incompatible pacemaker, ICD, pacing wires and loop records
    - metallic foreign bodies (e.g. intra-ocular)
    - prosthetic heart valves
    - blood vessel clips, coils or stents

- an implanted electronic and/or magnetic implant or pump (e.g. neurostimulator)
- cochlear implants
- mechanical implants (implanted less than 6 weeks ago)
- a copper intrauterine device

- Participation in any medical or surgical therapeutic trial other than DUMAS (or MR ASAP[28]/ARTEMIS[29])

4.4 Sample size calculation

A sample size of 200 patients with a discharge diagnosis of ischemic stroke randomized 1:1 to either standard treatment or dual thrombolytic treatment will provide us with a power of 0.8 to detect a statistically significant effect on the primary outcome, which we assume to occur with a probability of 20%[30] with standard treatment and an overall probability of 7% overall, in the patients treated with dual thrombolytic therapy, for an overall effect (OR) of 0.3. This estimate does not take into account the use of multivariable adjustment in the primary analysis. To account for inclusion of patients with a discharge diagnosis other than ischemic stroke (e.g. stroke mimic), we will include one extra patient for each included patient with a discharge diagnosis other than ischemic stroke. We estimate that 20% of the included patients will not have a diagnosis of ischemic stroke at discharge. In case a patient did not receive the assigned medication for any reason, one extra patient will be included as well.

5. TREATMENT OF SUBJECTS

5.1 Investigational product

The investigational treatment is dual thrombolytic therapy with low dose alteplase pre-treatment followed by m-pro-urokinase. In this study patients will receive a bolus of IV alteplase (5 mg), as part of usual care, followed by a continuous infusion of m-pro-urokinase. The study has an open label design. The study medication (m-pro-urokinase) will be compared with usual care (alteplase alone), no placebo will be used.

5.2 Use of co-intervention

No standard co-medication is advised by the Steering Committee. However, as described earlier, patients in the intervention group should receive a bolus of 5mg alteplase prior to infusion with m-pro-urokinase, No rescue medication is available. If a patient is randomized for treatment with m-pro-urokinase, it is not possible to also treat a patient with standard dose alteplase, due to the risk of hemorrhage. If an anaphylactoid reaction occurs with either alteplase or mutant pro-urokinase, treatment will be stopped immediately and appropriate anaphylactoid treatment will be given according to local guidelines.

5.3 Monitoring of subject compliance

We will monitor if patients received full dosages of the thrombolytic treatment or not at the emergency and neurology department. When thrombolytic treatment is stopped early, the causes and total dosage thrombolytic therapy received will be collected.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

The investigational treatment is m-pro-urokinase. Patients in the intervention group will be treated with a bolus of IV alteplase (5 mg) followed by a continuous infusion of m-pro-urokinase. Pre-treatment with alteplase is needed, because m-pro-urokinase only binds degraded fibrin. This therapeutic scheme has the potential to be safer, because alteplase will have almost completely disappeared from the systemic circulation within 20 minutes, as alteplase has a plasma half-life of 4-5 minutes),[31] and in the absence of alteplase, m-pro-urokinase will not be activated. On the other hand, alteplase binds to PAI-1, by which it is de-activated, and to the plasminogen – fibrin complex, where it will promote release of plasmin, which in its turn breaks down fibrin, but also fibrinogen.[32] The half-life of the alteplase-plasminogen complex is not well known, but it is considerably longer than the half-life of alteplase in the systemic circulation.[33] Therefore, the beneficial effect of m-pro-urokinase over alteplase is by no means certain.

HisprouUK is a single chain polypeptide of 411 amino acids. It has the sequence of human prourokinase with a single point mutation Lys300→His and a molecular weight of 46376.7 Da. M-pro-urokinase is predominantly cleared by the liver and has a half-life of 11-12 minutes. It is packed in vials of 20 mg and must be stored at -70°C to -80°C. It is possible to store at 5°C, however, than it has an expiry date of 6 months. Detailed information can be found in the investigator's brochure (IB).

6.2 Summary of findings from non-clinical studies

M-pro-urokinase is a Lys300 > His mutation of pro-urokinase with less susceptibility to non-specific (systemic) activation to urokinase, due to lessened intrinsic proteolytic activity.[25] A study in dogs showed a better clot-specific lysis, with less systemic bleeding.[23] Another experimental study with m-pro-urokinase in dogs, suggest a higher fibrinolytic effect and confirm that m-pro-urokinase by itself, in the absence of alteplase in the systemic circulation does not lyse hemostatic fibrin and will not deplete levels of circulation fibrinogen.[24] Intact fibrin contains only the D-domain plasminogen, which is the favored substrate of alteplase. Partially degraded fibrin bears three C-terminal lysines on the fibrin fragment E domain providing a high affinity-binding site for plasminogen, which induces a conformational change. This is the favored substrate of m-pro-urokinase (and pro-urokinase).[26] Detailed

information can be found in the investigational medicinal product dossier (IMPD) and investigator's brochure (IB).

6.3 Summary of findings from clinical studies

M-pro-urokinase has only been studied in healthy male volunteers. This phase 1 study of IV administration of m-pro-urokinase at therapeutic dosages showed that m-pro-urokinase was safe and does not result in bleeding or fibrinogen depletion in healthy volunteers (see separate appendix: 'Phase 1 trial of mutant proUK (HisproUK), version 3.0, d.d. 28-03-2018).

Pro-urokinase, however, is well studied. The structural and physical characteristics of m-pro-urokinase are similar to pro-urokinase, therefore the specific activation on the fibrin clot is equal. Two randomized trials of intra-arterial treatment in patients with acute ischemic stroke with pro-urokinase have been carried out.[34, 35] More patients in the intervention arm of the trial reperfused and more patients had a favorable outcome than controls, despite an increased rate of intracerebral hemorrhage.

A single arm study of sequential treatment with a 5-10 mg alteplase bolus followed by a 90 minutes continuous infusion of pro-urokinase at a rate of 40 mg/hr in 101 patients with ST elevation myocardial infarction, reported a 77% TIMI 2-3 reperfusion rate, with 60% of patients reaching TIMI 3,[36] which compares favorably to the effect of other fibrinolytics (alteplase, tenecteplase) in acute MI.[37-39]

6.4 Summary of known and potential risks and benefits

The potential benefits of the intervention have been described in Section 1. The potential risks of thrombolytic therapy consist of hemorrhage, in particular symptomatic intracranial hemorrhage. In the SITS-MOST, an international registry of patients treated with IV alteplase, the incidence of symptomatic hemorrhage was 4.6%, and the incidence of any hemorrhage was 17%.[30] In a similar Canadian registry (CASES), the incidence of any hemorrhage was 27%.[40]

Severe extracranial hemorrhage occurs in about 1% of patients who receive alteplase.[31] Dual thrombolytic therapy with low dose alteplase followed by m-pro-urokinase have a potential to be safer, because of the result of preclinical and clinical studies (described in Section 6.2 and Section 6.3). Adverse events of m-pro-urokinase are displayed in Table 6 of the phase 1 study of m-pro-urokinase (see appendix).

6.5 Description and justification of route of administration and dosage

Alteplase and m-pro-urokinase will be administered intravenously, since it is the only currently available effective route. The half-life of m-pro-urokinase is around 11 minutes and will therefore be administered with a continuous infusion.

Trials of fibrinolytic treatment that used similar doses of the drug as were used in trials of fibrinolytic treatment of acute myocardial infarction reported high rates of intracranial hemorrhage, and no beneficial effect of treatment on functional outcome.[41, 42] That prompted investigators of thrombolytic therapy for ischemic stroke to use doses of 60% to 90%of the dose used in MI. For example, in GUSTO, a randomized controlled trial in patients with acute myocardial infarction, the most effective thrombolytic regimen was accelerated tPA in a bolus of 15 mg, 0.75 mg/kg in 30 minutes, not to exceed 50 mg, and 0.5 mg/kg, up to 35 mg, over the next 60 minutes combined with intravenous heparin. This means that an average patient, weighting 75 kg, would receive a total of 100 mg alteplase (the maximum dose).[37] The total dose used in the effective landmark alteplase trials for ischemic stroke was 0.9 mg/kg, including a 10% bolus. An average patient, weighting 75 kg, would receive a total of 67.5 mg, which comes down to 67.5% of the GUSTO dose in an average person.[2 10] Considering the intrinsically increased risk of intracranial hemorrhage after thrombolytic treatment in patients with ischemic stroke compared to patients with MI, we consider it wise to reduce the cumulative dose of pro-urokinase with 33% by limiting the total duration of infusion to 60 instead of the 90 minutes in the PATENT trial.[36]

6.6 Dosages, dosage modifications and method of administration

A bolus of IV alteplase (5 mg), as part of usual care, will be followed by continuous infusion of m-pro-urokinase, either 40 mg/hr during 60 minutes (=40 mg in total) (initial dose) or an alternate dose. Depending on the result of interim analyses, the m-pro-urokinase dosage may be revised to:

- Higher than the initial dose, by 25% (i.e. 50 mg/hr during 60 minutes)
- Lower than the initial dose, by 25% (i.e. 30mg/hr during 60 minutes)

Standard treatment consists of alteplase alone (0.9mg/kg, with 10% of the total dosage given as a bolus).

