Safety and efficacy of dual thrombolytic therapy with mutant pro-urokinase and small bolus alteplase for ischemic stroke (DUMAS): a randomized clinical trial

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Key Points

Question: Is dual thrombolytic treatment with small bolus alteplase and mutant pro-urokinase safer than treatment with alteplase alone in patients with ischemic stroke.

Findings: In this randomized phase 2 clinical trial that included 238 participants with ischemic stroke who provided deferred consent, an ICH occurred in 16/121 (13.2%) patients in the intervention group and 16/117 (13.7%) patients in the control group (adjusted OR: 0.98 [95% CI:0.46-2.12]). Mutant pro-urokinase led to a non-significant shift towards better modified Rankin Scale scores at 30 days (adjusted cOR: 1.16 [95% CI:0.74-1.84]).

Meaning: In patients with minor ischemic stroke who were not eligible for endovascular therapy, thrombolytic treatment with a small bolus alteplase and m-proUK could not be proven safer than treatment with alteplase alone.

Abstract

Importance: Dual thrombolytic treatment with small bolus alteplase and mutant pro-urokinase (m-proUK) has the potential to be a safer and more efficacious treatment for ischemic stroke than alteplase alone, because m-proUK is designed to act only on degraded fibrin, without affecting circulating fibrinogen.

Objective: To assess the safety and efficacy of this dual thrombolytic treatment compared with alteplase.

Design: This randomized controlled open-label trial with blinded end-point was conducted from August 2019 to May 2022 with a total follow-up of 30 days.

Setting: Patients were included from four hospital in the Netherlands.

Participants: We enrolled adult patients with ischemic stroke, eligible for treatment with intravenous alteplase. Patients eligible for endovascular therapy were excluded.

Interventions: Randomly assignment (1:1) to receive a bolus of 5mg intravenous alteplase and 40mg intravenous infusion of m-proUK (intervention) or usual care with 0.9mg/kg intravenous alteplase (control).

Main outcomes and measures: The primary outcome was any intracranial hemorrhage (ICH) on neuroimaging at 24 hours. Secondary outcomes included functional outcome at 30 days, symptomatic ICH, and fibrinogen levels within 24 hours. Analyses were by intention to treat. Treatment effects were adjusted for baseline prognostic factors.

Results: Between August, 2019, and April, 2022, we randomized 268 patients; 238 provided deferred consent and were included in the intention-to-treat population (n=121 intervention, n=117 control). Median age was 69 years (IQR 59-77) and median baseline score on the NIHSS was 3 (IQR 2-5). Any ICH occurred in 16/121 (13.2%) patients in the intervention group and 16/117 (13.7%) patients in the control group (adjusted OR: 0.98 [95% CI: 0.46-2.12]). M-proUK led to a non-significant shift towards better modified Rankin Scale scores (adjusted cOR: 1.16 [95% CI:0.74-1.84]). sICH occurred in 0/121 (0%) and 3/117 (2.6%) of patients in

the intervention and control group, respectively. Plasma fibrinogen levels at 1 hour remained constant in the intervention group, but decreased in the control group (difference [β]: 0.65 g/L [95% CI :0.26-1.05]).

Conclusion and relevance: Dual thrombolytic treatment with a small bolus alteplase and m-proUK is safe and does not result in fibrinogen depletion. Further evaluation of thrombolytic treatment with m-proUK in larger trials to improve outcome in patients with larger ischemic strokes is needed. Overall, in patients with minor ischemic stroke meeting indications for treatment with intravenous thrombolytics, but who are not eligible for treatment with EVT, we could not prove superiority of dual thrombolytic therapy with IV m-proUK is over treatment with IV alteplase alone.

Trial registration: NL7409 (November 26, 2018) / NCT04256473 (February 5, 2020)

Background

Thrombolytic treatment with recombinant tissue plasminogen activator alteplase in patients with ischemic stroke leads to improved reperfusion and increases the likelihood of good clinical outcome.[1] Apart from its limited efficacy, it carries a risk of symptomatic intracranial hemorrhage (ICH) of 6-7%.[2]

Treatment with endovascular therapy (EVT) is effective in patients with ischemic stroke due to a large-vessel occlusion in the anterior circulation,[3] which is present in around 10-15% of ischemic stroke patients presenting at the emergency department.[4] For patients with ischemic stroke without a large-vessel occlusion, thrombolytic treatment is the only available reperfusion therapy.[5] Yet, there is a need for a better and safer thrombolytic treatment, which expands the number of patients that can be treated safely and successfully.

