DUMAS - Statistical Analysis Plan

Nadinda van der Ende, Bob Roozenbeek, Aad van der Lugt, Diederik Dippel, on behalf of the DUMAS investigators.

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Introduction

This document summarizes the statistical analysis of the DUMAS (DUal thrombolytic therapy with Mutant pro-urokinase and low dose Alteplase for ischemic Stroke) trial. We will describe how missing data will be handled, the methodology to assure adequate blinding, and the statistical procedures to estimate the effect of this dual thrombolytic treatment. Additionally, we predefined the most important subgroup analyses. Please note that, it is possible that not all pre-specified analyses listed in this statistical analysis plan will be included in the publication on the main outcomes of the trial, due to word count restrictions. Those analyses will be made available in subsequent publications or online. This document should be read as an adjunct to the study protocol, which can be found on the DUMAS website (https://dumas-trial.nl/trial-protocols-and-documents.html).

Aim of DUMAS

The aim of DUMAS is to assess the safety and efficacy of dual thrombolytic treatment consisting of a small bolus of alteplase followed by mutant pro-urokinase (m-proUK) against usual treatment with alteplase in patients presenting with ischemic stroke.

Trial design

DUMAS (NCT04256473) is a multicenter, phase II trial with prospective randomized open-label blinded end-point (PROBE) design, and adaptive design for dose optimization. Patients are randomly assigned (1:1) to receive a bolus of IV alteplase (5mg) followed by continuous IV infusion of m-proUK (40mg/hr during 60 minutes) or usual care with alteplase (0.9mg/kg).

During the study, the m-pro-urokinase dosage may be revised, randomization will remain the same. 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.

Study population

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

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 is possible
    - within 4.5 hours from symptom onset or last seen well, or

    - between 4.5 to 12 hours from symptom onset or last seen well, and
        - the infarct core is less than 25 mL **and** a penumbra is at least the same size as the infarct core (i.e. total ischemic volume/infarct core mismatch ≥ 2.0),[1]
        - or in case of lacunar syndrome,[2] if there is a diffusion-weighted imaging and FLAIR mismatch[3];
- The criteria for standard treatment with IV alteplase according to national guidelines[4] are met;
- Patient age is 18 years or older;
- Patient or legal representative has provided written informed consent (deferred).

Exclusion criteria

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

- The subject is eligible for endovascular thrombectomy (i.e. has a proximal intracranial large artery occlusion on CTA or MRA);
- Contra-indication for treatment with IV alteplase according to national guidelines[4]:
    - 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