6.7 Preparation and labeling of Investigational Medicinal Product

Commercially available preparations of alteplase will be used for bolus and continuous infusion in 60 minutes, both as part of usual care. The hospital pharmacy of Erasmus MC will label and store alteplase according to the Good Manufacturing Practice Guideline (2003/94/EG), as standard protocol for usual care. M-pro-urokinase will be prepared and labeled by Thrombolytic Science LLC, Boston, USA (TSI). TSI will label the IMP according to regulations under supervision of the hospital pharmacy of Erasmus MC. M-pro-urokinase will be labeled as HisproUK (brand name). In case new labels are needed for any reason (e.g. to update the retest date), Erasmus MC will label the IMP according to regulations.

6.8 Drug accountability

M-pro-urokinase will be distributed by the hospital pharmacy of Erasmus MC as described in appendix 1. Each participating hospital will store the investigational medicinal product (IMP) under prespecified, secured conditions. The local pharmacies of the participating hospitals will maintain patient-level drug accountability records for all locally enrolled patients. The central pharmacy of Erasmus MC will maintain patient-level drug accountability records for patients enrolled at Erasmus MC and a center-level drug accountability record for the full trial. Not used m-pro-urokinase will be returned to TSI and used medication will be destructed by each participating hospital after being accounted for by the study monitor.

7. NON-INVESTIGATIONAL PRODUCT

This is not applicable for this study.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

The primary outcome is any post-intervention intracerebral hemorrhage/hematoma on MRI (SWI) according to the Heidelberg Bleeding Classification at 24 (to 48 hours) of study drug administration. A detailed classification of the Heidelberg Bleeding Classification is provided in Table 1.[9] Assessment of any intracerebral hemorrhage on the Heidelberg Bleeding Classification will be performed by an independent central core laboratory.

8.1.2 Secondary study parameters/endpoints

Secondary clinical outcomes

- Score on the National Institute of Health Stroke Scale (NIHSS) assessed at 24 hours and at 5-7 days post-treatment.[43]
- Improvement of at least 4 points on NIHSS at 24 hours compared to baseline, or (near) complete recovery (NIHSS 0 or 1).
- Score on the modified Rankin Scale (mRS) assessed at 30 days (-7 days or +14 days) post-treatment.[44]
- All possible dichotomizations of the mRS as assessed at 30 days (-7 or +14 days) post-treatment. This includes complete recovery (mRS 0 vs 1-6), excellent functional outcome (mRS 0-1 vs 2-6), good functional outcome (mRS 0-3 vs 4-6), and handicapped survival (mRS 0-4 vs 5-6) and survival in any condition (mRS 0-5 vs 6).

Secondary neuroimaging outcomes

- Infarct volume measured with MRI (DWI) at 24 (to 48 hours) post-treatment.
- Change (pre-treatment vs. post-treatment) in abnormal perfusion volume based on TTP/MTT maps measured with CT perfusion at baseline and MRI at 24-48 hours post treatment.

Secondary blood biomarker outcomes

- Secondary blood biomarkers of thrombolysis within 1 hour post-treatment, after 3 hours and after 24 hours post-treatment, including d-dimers and fibrinogen.
- Change in blood biomarkers of thrombolysis from baseline to 24 hours, including d-dimers and fibrinogen.

Safety outcomes

- Symptomatic intracranial hemorrhage (sICH) according to the Heidelberg Bleeding Classification[9]
- Death from any cause including intracranial hemorrhage within 30 days (-7 days or +14 days) (this is equivalent to handicapped survival (mRS 0-4 vs 5-6) and survival in any condition (mRS 0-5 vs 6).
- Major extracranial hemorrhage according to the ISTH criteria within 24 hours of study drug administration.[45]

8.1.3 Other study parameters

Baseline parameters that will be recorded include age, sex, pre-stroke mRS; previous stroke; conditions such as hypertension, diabetes mellitus, atrial fibrillation, myocardial infarction; smoking status; medication including antihypertensive treatment, antiplatelet agents and anticoagulants; vital parameters such as blood pressure, heart rate, body temperature; weight and height; neurological examination including NIHSS; laboratory examination including INR, APTT, C-reactive protein, glucose, creatinine, fibrinogen, plasminogen, alpha-2-antiplasmine, D-dimers, and imaging results on admission including ASPECTS on NCCT and CT-perfusion parameters.
We will record the administered dose of alteplase and timing of IVT medication. To monitor the workflow we will record time of symptom onset, time from symptom onset to: ER, imaging, randomization and IVT.

8.2 Randomization, blinding and treatment allocation

Patients will be randomized to standard treatment with alteplase alone vs. dual thrombolytic treatment with bolus alteplase + m-pro-urokinase (1:1). During the study, the m-pro-urokinase dosage may be revised, randomization will remain the same. Patients will be

randomized after CTA (exclusion of a large vessel occlusion) at the emergency department. The randomization procedure will be computer- and web-based, using permuted blocks. Block size will not be revealed to investigators and study personnel. Back-up by telephone will be provided. Randomization will be stratified for center.

8.3 Study procedures

All patients will undergo assessment of the NIHSS at baseline, 24 hours and 5-7 days (or discharge if earlier), which is routine in clinical procedure. It will be carried out by certified assessors. All patients will undergo NCCT, CT-angiography and CT-perfusion or MRI/MRA of the brain at baseline, as part of routine clinical care. The CT-perfusion should be focused on the anterior circulation or posterior circulation depending on the suspected location of the ischemic stroke as determined by the neurology assistant or neurologist. For follow-up, all patients will undergo an MRI-scan of the brain at 24 (to 48) hours. The MRI-scan will include the following sequences: 1) T2w-TSE, 2) 3D-T2w-FLAIR, 3) DWI/ADC, 4) SWI. 5) DSC-PW MRI, 6) 3D-T1w without and with gadolinium. In the event of any contra-indication for an MRI after randomization (e.g. because the contra-indication was not known at the time of inclusion or the patient has a new contra-indication due to an intervention during hospital admission or stay), a follow-up NCCT and CT-perfusion at 24-48 hours will be performed instead. Intracranial hemorrhage will be assessed on SWI. Infarct volume will be assessed on DWI. Follow-up with MRI is not part of usual care in every hospital. Blood samples will be taken at baseline, one tube EDTA (+/- 5 mL), one tube without anticoagulant (+/- 7mL) and two tubes citrated blood (2.7 mL) will be drawn. Additional blood samples will be taken (two tubes citrated blood of 2.7 mL) within 1 hour, after 3 hours and 24 hours post treatment. Biomaterials will not be collected for all patients. This will only be collected for patients in some participating centers. Plasma samples will be stored at -80 degrees Celsius for later analysis. A schedule of all activities is shown in Table 2.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons. Data and biomaterials from non-consenting subjects will not be used when there is a written objection from the subject or representative. In an effort to describe the non-consenting population we will ask the subject or his/her representative to allow the use of routinely collected data and materials in a coded manner. If no consent for the use of these data is obtained, only study number, treatment allocation and refusal will be noted. Safety parameters of these withdrawn subjects will also be collected and analyzed. Other missing data, including any intracerebral hemorrhage, will be imputed for the main analysis, by multiple imputation.

8.5 Replacement of individual subjects after withdrawal

Patients who refused consent will be replaced in the study.

8.6 Follow-up of subjects withdrawn from treatment

All patients in the study will be followed until final assessment at 30 days. For patients who do not give or have withdrawn consent only safety parameters will be assessed.