Tenecteplase might be a promising alternative, as it is a genetically modified variant of alteplase, engineered to improve efficacy through greater fibrin specificity and longer plasma half-life. Recently, non-inferiority has been demonstrated, but not superiority.[6,7] Also, the rates of ICH in patients treated with tenecteplase and alteplase are similar.[6,7]

Preclinical and clinical studies have indicated that dual thrombolytic therapy with a small bolus of alteplase followed by a mutant pro-urokinase (m-proUK) has a noteworthy potential to be safer and more efficacious than standard dose alteplase alone (0.9 mg/kg).[8-10] Intra-arterial treatment with pro-urokinase in patients with ischemic stroke resulted in better reperfusion and more patients with favorable outcome than controls, despite an increased rate of intracerebral hemorrhage.[11,12] M-proUK is a single-point mutation of pro-urokinase, that makes it less susceptible to non-specific activation into its enzymatic, two-chain form, urokinase.[10] M-proUK by itself does not lyse hemostatic fibrin, only partially degraded fibrin.[9,13] M-proUK is designed to specifically continue intravascular clot lysis, and spare hemostatic fibrin, after alteplase is cleared from the systemic circulation. Administration of m-proUK at therapeutic dosages in healthy volunteers did not result in fibrinogen depletion. A fibrinogen depletion below 2g/L is associated with bleeding after thrombolysis for ischemic stroke.[14-16]

The current study – dual thrombolytic therapy with mutant pro-urokinase and small bolus alteplase for ischemic stroke (DUMAS) – aimed to assess the safety and efficacy of treatment with a dual plasminogen activator, which consists of a small bolus of IV alteplase followed by IV infusion of m-proUK against usual treatment with IV alteplase in patients presenting with ischemic stroke.

Methods

Study design and participants

We conducted an open-label, multicenter, randomized controlled phase II trial with blinded endpoint assessment and an adaptive design for dose optimization in one primary stroke center and three comprehensive stroke centers in the Netherlands. Patients were eligible if they were 18 years or older, had a clinical diagnosis of ischemic stroke with a deficit on the National Institutes of Health Stroke Scale (NIHSS) of at least 1 point, and met the criteria for standard treatment with IV alteplase according to national guidelines.[17] Patients eligible for EVT (i.e., patients with a proximal large-artery occlusion on computed tomography (CT) angiography or magnetic resonance (MR) angiography), with pre-stroke disability (i.e., modified Rankin Scale (mRS) scores > 2), as this interferes with assessment of functional outcome at 30 days. In addition, eleigible patients with known pregnancy, or a contra-indication for MRI were excluded.

The study protocol was approved by the central medical ethics committee at Erasmus MC University Medical Center.[18] We used a deferred informed consent procedure, because this study evaluated an acute intervention in an emergency situation concerning a life-threatening disorder, in accordance with national legislation.[19] Patients or their legal representatives provided written deferred informed consent. If no deferred informed consent was given, only the following characteristics were collected for a safety registry: study number, treatment allocation, in-hospital symptomatic ICH, and in-hospital death. If a patient died before deferred consent could be obtained, all data were used.

Randomization, masking, and procedures

Patients were randomized 1:1 to standard treatment with alteplase alone or dual treatment with a bolus alteplase and m-proUK by treating physicians or local investigators. The randomization procedure was web-based, using permuted blocks, and was stratified for center. The allocation sequence was generated by the independent trial statistician and was unknown to all investigators.

Clinical outcomes, such as NIHSS scores, were collected by trained research personnel unaware of treatment allocation. Neuroimaging was assessed by an imaging core laboratory blinded to study treatment allocation. Members of the imaging core laboratory were unaware of clinical data including treatment allocation, but were informed about baseline clinical symptoms, i.e.. side of the hemiparesis, presence of aphasia, or other symptoms for the patients without hemiparesis or aphasia. Follow-up neuroimaging on which the primary outcome was assessed, was evaluated by two independent members of the imaging core laboratory. If the two imaging core laboratory assessments did not match, disagreements were resolved by consensus.