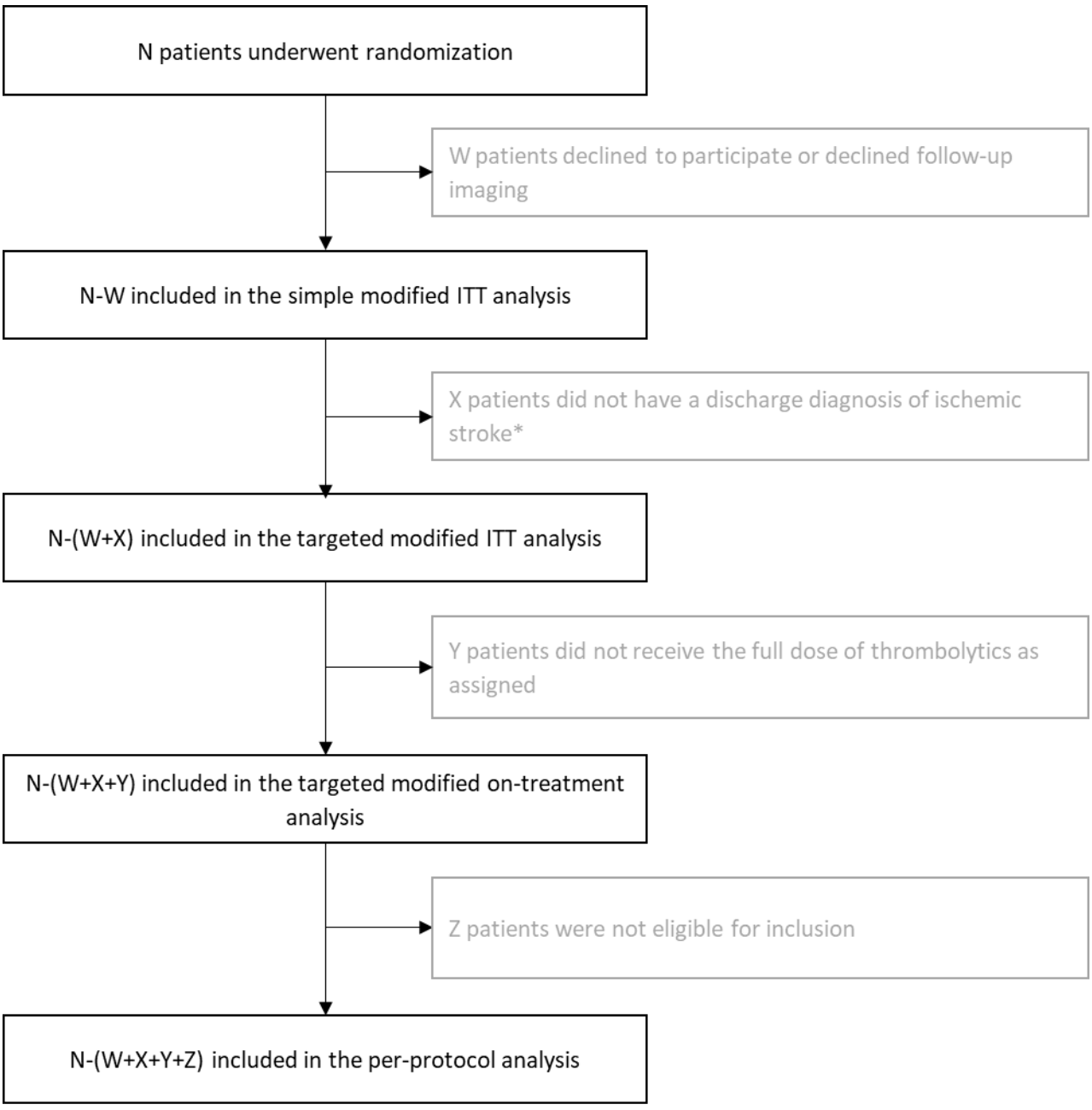
    - Major surgery or serious trauma in the previous 2 weeks
    - Gastrointestinal or urinary tract hemorrhage in the previous 2 weeks
    - Previous intracerebral hemorrhage
    - Use of anticoagulant with INR exceeding 1.7 or APTT exceeding 50 seconds
    - Known thrombocyte count less than 90 x 109/L. 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., 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 not confirmed to be MRI compatible
    - 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[5]/ARTEMIS.[6]

Sample size

We will include 200 patients with a discharge diagnosis of ischemic stroke randomized 1:1 to either standard thrombolytic treatment or dual thrombolytic treatment. We assume that the primary outcome, any ICH, will occur with a probability of 20%[7] with standard thrombolytic treatment and a probability of 7% in the patients treated with dual thrombolytic therapy, for an overall effect (OR) of 0.3. This sample size will provide us with a power of at least 77% to detect a statistically significant effect on the primary outcome. This estimate does not take into account the use of multivariable adjustment for differences in baseline characteristics in the primary analysis, which will increase the power by 10-25%. To compensate for inclusion of patients with a discharge diagnosis other than ischemic stroke (e.g. stroke mimic) and patients who did not receive the full dose of thrombolytics as assigned, we will include additional patients on a 1 to 1 basis. This implies that we expect that the intention to treat population will be larger. We estimate that 20% of the included patients will not have a diagnosis of ischemic stroke at discharge.

Figure 1: selection of patients into the simple modified intention-to-treat, targeted modified intention-to-treat, and targeted modified on-treatment groups, as well as per protocol groups.



* Based on discharge diagnosis or as discussed in the Steering committee

Study treatment

The intervention arm will receive a bolus of IV alteplase 5 mg, which will be followed by a continuous infusion of m-proUK, either 40 mg in 60 minutes (initial dose) or an alternate dose. Depending on the result of interim analyses, the m-proUK dosage may be revised to:

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

A detailed description of this adaptive design for dose optimization can be found in Appendix 6 of the DUMAS research protocol on the website (https://dumas-trial.nl/trial-protocols-and-documents.html).

The control arm will receive standard treatment with IV alteplase alone in a dose of 0.9 mg/kg (10% bolus + 90% infusion in 60 minutes), maximum dose 90 mg.