8.7 Premature termination of the study

The study will only be terminated prematurely if the Data and Safety Monitoring Board recommends discontinuation of the study, see Section 9.5. In case of premature termination of the study the database will be closed 90 days after assessment of the last enrolled patient and results will be reported.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardize subject health or safety. The assessment of sufficient ground will be based on the advice of the DSMB. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product. All adverse events reported spontaneously by the subject or observed by the investigator or his staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect;
- that required medical or surgical intervention.

Any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate medical judgement. An elective hospital admission will not be considered as a serious adverse event.

Serious adverse events will be immediately after coming to notice of the investigator reported to the trial coordinator, who is 24/7 available.

The investigator will report the following SAEs occurring in the study period to the sponsor without undue delay of obtaining knowledge of the events: Death from any cause; symptomatic intracranial hemorrhage, extracranial hemorrhage, cardiac ischemia, pneumonia, allergic reactions, new ischemic stroke in a different vascular territory.

Technical complications that do not lead to clinically detectable SAE and neurological deterioration not caused by intracranial hemorrhage, new ischemic stroke, are considered as consistent with the natural course of the ischemic stroke, will not be reported immediately.

The sponsor will report the SAEs through the web portal *ToetsingOnline* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Adverse reactions are all untoward and unintended responses to an investigational product related to any dose administered.

Unexpected adverse reactions are SUSARs if the following three conditions are met:

1. the event must be serious (see chapter 9.2.2);
2. there must be a certain degree of probability that the event is a harmful and an undesirable reaction to the medicinal product under investigation, regardless of the administered dose;
3. the adverse reaction must be unexpected, that is to say, the nature and severity of the adverse reaction are not in agreement with the product information as recorded in:
   - Summary of Product Characteristics (SPC) for an authorised medicinal product;
   - Investigator's Brochure for an unauthorised medicinal product.

The sponsor will report expedited the following SUSARs through the web portal *ToetsingOnline* to the METC:

- SUSARs that have arisen in the clinical trial that was assessed by the METC;

SUSARs that have arisen in other clinical trials of the same sponsor and with the same medicinal product, and that could have consequences for the safety of the subjects involved in the clinical trial that was assessed by the METC.

The remaining SUSARs are recorded in an overview list (line-listing) that will be submitted once every half year to the METC. This line-listing provides an overview of all SUSARs from the study medicine, accompanied by a brief report highlighting the main points of concern. The expedited reporting of SUSARs through the web portal Eudravigilance or ToetsingOnline is sufficient as notification to the competent authority.

The sponsor will report expedited all SUSARs to the competent authorities in other Member States, according to the requirements of the Member States.

The expedited reporting will occur not later than 15 days after the sponsor has first knowledge of the adverse reactions. For fatal or life threatening cases the term will be maximal 7 days for a preliminary report with another 8 days for completion of the report.

9.3 Annual safety report

In addition to the expedited reporting of SUSARs, the sponsor will submit, once a year throughout the clinical trial, a safety report to the accredited METC, competent authority, and competent authorities of the concerned Member States.

This safety report consists of:

- a list of all suspected (unexpected or expected) serious adverse reactions, along with an aggregated summary table of all reported serious adverse reactions, ordered by organ system, per study;
- a report concerning the safety of the subjects, consisting of a complete safety analysis and an evaluation of the balance between the efficacy and the harmfulness of the medicine under investigation.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported until end of study within the Netherlands, as defined in the protocol

9.5 Data Safety Monitoring Board (DSMB)

In order to increase the safety of the intervention, the trial will be monitored by an independent data safety monitoring board (DSMB). The DSMB, consisting of a neurologist with sufficient neuroradiological expertise, neuroradiologist, and hematologist, will advise the chairman of the Steering Committee if analyses of safety and efficacy raise an ethical concern with regard to continuation of the trial. The DSMB will advise the chairman of the Steering Committee if, in their view, the randomized comparisons have provided both (i)

'proof beyond reasonable doubt' that for all, or some, the treatment is clearly indicated or clearly contra-indicated and (ii) evidence that might reasonably be expected to materially influence future patient management. Appropriate criteria of proof beyond reasonable doubt cannot be specified precisely, but the DMSB will work on the principle that a difference of at least 3 standard errors in an interim analysis of a major outcome event (e.g. any intracranial hemorrhage, death) may be needed to justify halting, or modifying, a study before the planned completed recruitment. This criterion has the practical advantage that the exact number of interim analyses would be of little importance, but we suggest safety analyses (death and symptomatic ICH) after inclusion of 20, 30 40 and 50 patients and after that with increments of 50, after start of the trial and after any dose change, until the trial is completed, unless the DSMB advises otherwise during the conduct of the trial. These analyses will also include measures of efficacy (NIHSS scores). Following a report from the DMSB, the steering committee will decide whether to modify entry to the study (or seek extra data) and inform the sponsor. Unless this happens however, the Steering Committee, the collaborators and central administrative staff will remain ignorant of these analyses and results.

Apart from these safety and efficacy reports, the DSMB will receive additional analyses from an independent statistician, that will inform the DSMB on the likelihood of success or failure of the study to reach a positive result as defined in the sample size calculation. This information will be used to advise the Steering Committee to adapt the dosing in the study according to pre-specified criteria, see section 10.3. The information provided by the interim analysis will not be used to discontinue the study for expected futility, as it is the intention of the steering committee to run the trial until 200 patients with a discharge patients with a discharge diagnosis of ischemic stroke have been included, as long as there are no safety or efficacy concerns, as described earlier.

The advice(s) of the DSMB will be sent to the chair of the Steering Committee, who will inform both the PIs and the sponsor of the study. Should the Steering Committee decide not to fully implement the advice of the DSMB, the Steering Committee will send the advice to the reviewing METC, including a note to substantiate why (part of) the advice of the DSMB will not be followed.

10. STATISTICAL ANALYSIS

Baseline data by treatment allocation will be reported with standard statistical procedures. Missing values for baseline characteristics will be reported. Missing baseline characteristics will be imputed using regression imputation. All analyses will be performed according to the intention-to-treat principle. Additionally, an on-treatment analysis and analysis of safety parameters with all patients, including all patients who had withdrawn from the study, will be performed. Finally, the study will be analyzed after exclusion of patients who were diagnosed as a ‘stroke mimic’.

10.1 Primary study parameter(s)

The effect of the study treatment on the primary outcome will be assessed with multivariable logistic regression modeling with study treatment as a binary independent variable (either dose of m-pro-urokinase vs. control). The effect parameter is an odds ratio (OR) with 95% confidence interval (CI). This effect estimate will be adjusted for important prognostic factors at baseline, which include age, pre-stroke mRS, time from onset of symptoms to randomization, stroke severity (NIHSS), systolic blood pressure, antiplatelet treatment and imaging based estimates of infarct core and penumbra. Whether the dosing (initial vs. higher) of the study treatment modifies the treatment effect, will be analyzed with a multiplicative interaction parameter in the main analysis. Adjusted and unadjusted effect estimates with corresponding 95% confidence intervals will be reported.

10.2 Secondary study parameter(s)

The effect of the study treatment on the secondary outcomes will be assessed with multivariable linear, logistic or ordinal regression modeling with study treatment as a binary independent variable (either dose of m-pro-urokinase vs. control). The effect parameter will be either a beta or (common) OR with 95% CI. This effect will be adjusted with the same adjustment variables as the primary outcome (see above).
Pre-specified subgroups will be performed by testing for interaction between the specific baseline characteristic and treatment.

10.3 Interim analysis

Interim analysis for dose opimization

The trial includes a pre-specified rule for adaptation of the IV m-pro-urokinase dose, with the goal of finding the optimal dose of m-pro-urokinase. After inclusion of 60 patients with a discharge diagnosis of ischemic stroke and every 20 patients with a discharge diagnosis of ischemic stroke thereafter, the DSMB will advise the Steering Committee about reverting to a second therapeutic regimen, i.e. alternate dose, see Section 6.6. Only a switch back to the original dose is allowed. The total number of different dosages used in the trial will therefore not exceed two, in order to retain sufficient precision in the estimate of dose related treatment effect.