The intervention group received a 5 mg bolus of IV alteplase followed by a continuous IV infusion of m-proUK, 40 mg in 60 minutes (initial dose) independent of patient weight, based on previous research with pro-urokinase.[20] Because the optimal dose of IV m-proUK for patients with ischemic stroke was still unknown, sequential interim analyses were performed allowing adaptation of the IV m-proUK dose. Depending on the result of interim analyses, the m-proUK dosage could be revised to a dose 25% higher than the initial dose (i.e. 50 mg in 60 minutes) or to a dose 25% lower than the initial dose (i.e. 30 mg in 60 minutes). A detailed description of this adaptive design can be found in Supplemental File 1 of the study protocol.[18]

The control group received 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.

All patients underwent neurological assessments by certified assessors at baseline, at 24 hours, and at 5–7 days (or hospital discharge if earlier). Patients underwent non-contrast CT, CT-angiography and CT-perfusion or MRI and magnetic resonance angiography of the brain at baseline. For follow-up imaging, patients underwent MR imaging of the brain at 24 hours (range: 12 to 48 hours). The MRI should include the

following sequences: 1) Susceptibility weighted imaging (SWI), 2) diffusion weighted imaging (DWI)/apparent diffusion coefficient (ADC), 3) dynamic susceptibility contrast MRI (DSC-MRI), 4) T2 weighted imaging (T2w) or fluid attenuation inversion recovery (FLAIR). In case of any contra-indication for MRI after randomization (e.g. because the contra-indication was not known at the time of inclusion or the patient had a new contra-indication due to an intervention during hospital admission), a follow-up non-contrast CT and CT-perfusion at 24 hours was performed instead. Blood samples were taken at baseline, at 1 hour, at 3 hours, and at 24 hours post treatment for patients in two participating centers. All patients were followed-up until the final assessment at 30 days. Standardized telephone interviews to assess the mRS score at 30 days were conducted from a central location by experienced research nurses, unaware of treatment allocation.[21,22] The assessors instructed patients or relatives before starting the interview not to mention anything about the acute stroke treatment or the admission in the hospital.

Outcomes

The primary outcome was any post-intervention ICH confirmed on SWI or non-contrast CT according to the Heidelberg Bleeding Classification at 24 hours (range: 12 to 48 hours) after study drug administration.[23] Secondary clinical outcomes were the NIHSS score at 24 hours, the NIHSS score at 5–7 days (or discharge if earlier), and the mRS at 30 days.[24,25] Secondary imaging outcomes were post-treatment abnormal perfusion volume (defined as tissue with a time-to-maximum (Tmax) >6 seconds on DSC or CTP Tmax maps) and infarct volume on DWI or non-contrast CT at 24 hours post-treatment. Secondary blood biomarker outcomes were fibrinogen at 1 hour, at 3 hours, and at 24 hours. Safety outcomes were symptomatic ICH according to the Heidelberg Bleeding Classification, death from any cause within 30 days, and major extracranial hemorrhage according to the ISTH criteria within 24 hours of thrombolytic treatment.[23,26]

Statistical analysis

We followed the study protocol and the statistical analysis plan, which were published before database lock.[18] For the sample size calculation, we assumed that the primary outcome, any ICH, would occur with a probability of 20% with standard thrombolytic treatment and a probability of 7% in the patients treated with

dual thrombolytic therapy.[27] A sample of 200 patients would provide us with a power of at least 77% to detect a statistically significant effect on the primary outcome. This estimate did not take into account increasing effect on power of the use of multivariable adjustment for differences in baseline characteristics in the primary analysis. To ensure sufficient power, an additional patient was randomized and included for each patient who did not give consent for participation in the study, or for any reason did not receive the full dose of thrombolytics as assigned, or had a final diagnosis other than ischemic stroke (i.e., stroke mimic). The steering committee discussed all patients about whom doubt existed concerning the discharge diagnosis of ischemic stroke or not, while being blinded for treatment allocation. Every local principal investigator could propose cases for discussion. We estimated that up to 20% of the included patients would not be having a final diagnosis of ischemic stroke.[28]

The effect of the study treatment on the primary outcome was assessed with multivariable logistic regression with study treatment as a binary independent variable (m-proUK vs. control). We reported adjusted and unadjusted effect estimates with corresponding 95% confidence intervals (CI). The effect estimate was adjusted for important prognostic factors at baseline, which included age, time from onset of symptoms to randomization, and stroke severity (NIHSS score). An odds ratio (OR) of more than 1 indicates an increased risk of any ICH of the intervention and an OR of less than 1 indicates a decreased risk of any ICH.