Outcomes

Primary outcome

The primary outcome is any post-intervention intracerebral hemorrhage/hematoma confirmed by neuroimaging according to the Heidelberg Bleeding Classification at 24 hours (range: 12 to 48 hours) of study drug administration preferably by MRI (SWI).[8]

Secondary outcome

Secondary clinical outcomes

- Score on the National Institute of Health Stroke Scale (NIHSS) assessed at 24 hours (range: 12 to 48 hours) and at 5-7 days post-treatment, or discharge if earlier.[9]

- Improvement of at least 4 points on NIHSS at 24 hours (range: 12 to 48 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.[10]
- 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 hours (range: 12 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 hours (range 12 to -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 within the follow-up period defined by the last follow-up contact at 30 days.[8]
- Death from any cause including intracranial hemorrhage within the follow-up period defined by the last follow-up contact at 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.[11]

Blinding

DUMAS has a PROBE design, which means both patient and treating physician will be aware of the treatment assignment. All imaging will be assessed in a blinded manner by an independent imaging committee. Members of the imaging committee will be blinded to all clinical information, except for clinical symptoms at baseline. Clinical symptoms will be defined as side of hemiparesis, presence of aphasia, or non-localizing symptoms for patients without hemiparesis or aphasia. Assessment of the mRS at 30 days will be assessed in a telephone interview through standardized forms and procedures from a central location, by a trained investigator unaware of treatment allocation. Information on treatment allocation will be kept separate from the outcome database. The steering committee will be kept unaware of the results of interim analyses of efficacy and safety. The independent trial statistician will combine clinical data with the outcome data in order to report to the data safety monitoring board (DSMB).

Missing data and death

Baseline data by treatment allocation will be reported with standard statistical procedures. Missing values will be reported. For descriptive analyses, only the crude, non-imputed data will be presented. For the regression analyses, missing values, except the primary outcome, will be imputed using multiple imputation (n=5). For patients who died within the study period we will assign the worst score for all unassessed clinical outcome measures and use those for analyses.

Statistical analysis

We will perform and report 4 analyses, of which the first is the main and primary, and will be reported as such:

1. Simple modified intention-to-treat analysis to assess overall safety and efficacy. This is a modified intention-to-treat analysis because we exclude patients who did not give consent to participate in the study. We will additionally report safety parameters based on the full cohort, including patients who did not give consent.
2. Targeted modified intention-to-treat analysis excluding patients with a discharge diagnosis other than ischemic stroke to assess safety and efficacy in the target population.
3. Targeted modified on-treatment analysis to assess the safety and efficacy in patients who actually received the treatment excluding patients with a discharge other than ischemic stroke.
4. Per-protocol analysis.

See also Figure 1 for selection of patients into simple modified intention-to-treat, targeted modified intention-to-treat, and targeted modified on-treatment groups, as well as per protocol groups.

Primary effect analysis

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 (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), lacunar syndrome (yes/no),[2] systolic blood pressure, pre-study antiplatelet treatment and indication for endovascular treatment (yes/no). Whether the dosing (initial vs. modified) 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.

Secondary, tertiary and safety analyses

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). Whether the dosing (initial vs. modified) of the study treatment modifies the treatment effect, will be analyzed with a multiplicative interaction parameter.

Subgroup analyses

The effect of intervention in subgroups will be analyzed on any ICH (i.e., primary outcome) and the NIHSS (i.e., efficacy outcome). We will perform subgroup analyses, by age, sex, systolic blood pressure, ASPECTS, time from onset to study treatment, NIHSS score, extracranial carotid or vertebral arterial occlusion, pre-study antiplatelet treatment, DWI lesion (yes/no), and lacunar syndrome (yes/no)0. In case of a change in the dose of m-proUK, we will also perform subgroup analysis in patients with the initial dose and patients with the alternate dose.

Time path of the analysis and locking of the database

After the follow-up of the final patient, the last records of the database will be cleaned and checked for completeness within one month. Data will be checked by the research coordinator and by an independent monitor according to the monitoring plan. Upon completion, the database will be locked. The final analysis will be done by the two study coordinators, and reported to the independent statistician who will do a third check for consistency and adherence to the SAP. The final results will then be shared for consideration with the Trial Steering Committee. Within 4 months after obtaining the final results, a manuscript describing the main results of the trial will be submitted for publication. The syntax and output will be made available upon request.

Status of the trial

As of this writing, a total of 4 centers have been initiated in the Netherlands, and a total of 195 patients have been included in DUMAS. No dose adaptations have occurred as advised by the DSMB until now. The database will be locked ultimately 3 months after the last patient has been included.

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