The decision to revert to an alternate dose will depend on the estimated likelihood that the intervention will not lead to safer treatment (i.e. lower rate of any ICH) and the estimated likelihood that the intervention will lead to decreased likelihood of good outcome compared to standard treatment, as measured by the change (decrease) in NIHSS. Computations will be based on a Bayesian analytic model, see appendix. We will not use an alpha spending approach, because the interim analysis will not be performed with the intention to terminate the trial at an early stage.

Interim analyses by the DSMB
See Section 9.5.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted in compliance with this protocol and according to the principles of the Declaration of Helsinki (October 2013),[46] ICH-GCP principles[47], and in accordance with the Medical Research Involving Human Subjects Act (WMO).

11.2 Recruitment and consent

For every 15 minutes of delay of IV thrombolytic treatment, the likelihood of a beneficial outcome is reduced by 1% (absolute risk difference). The new treatment is comparable to the standard treatment, alteplase. It has an improved safety profile in ex- and in-vivo experimental studies and in a clinical study in myocardial infarction and similar effectiveness. The informed consent procedure takes on average one hour, both in proxies and in stroke patients themselves. Additionally, approximately all patients with ischemic stroke have neurological deficits interfering with their decision-making capacity. Representatives are often not directly on the scene, and if they are, there is no time for a proper informed consent procedure, which takes at least 1 hour. Also, it is almost never possible for a relative to make a well thought-through decision in this emergency situation, which is characterized by high emotional strain. We will therefore defer consent and ask for written informed consent as early as deemed appropriate according to the treating physician. We aim to ask for written informed consent as early as possible.

At the time of deferred consent, subjects or their representatives will be provided with a patient information form and verbal explanation of the purpose of the study. They will be informed about the inclusion in the trial, data and biomaterials that have been collected, and treatment they may have received. They will be asked for consent in follow-up and data usage. Participation in this trial is voluntary. Patients or their legal representatives will have ample time (several hours) to decide whether they want to continue participation in the study. When the patient is not competent and no representative is available or present, we will stop the study procedures until we can inform the representative and ask for consent. When consent by proxy (i.e., legal representative) has been obtained and the patient recovers, we will again ask for written consent from the patient (Figure 3). The patient or representative may, at any given time, withdraw informed consent. An explanation is not needed. If a patient has died before deferred consent has been obtained, his/her representative will be informed about the study treatment the patient may have received, trial procedures and use of the

collected data and biomaterials. These patients will be included in all analyses, there is no opt-out option since that may bias results. A separate information form will be sent to the representative by the medical center where the patient last resided.

11.3 Objection by minors or incapacitated subjects

Minors (patients of 18 years old and less) will not be included in the trial. Patients eligible for the trial have acquired neurological deficits due to the stroke, which may interfere with their decision-making capacity. We will follow the procedure as described in 11.2. In the situation that a legally incompetent patient shows behavior suggesting objection to participate in the trial, the patient will be not be included in the study. The investigators will adhere to the following code of conduct: 'Verzet bij wilsonbekwame (psycho) geriatrische patiënten in het kader van de Wet Medisch-Wetenschappelijk Onderzoek met Mensen'(http://wetten.overheid.nl/BWBR0009408/2017-03-01).

11.4 Benefits and risks assessment, group relatedness

All patients included in the trial will receive usual care, including indicated interventions. The main complication of thrombolytic therapy for acute ischemic stroke is intracranial hemorrhage. Dual thrombolytic therapy with m-pro-urokinase and a small bolus of alteplase has a significant potential to be safer and more efficacious than alteplase alone. The Executive Committee expects that the potential benefits of dual thrombolytic therapy outweigh the limited risk of harm of the study treatment. We refer to the chapters 6 and 13.1 for more details on these potential benefits and harms.

11.5 Compensation for injury

Each participating center has a liability insurance, which is in accordance with article 7 of the WMO. The sponsor, Erasmus MC, also has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study. The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

All data will be entered into a web-based database (OpenClinica), by local research personnel. Subject records are coded by a unique study number. The local investigators will keep a list showing codes and names. Unique documents with identifying information will be stored separately from the study database in digital files, categorized by study number on a secure drive system, only accessible to the study coordinator.

12.2 Monitoring and Quality Assurance

The level of monitoring meets the standards set by CCMO (Central Committee for Research in Humans) and Erasmus MC. As required, per GCP, the investigator(s)/institution(s) will permit trial-related monitoring, audits, METC review, and regulatory inspection(s), and will provide direct access to source data/documentation to monitor, regulatory agency and DSMB. This trial qualifies as a moderate risk study, i.e. a study with a small risk of serious adverse events compared to standard treatment. This implies that the level of monitoring should be at least as follows:

Monitoring frequency	At least 2-3 visits per center annually
Monitoring of Patient inclusion	Rate of inclusions
Trial Master File/ investigator file	Completeness
Informed consent	In 100% of cases
In and exclusion criteria	in 100% of cases
Source data verification	In 100%, based on a predefined list of variables.
Protocol compliance	in 100% of cases, based on a predefined item list.
SAE and SUSARs	100% SAEs + SUSARs: screening for missed SAEs and verification of procedures.
Study medication	Dosing and completion of infusion in 100% of cases.
Study procedures	Check instructions for personnel
Laboratories and pharmacy	Check GLP/GMP certification
Biological samples (blood)	Check admin, labeling and storage conditions

Source data verification and protocol compliance includes deferred informed consent, NIHSS at baseline and performance of baseline and follow-up imaging (includes the primary endpoint), blood sampling and clinical assessment.

12.3 Amendments

A ‘substantial amendment’ is defined as an amendment to the terms of the METC application, or to the protocol or any other supporting documentation, that is likely to affect to a significant degree:

- the safety or physical or mental integrity of the subjects of the trial;
- the scientific value of the trial;
- the conduct or management of the trial; or

- the quality or safety of any intervention used in the trial.

All substantial amendments will be notified to the METC and to the competent authority. Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The sponsor will notify the accredited METC and the competent authority of the end of the study within a period of 90 days. The end of the study is defined as the last patient's last visit. The sponsor will notify the METC immediately of a temporary hold of the study, including the reason of such an action. In case the study is ended prematurely, the sponsor will notify the accredited METC and the competent authority within 15 days, including the reasons for the premature termination. Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC and the Competent Authority.

12.6 Public disclosure and publication policy

The trial is registered as NTR 7634.

The study database will be closed within one month after the last scheduled follow-up date of the last included patient. A first report of final results will be drafted within 2 months after completion of follow-up of the last patient and presented to the Sponsor, Erasmus MC, who may comment on it but cannot alter its contents or decide on publication. The manuscript will be submitted for publication 3 months after presentation to the Sponsor.

Anonymous data can be requested from the PI with a detailed description containing the aims and methods of the study for which the data are intended to be used. Data will be made available for this purpose at least 18 months after publication of the main report. Data may also be shared with non-commercial parties for scientific purposes, including individual patient meta-analyses, and with commercial parties for FDA approval. Consent will be asked specifically for these purposes.

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

a. Level of knowledge about mechanism of action

The intervention concerns dual thrombolytic therapy (low dose alteplase and m-pro-urokinase) for acute ischemic stroke. M-pro-urokinase is more stable than pro-urokinase and therefore less likely to convert to nonspecific urokinase. M-pro-urokinase targets primarily degraded fibrin, which is why previous administration with alteplase is necessary. Experimental studies with m-pro-urokinase, suggest a higher fibrinolytic effect and confirm that, m-pro-urokinase by itself, in the absence of alteplase in the systemic circulation does not lyse hemostatic fibrin. However, everywhere where alteplase is bound to plasminogen, activation of m-pro-urokinase may occur.

The main risk with alteplase in acute ischemic stroke is hemorrhage. Dual thrombolytic therapy has the potential to be safer, because alteplase will have almost completely disappeared from the systemic circulation within 20 minutes, as alteplase has a plasma half-life of 4-5 minutes),[31] and in the absence of alteplase, mutant pro-urokinase will not be activated. On the other hand, alteplase binds to PAI-1, by which it is de-activated, and to the plasminogen – fibrin complex, where it will promote release of plasmin, which in its turn breaks down fibrin, but also fibrinogen.[32] The half-life of the alteplase-plasminogen complex is not well known, but it is considerably longer than the half-life of alteplase in the systemic circulation.[33]

The exact side effects of dual thrombolytic therapy with low dose alteplase and m-pro-urokinase, as applied in this trial, are unknown but their frequency is expected to be low as described above. Treatment benefit is expected to outweigh the occurrence and severity of this potential side effect. Detailed information is described in the investigator's brochure and the investigational medicinal product dossier.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

See Section 6.3.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

M-pro-urokinase has a high affinity for plasminogen, after plasminogen has undergone a conformational change by binding to fibrin fragment E domains. The fibrin fragment E domains are only present on degraded fibrin. When tissue plasminogen activator binds to (intact) fibrin, it forms a ternary complex with plasminogen and initiates fibrinolysis. This creates new plasminogen binding sites, principally the one of the fibrin fragment E domain. Effective clot lysis with low dose alteplase and m-pro-urokinase has been shown in human plasma in vitro.[24] A study in dogs showed a better clot specific lysis and less bleeding from hemostatic sites compared with tissue plasminogen activator.[23] Moreover, pro-urokinase is well studied and has shown good reperfusion rates in both myocardial infarction and as intra-

arterial treatment in ischemic stroke, despite an increased rate of intracranial hemorrhage.[34-36]

d. Selectivity of the mechanism to target tissue in animals and/or human beings

See Section 13.1c.

e. Analysis of potential effect

See Section 13.1c.

f. Pharmacokinetic considerations

M-pro-urokinase shares the basic physical, biochemical and pharmacokinetic properties as pro-urokinase. However, it is more stable in plasma at higher concentrations than pro-urokinase, due to the mutation which reduces the intrinsic activity. M-pro-urokinase is predominantly cleared by the liver with an half-life of 11-12 minutes. IV administration of m-pro-urokinase at therapeutic dosasges healthy volunteers has been shown safe and does not result in bleeding or fibrinogen depletion (see appendix).

g. Study population

All included patients are suffering from ischemic stroke, which is a life threatening disease. Detailed information is described in Section 4.

h. Interaction with other products

Not applicable.

i. Predictability of effect

Any intracranial hemorrhage will be assessed with MRI (SWI), which is more sensitive for hemorrhage compared with CT. All neuro-imaging will be evaluated by an imaging committee. Also, blood biomarkers of thrombolysis will be determined for safety.

j. Can effects be managed?

No antidotes or antagonists are available, however these are not available for usual treatment with alteplase either. Also, the half-life of both drugs is short, so it is unknown whether an antidote or antagonist would be beneficial for the patient.

If a patient has neurological deterioration based on intracranial hemorrhage, while still receiving the infusion of m-pro-urokinase or alteplase, the infusion will be stopped.

13.2 Synthesis

The only FDA-approved thrombolytic agent for thrombolytic treatment of ischemic stroke, alteplase, has a limited effectiveness and carries a risk of symptomatic intracerebral hemorrhage of 6-7%.[1, 2, 48] There is a need for a better and safer thrombolytic therapy, that expands the number of patients that will be treated safely and successfully. Since dual thrombolytic therapy has a significant potential to be safer and more efficacious than alteplase alone, it is important to assess this thrombolytic therapy.

The dose of m-pro-urokinase will be reduced with 33% and the total duration will be limited to 60 minutes instead of 90 minutes, compared with the PATENT trial which evaluated pro-urokinase in myocardial infarction.[36] Because trials of fibrinolytic treatment that used similar doses of the drug as were used in trials of fibrinolytic treatment of acute

myocardial infarction reported high rates of intracranial hemorrhage, and no beneficial effect of treatment on functional outcome.[41, 42] Also, blood biomarkers of thrombolysis will be measured, including d-dimers and fibrinogen levels.

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15. TABLES

15.1 Table 1: Classification of intracranial hemorrhage according to location, severity and causal relation with neurological deterioration

NINDS	
sICH	Any hemorrhage associated with neurological deterioration, not further defined
ECASS I	
HI 1	Small petechiae along the margins of the infarct
HI 2	Confluent petechiae within the infarcted area, without space-occupying effect
PH 1	A clot not exceeding 30% of the infarcted area with some mild space-occupying effect
PH 2	Represented dense blood clot(s) exceeding 30% of the infarct volume with significant space-occupying effect
sICH	Not defined
ECASS II	
HI 1	Small petechiae along the margins of the infarct
HI 2	Confluent petechiae within the infarcted area, without space-occupying effect
PH 1	A clot not exceeding 30% of the infarcted area with some mild space-occupying effect
PH 2	Represented dense blood clot(s) exceeding 30% of the infarct volume with significant space-occupying effect
sICH	Neurological deterioration of NIHSS ≥ 4 + any hemorrhage on CT
ECASS III	
sICH	Any hemorrhage with neurological deterioration, as indicated by an NIHSS score that was higher by ≥4 points than the value at baseline or the lowest value in the first 7 days or any hemorrhage leading to

	death. In addition, the hemorrhage must have been identified as the predominant cause of the neurological deterioration.
SITS-MOST	
sICH	Local or remote PH2 on 22– to 36-hour post-treatment imaging, combined with a neurological deterioration of ≥4 points on the NIHSS from baseline, from the lowest NIHSS value between baseline and 24 hours, or leading to death.
Heidelberg Bleeding Classification	
1	Hemorrhagic transformation of infarcted brain tissue
1a – HI 1	Scattered small petechiae, no mass effect
1b – HI 2	Confluent petechiae, no mass effect
1c – PH 1	Hematoma within infarcted tissue, occupying <30%, no substantive mass effect
2	Intracerebral hemorrhage within and beyond infarcted brain tissue;
PH 2	Hematoma occupying 30% or more of the infarcted tissue, with obvious mass effect
3	Intracerebral hemorrhage outside the infarcted brain tissue or intracranial-extracerebral hemorrhage
3a	Parenchymal hematoma remote from infarcted brain tissue
3b	Intraventricular hemorrhage
3c	Subarachnoid hemorrhage
3d	Subdural hemorrhage
sICH	Any intracranial hemorrhage followed by a neurological deterioration that can be attributed to that hemorrhage, defined as an increase of ≥4 points on the NIHSS or ≥2 points on a specific NIHSS item.

Glossary: HI, hemorrhagic infarction; PH, parenchymatous hematoma; sICH, symptomatic intracranial hemorrhage

15.2 Table 2: Schedule of al study activities

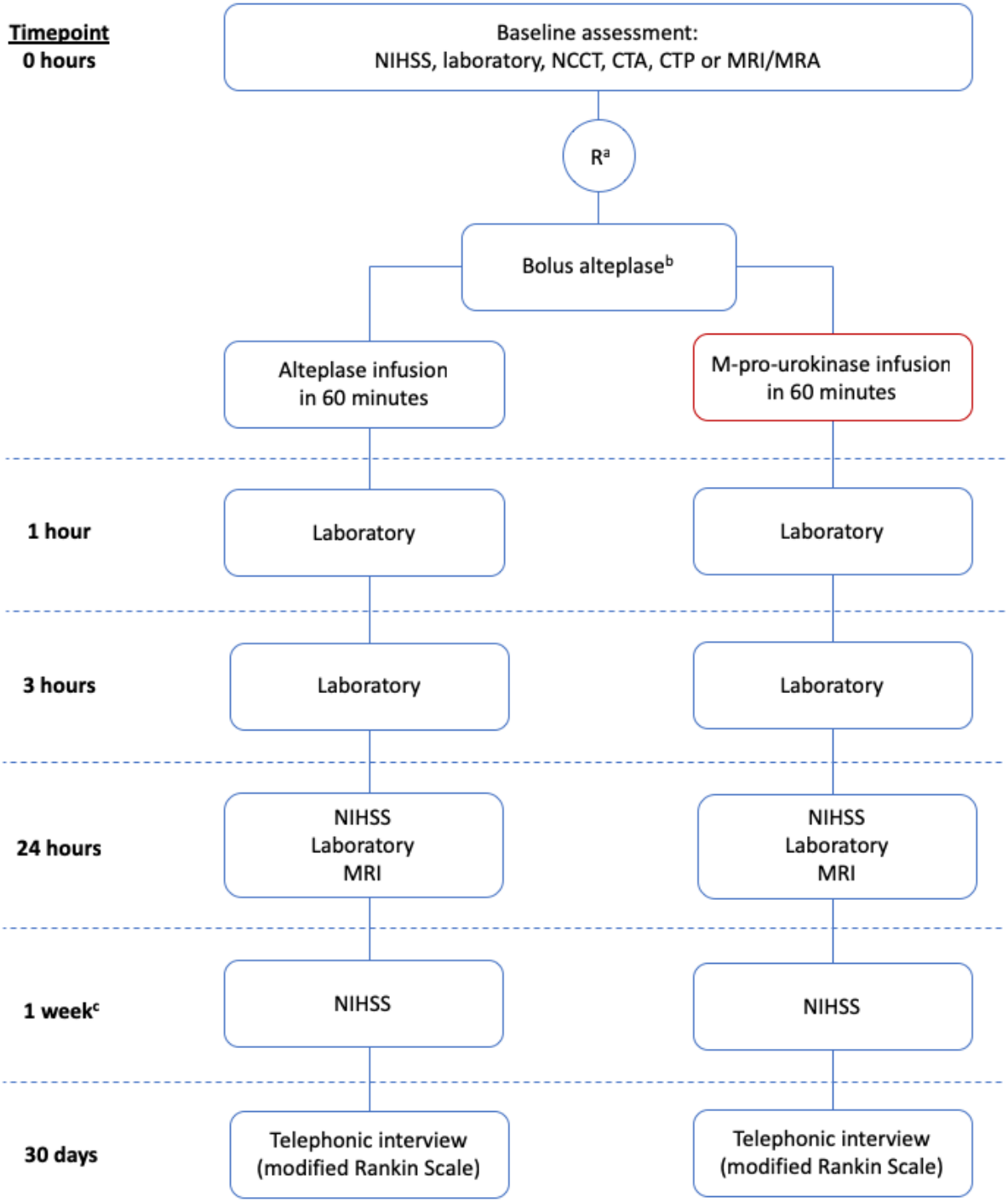
	Baseline	< 1 hour	3 hours	24 hours	Day 5-7*	Day 30
NIHSS	x			x	x	
Laboratory	x	x	x	x		
CT/CTA/CTP or MRI/MRA	x					
MRI				x		
Modified Rankin Scale						x

** or discharge if earlier*

Glossary: CT, computed tomography; CTA, computed tomography angiography; CTP computed tomography perfusion; MRI, magnetic resonance imaging; NIHSS, National Institute of Health Stroke Scale

16. FIGURES

16.1 Figure 1. Patient flow in the trial



[a] Randomization 1:1. DUMAS uses a deferred consent procedure. Written informed consent will be asked as early as deemed appropriate according to the treating physician. If a patient has died before deferred consent has been obtained, his/her representative will be informed about the study. For these patients, there is no opt-out option since that may bias results.
[b] The control group will receive 10% of the total alteplase dose as a bolus. The intervention group will receive a standard bolus of 5 mg alteplase.
[c] Or discharge, if earlier

Glossary: CTA, Computed tomography angiogram; CTP, Computed tomography perfusion; m-pro-urokinase, Mutant pro-urokinase; MRA, Magnetic Resonance Angiogram; MRI, Magnetic Resonance Imaging; NCCT, Non contrast computed tomography; NIHSS, National Institutes of Health Stroke Scale;

17. APPENDICES

17.1 Appendix 1: Distribution of study medication (m-pro-urokinase)

M-pro-urokinase will be distributed by the hospital pharmacy of Erasmus MC. As long as the dose of m-pro-urokinase stays the initial dose of 40mg/hour (means 2 vials per patient), Erasmus MC will distribute each hospital with 25 vials (= 1 carton) of m-pro-urokinase at the start of the trial. If a center has only 6 vials left, they will notify Erasmus MC and another carton with 25 vials will be send. When 150 patients are included, Erasmus MC will only distribute an amount of 10 vials each time.
In this trial, a switch to a second therapeutic regimen is possible, which may affect the number of vails per patient. When switched to a lower dose, the distribution scheme stays the same (still 2 vails per patient needed). When switched to a higher dose (50mg/hour), 3 vials per patient are required. In this case, Erasmus MC will distribute 30 vials to each center, and 12 vials after inclusion of 150 patients. Centers will notify the Erasmus MC when only 9 vials are left.

17.2 Appendix 2: Study personnel

Principal investigators
Diederik Dippel, MD, PhD; neurologist; Erasmus MC Rotterdam
Aad van der Lugt, MD, PhD; neuroradiologist; Erasmus MC Rotterdam

Coordinating investigators
Bob Roozenbeek, MD, PhD; neurologist; Erasmus MC Rotterdam
Nadinda van der Ende, MD; PhD-student; Erasmus MC Rotterdam

Local investigators
Leo Aerden, MD, PhD, Reinier de Graaf, Delft
Ido R van den Wijngaard, Haaglanden MC, The Hague
Heleen den Hertog, Isala, Zwolle

17.1 Appendix 3: Study organization and study committees

Steering Committee
The Steering Committee, consisting of the Principal Investigator and the Local Principal Investigators of each study center, and one independent expert in acute thrombolytic therapy for ischemic stroke (professor Gregory del Zoppo, Seattle, University Washington), will be responsible for the overall supervision of the trial. Additionally, the steering committee will discuss all patients about whom doubt exists concerning the discharge diagnosis of ischemic stroke or not (i.e. stroke mimic). Every Steering Committee member can propose cases for discussion. This concerns at least all patients without a DWI lesion on follow-up MRI. The Steering Committee will be chaired by the central PI.

Executive Committee and staff
The Executive committee keeps track of trial progress and makes the strategic decisions on a weekly basis. The Executive committee consists of the central PIs (neurologist), a neuroradiologist, the study coordinator (postdoc) and an MD/PhD student. The central PI will act as overall supervisor. The study coordinator will supervise day to day conduct of the trial. An MD (PhD student) will take care of all contacts with participating centers, write reports and check incoming data. The Executive committee will report to the Steering committee at least on a 3-montly basis. They will be supported by experienced administrative staff. The participating centers will be reimbursed for employment of part-time trial staff.

Writing Committee
The Writing committee consists of the Executive committee and local PIs. The task of the Writing committee is to prepare the main publication which will be drafted by the study coordinators, supervised by the two central PIs. Typically, the main paper will be authored by the study coordinators (first), the local PIs, the committee members, and the central PIs.

Neuroimaging Central Reading Committee
All CT and MRI scans will be assessed by a Neuroimaging Central Reading Committee that is blinded to treatment allocation and other clinical information, except expected lesion side.

Adverse event Committee
The Adverse event committee consists of at least 3 members, including a neurologist. Their task is to oversee the review and reporting process of all reported serious adverse events. The committee will regularly report to the Steering committee.

Data Safety and Monitoring Board
A Data Safety and Monitoring Committee (DSMB), consisting of a neurologist, hematologist and neuroradiologist, will advise the chairman of the Steering Committee on the basis of unmasked reports about continuation of the trial at intervals proposed above.
Members:
Michael Hill, MD, neurologist, chair of the DSMB
Ann Lowe, MD, hematologist
Jeremy Rempel, MD, neuroradiologist

Independent statistician
Daan Nieboer, PhD (Erasmus MC)

Independent statistician for Bayesian adaptive analysis team
William Meurer MD and Scott Berry, PhD

Advisory Board
The Advisory Board consists of experts in the field of thrombosis, hemostasis and thrombolytics. The Advisory Board will provide non-binding strategic advice to one member

of the Steering Committee (e.g., Prof. dr. Gregory del Zoppo). Members: Dr. Dick Rijken, Prof. dr. Victor Gurewich, Prof. dr. Koos Burggraaf, and Prof. dr. Adam Cohen.

Trial statistician and methodologist
Hester Lingsma, PhD (Erasmus MC)

17.2 Appendix 4: Core data set

Inclusion check list
A clinical diagnosis of ischemic stroke
A score of at least 1 on the NIH Stroke Scale
CT ruling out intracranial hemorrhage
Treatment possible within 4.5 hours from symptom onset or last seen well
Meet the criteria for standard treatment with IV alteplase according to national guidelines
Age of 18 years or older
Written informed consent (deferred)

Baseline characteristics	
Demographics	Age, sex
Clinical	NIHSS, pre-stroke mRS, systolic and diastolic blood pressure, Glascow coma scale, weight, height, body temperature, heart rate
Medical history and intoxications	Previous stroke, myocardial infarction, hypertension, hypercholesterolemia, peripheral arterial disease, diabetes mellitus, atrial fibrillation, chronic heart failure, intra-cranial hemorrhage, smoking (current or stopped within 6 months), mechanical aortic and/or mitral valve replacement
Medication	Antiplatelet agents (and if yes, subtypes: acetylsalicylic acid, clopidogrel, dipyridamole, ticagrelor, other), coumarines, direct

	oral anticoagulansts (DOAC), therapeutic heparin(oids), statins, NSAIDs
Laboratory	INR, serum creatinine, GFR (Cockroft-Gault), serum glucose, C-Reactive Protein, triglycerides, cholesterol status, HbA1c, thrombocyte count, fibrinogen, plasminogen, alpha2-antiplasmine, d-dimer, when available APTT, DTT, anti-Xa
Neuro-imaging*	CT-brain: severity of ischemia with ASPECTS CT-angiography: status extracranial carotid artery CT-perfusion: infarct core, ischemic penumbra

*Neuro-imaging parameters will be assessed by a central subcommittee

Intravenous treatment	
General information	Date of IVT
Time registration	Time of start IVT
Pre-treatment	Final systolic and diastolic blood pressure before bolus alteplase
Blood pressure	Delay in IVT due to hypertension, medication given to lower blood pressure (if yes, which and how much, if no, why explain why not)

Workflow	
Pre-hospital	Time of symptom onset, if no: time of last seen well and time of symptoms noticed
In-hospital	Time of arrival at hospital, time of NCCT, time of randomization

Follow-up	
Laboratory within 1 hour	Fibrinogen, plasminogen, alpha2-antiplasmine, d-dimer

Laboratory at 3 hours	Fibrinogen, plasminogen, alpha2-antiplasmine, d-dimer
Clinical assessment at 24 hours	NIH Stroke Scale
Laboratory at 24 hours	Fibrinogen, plasminogen, alpha2-antiplasmine, d-dimer
Neuro-imaging with MRI at 24-48 hours	Infarct size and location, hemorrhagic transformation (Heidelberg Bleeding Classification)
Clinical assessment at 5-7 days or discharge	NIH Stroke Scale
Clinical assessment at 30 days (-7 days or +14 days) via telephone interview	Modified Rankin Scale score
(Serious) adverse events (at any given time)	Name investigator; date of report; date of (S)AE onset; description of (S)AE; SAE category: an adverse event is considered serious when it: causes mortality, is life-threatening, results in required or prolonged hospitalization, results in risk of persistent or significant disability or incapacity, results in medical or surgical intervention; Most likely cause for (S)AE and other causes: 1. Stroke progression 2. New ischemic stroke 3. Intracranial hemorrhage 4. Extracranial hemorrhage 5. Cardiac ischemia 6. Allergic reaction 7. Pneumonia 8. Other infection and description 9. Other cause for (S)AE and description;

	Relationship with the study treatment: none, unlikely, possible, probable, definite; Actions regarding the study treatment: none, unlikely, possible, probable, definite; Outcome and date: resolved without sequela(e); resolved with sequela(e) and description, death

17.3 Appendix 5: Imaging requirements

- Minimum baseline requirements

  WHEN

  1. Before randomization a NCCT, CTA and CTP or MRI/MRA should be performed to assess eligibility of the study.

  HOW

  1. Pre-randomization NCCT:
     - I. The NCCT should contain both thick (5mm) and thin slices (maximum of 2.5mm).
     - II. The NCCT should include the whole head.
  2. Pre-randomization CTA:
     - I. The CTA should cover the area from the aortic arch to the vertex.
     - II. The CTA should include thin slices (maximum of 1.0mm, overlap 50%).
     - III. The CTA should include the following reconstructions:
       1. Axial maximum intensity projection (MIP):
          a. MIP slab thickness: 25mm
          b. Overlap: 5mm
       2. Coronal MIP:
          a. MIP slab thickness 25mm
          b. Overlap: 5mm
  3. Pre-randomization CTP:
     - I. The CT-perfusion should be focused on the anterior circulation or posterior circulation depending on the suspected location of the ischemic stroke as determined by the neurology assistant or neurologist
  4. Pre-randomization MRI/MRA:
     - I. The study should include the following sequences
       1. Axial DWI and ADC maps
       2. Axial FLAIR
       3. Axial T2*
       4. 3D TOF
       5. Contrast Enhanced MRA (CEMRA)
     - II. The MRI study should cover the whole head (i-iv)
     - III. The CEMRA study should cover the whole area from the aortic arch to the vertex (v)
  5. After acquisition:
     - I. All images (NCCT, CTA and CTP or MRI/MRA) should be saved to the DICOM format.
     - II. All available series should be sent to the core lab for assessment.
- Minimum follow-up requirements

  WHEN

1. 24 hours after intravenous treatment a MRI/MRA (24-48 h) should be performed to assess any intracranial hemorrhage (primary outcome).
2. If clinically required (i.e. in case of clinical deterioration of the patient) additional imaging as needed, at the discretion of the treating physician is acquired.

HOW

1. 24(-48) hours MRI:
    I. The MRI study should cover the whole brain.
    II. MRI study should include thin slices (maximum of 1.0 mm).
    III. The MRI study should include the following sequences:
        1. SWI (susceptibility weighted imaging)
        2. DWI and ADC maps
        3. T2w-TSE (turbo spin echo, also known as fast spin echo (FSE))
        4. 3D-T2w-FLAIR
        5. DSC-PW MRI (dynamic susceptibility contrast perfusion weighted)
        6. 3D-T1w with and without gadolinium
2. Additional, clinically required imaging:
    I. At the discretion of the treating physician

3. After acquisition:
    I. All images (MRI, MRA and additional imaging) should be saved to the DICOM format
    II. All available series should be sent to the core lab for assessment

17.4 Appendix 6: Adaptive design – Design and Simulation Report

17.4.1 Introduction

Background

This document describes the features of the simulated design, including the statistical models, decision rules, and simulation scenarios as input into the FACTS (Fixed and Adaptive Clinical Trial Simulator) software. A small set of operating characteristics for the simulations is also summarized. The goal of this design is to provide a set of prospectively defined, quantitative decision rules to guide interim analyses in the DUMAS trial. In this design, the DUMAS trial can either proceed to the maximum sample size of 200 without any changes, or it can transition to a lower or higher dose of the investigational drug at an interim analysis.

Endpoints

The primary endpoint is freedom from any intracranial hemorrhage (NoICH) after stroke thrombolysis (dichotomous) and is measured within 24 (to 48) hours. The secondary endpoint is clinical improvement within 24 hours (Clin), also dichotomous. A positive outcome is indicated by a value of 1, and a negative outcome (presence of ICH or failure to clinically improve) is indicated by a value of 0. Clinical improvement is defined as improvement of at least 4 points on the National Institute of Health Stroke Scale (NIHSS) at 24 hours compared to baseline, or (near) complete recovery (NIHSS 0 or 1).

Treatment Arms

The trial will enroll up to a maximum of 200 subjects with a discharge diagnosis of ischemic stroke, randomized among 2 arms, including a control arm. We have 1 treatment arm which we label generically by their arm index as: $d = 0$ (control – standard alteplase dosing), 1 (treatment – investigational thrombolytic regimen – also known as mutant pro-urokinase (mproUK; HisproUK).

17.4.2 Statistical Modeling

This section describes the statistical modeling used in the design. The modeling is Bayesian in nature.

Final Endpoint Model

The following models are fit separately for the primary and secondary endpoint.
Let Y_i be the primary outcome measured at 24 hours for the i^{th} subject. We model the outcomes as

$$Y_i \sim \text{Bernoulli}(P_{d_i})$$

where P_d is the underlying response rate for arm d. We transform the response rates onto the log-odds scale to allow modeling on a continuous scale:

$$\theta_d = \log\left(\frac{P_d}{1 - P_d}\right).$$

The mean response is modeled independently for each dose as:

$$\theta_0 \sim N(0, 2^2),$$

$$\theta_1 \sim N(0, 2^2).$$

Thus, θ_d for each dose is estimated separately using only data from that dose.

Evaluation of Posterior Estimates
Posterior estimates are independently calculated for each endpoint.

The Bayesian final endpoint model is fitted to the data at each update. The posterior is calculated as:

$$p(\omega|Y) \propto \prod_{i=1}^{n} p\,(y_i|\varphi)p(\varphi)$$

where φ is the set of parameters for the final endpoint model, $p(\varphi)$ is the prior for those parameters, y_i is the final response for each subject, and n is the number of subjects. The posterior is evaluated using MCMC with individual parameters updated by Metropolis Hastings (or Gibbs sampling where possible), using only the y_i data available at the time of the update.

Quantities of Interest
We define a number of quantities that will be tracked and may be used to make decisions during the trial.

Posterior Probabilities
For each dose, we calculate the following quantities from the posterior:

- For the primary endpoint (NoICH), the probability that the mean response on dose d is greater than on control by at least 0.05:
$$Pr(\theta_d - \theta_0 > 0.05)$$

- For the secondary endpoint (Clin), the probability that the mean response on dose d is greater than on control by at least 0.1:
$$Pr(\theta_d - \theta_0 > 0.1)$$

Decision Quantities
Throughout the trial, decisions may be based on the following quantities:

- NoICH endpoint $Pr(\theta_d - \theta_0 > 0.05)$for $dose = \text{mproUK}$
- Clin endpoint: $Pr(\theta_d - \theta_0 > 0.1)$for $dose = \text{mproUK}$

Conventions for Missing Data
At any analysis, some subjects may have missing data for the final endpoint. The missing data could result from the subject dropping out of the study, or because the subject simply has not yet reached the final visit.

If the subject has not yet reached the final visit, the endpoint value is imputed from the estimate of the response for the subjects treatment arm (effectively contributing no information to the update of that estimate).

For any subject whose final endpoint is unknown due to drop out, the final outcome will be multiply imputed from the Bayesian model.

17.4.3 Study Design

Timing of Interim Analyses for dose adaptation

The first interim will occur after 60 subjects with a discharge diagnosis of ischemic stroke have data up to 48 hours. Subsequent interims will be conducted after inclusion of every 20 patients with a discharge diagnosis of ischemic stroke and will continue until full accrual. Since interims are defined by calendar time, the total number of planned interims, I, is random and will depend on the rate at which subjects accrue to the trial. Note that in the initial phase of the trial, mixed quantitative-qualitative review for safety will be carried out by the DSMB, after inclusion of every 10 patients.

Allocation

The trial will enroll 200 subjects with a discharge diagnosis of ischemic stroke that will be randomized to the treatment arms in a fixed ratio. Randomization will occur in blocks of variable sizes.

Criteria for Changing Dose

Changing to a lower dose

For interim 1-I, the trial may transition to a lower dose if BOTH of the following criteria are satisfied:

- $NoICH\ endpoint$: $\Pr(\theta_d - \theta_0 > 0.05) < 0.5$ for $d = \text{mproUK}$
- $Clin\ endpoint$: $\Pr(\theta_d - \theta_0 > 0.1) > 0.5$ for $d = \text{mproUK}$

Changing to a higher dose

For interim 1-I, the trial may transition to a higher dose if all of the following criteria are satisfied:

- $NoICH\ endpoint\ Pr(\theta_d - \theta_0 > 0.05) > 0.5$ for $d = \text{mproUK}$
- $Clin\ endpoint\ Pr(\theta_d - \theta_0 > 0.1) < 0.5$ for $d = \text{mproUK}$

Note that, as per protocol, the results of the interim analysis will be presented to the DSMB, who will advise the chair of the Steering Committee.

Final Evaluation Criteria

At the final analysis, the trial will be considered successful based on the primary endpoint analysis defined in the statistical analysis plan and in the main clinical protocol.

17.4.4 Simulation Scenarios

We evaluate the proposed design through trial simulation. We hypothesize several possible underlying truths for the mean response, as well as for trial execution variables such as accrual and dropout. For each of these scenarios, we generate data according to those truths and run through the design as specified above. We repeat this process to create multiple “virtual trials” and we track the behavior of each trial. In this section, we describe the parameters used to generate the virtual subject-level data. Simulations provided below

provide what happens until either the trial reaches the maximum sample size without triggering a dose adjustment OR whether a dose change rule is triggered. For example, if a dose increase is recommended at 120 patients, the last 80 patients would be randomized 1:1 to the new dose versus control.

Virtual Subject Response Profiles

We consider 7 profiles for which subject outcomes for the final endpoints are simulated to have response rates as shown in Table 1.

Table 1: Virtual subject response rates

Scenario	**NoICH**		**Clin**	
	Control	mproUK	Control	mproUK
BetterBetter	0.8	0.93	0.4	0.6
ICHbetterClinNull	0.8	0.93	0.4	0.4
ICHNullClinBetter	0.8	0.93	0.4	0.6
NullNull	0.8	0.8	0.4	0.4
ICH5betterClinNull	0.8	0.85	0.4	0.4
ICHnullClin10better	0.8	0.8	0.4	0.5
ICH5betterClin10better	0.8	0.85	0.4	0.5

Accrual Profiles

We assume two patients per week for just under 2 years. We simulate the random arrival of subjects into the trial from a Poisson process with the mean weekly rates specified in Table 2. Within each accrual profile, there may be differential recruitment rates over time and across regions. Currently, we simulated only one region for recruitment. Thus, for each region, we specify:

- the mean number of subjects per week at peak accrual,
- the start date (in weeks from the start of the trial),
- whether the region will have a ramp up phase, and if so, when the ramp up will be complete, and
- whether the region will have a ramp down phase, and if so, when the ramp down will begin and when it will be complete. Ramp up and ramp down define simple linear increases and decreases in the mean recruitment rate from the start to the end of the ramp. Thus some simulated trials recruit more quickly than this and some more slowly.

Table 2: Accrual Profiles

Profile Name	Region Index	Peak Rate	Start Week	Ramp Up	Ramp up Complete	Ramp Down	Start Ramp Down	Ramp Down Complete
Acc 1	1	2	0	NA	NA	NA	NA	NA

Dropout Profiles

We assume no dropouts for the purpose of this simulation.

17.4.5 Operating Characteristics

For the scenarios described above, we simulate multiple virtual trials and track the behavior of each trial, including the preliminary or final outcome of the trial, the estimated mean response, etc. In this study, the trial will continue with a new dose replacing the initial dose in the event a decision rule is triggered. The results in this section are summarized across all simulated trials for each scenario.

Overall

This section gives a high-level description of the operating characteristics. Table 3 shows the following information per scenario:

- N sim: the number of simulated trials
- E[N]: the expected sample size at the time a dose adaptation is recommended
- Pr(max): the proportion of trials that enroll fully without any interim analysis recommending a dose change
- E[duration]: the expected time until the first dose adaptation trial in weeks.

Table 3:Operating Characteristics Up To First Dose Adaptation

Accrual	Dropout	VSR	N sim	E[N]	Pr(Max)	E[duration]
Acc1	Drop1	BetterBetter	10000	125	0.42	63
Acc1	Drop1	ICHbetterClinNull	10000	68	0.02	35
Acc1	Drop1	ICHNullClinBetter	10000	73	0.04	36
Acc1	Drop1	NullNull	10000	75	0.07	38
Acc1	Drop1	ICH5betterClinNull	10000	71	0.03	36
Acc1	Drop1	ICHnullClin10better	10000	68	0.02	34
Acc1	Drop1	ICH5betterClin10better	10000	74	0.05	37

Trial Outcomes

This section summarizes the outcomes of the simulated trials. For each scenario in Table 4, the columns represent the proportion of simulated trials meeting each of the following definitions:

- Early Dose Increase (EDI): recommended increase in dose at interim analysis
- Early Dose Decrease (EDD): recommended decrease in dose at interim analysis

Table 4: Trial Outcomes Up To First Dose Adaptation

Accrual	Dropout	VSR	EDI	EDD
Acc1	Drop1	BetterBetter	0.30	0.28
Acc1	Drop1	ICHbetterClinNull	0.82	0.16
Acc1	Drop1	ICHNullClinBetter	0.09	0.86
Acc1	Drop1	NullNull	0.32	0.60
Acc1	Drop1	ICH5betterClinNull	0.51	0.45
Acc1	Drop1	ICHnullClin10better	0.21	0.76
Acc1	Drop1	ICH5betterClin10better	0.35	0.59

17.4.6 Computational Details

This report reflects the design parameters contained within the TSIdualendpointDec3.facts file. The simulations were run using FACTS (Berry Consultants, LLC, Austin, TX) version 6.2.4. The R software package was used to summarize the simulation output and to create tables for this report.