The effects of the study treatment on the secondary outcomes was assessed with multivariable linear, logistic or ordinal regression models. The effect parameter was either a beta, OR, or common OR. These effects were adjusted with variables that are predictive of the specific outcome measure.

We performed and reported four analyses, of which the first is the primary:

1. Simple modified intention-to-treat (mITT) 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.
2. Targeted modified intention-to-treat (tmITT) analysis excluding patients with a final diagnosis other than ischemic stroke to assess safety and efficacy in the target population.

3. Targeted modified on-treatment (tmOT) analysis to assess the safety and efficacy in patients who actually received treatment excluding patients with a final diagnosis other than ischemic stroke.
4. Per-protocol (PP) analysis.

Additionally, we performed pre-specified subgroup analyses on categorized baseline variables including 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). Subgroup analyses were done by testing for interaction of the subgroup indicator with treatment.

An independent trial statistician (DN) performed the interim analyses. Interim analyses were planned after inclusion of 20, 30, 40, and 50 patients with a discharge diagnosis of ischemic stroke and after that with increments of 50, after start of the trial and after any dose change, until the trial was completed. Additional analyses to adapt the dosing in the study were planned after inclusion of 60 patients with a discharge diagnosis of ischemic stroke, and every 20 patients thereafter.

For regression analyses, we assigned the worst score for all unassessed clinical outcome measures for patients who died within the study period. All other missing values, except the primary outcome, were imputed using multiple imputation (n=5). Statistical analyses were performed using R (version 4.0.5). The trial was registered at Netherlands Trial Register (NL7409) and ClinicalTrials.gov (NCT04256473).

Results

Between August 10, 2019, and March 26, 2022, 268 patients were randomly assigned, of whom 15/268 (5.6%) patients did not provide deferred consent and 15/268 (5.6%) patients declined follow-up imaging (Figure 1). A total of 238 patients were included in the modified intention-to-treat population; 121/238 (50.8%) were allocated to receive dual thrombolytic treatment and 117/238 (49.2%) were allocated to receive standard treatment with alteplase alone.

Median age in the modified intention-to-treat population was 69 years (IQR 59-77); 147/238 (61.8%) were male; median time from symptom onset or last seen well to randomization was 114 minutes (IQR 80-156); and median NIHSS at baseline was 3 (IQR 2-5). Baseline demographic and clinical characteristics of patients were similar between the intervention and control group in the modified intention-to-treat population (Table 1).

Of the included patients, 222/238 (93%) patients had MRI as follow-up imaging. The primary outcome (any ICH) occurred in 16/121 (13.2%) patients assigned to the intervention group and 16/117 (13.7%) patients assigned to the control group (unadjusted OR 0.96 [95% CI 0.45 to 2.03], adjusted OR 0.98 [95% CI 0.46 to 2.12]; table 2). Results were similar in the tmITT, tmOT, and PP analyses. A detailed overview of the ICH subtypes according to the Heidelberg Bleeding Classification is shown in Supplemental Table S1.

Occurrence of secondary clinical and imaging outcomes did not differ between the intervention and control group in the modified intention-to-treat population (Table 3). The intervention group had higher fibrinogen levels than the control group at 1 hour (adjusted β 0.65g/L [95% CI 0.26 to 1.05]), at 3 hours (adjusted β 0.47 g/L [95% CI 0.02 to 0.93]), and at 24 hours (adjusted β 0.51 g/L [95% CI 0.10 to 0.92]) (Table 3 and Supplemental Figure S1). There were no significant differences in safety outcomes between the intervention and control group (Table 3). Symptomatic ICH occurred in 0/121 (0%) patients assigned to the intervention group and 3/117 (2.6%) patients in the control group. In the intervention group, 2/121 (1.7%) patients died and 4/117 (3.4%) patients died in the control group. Sensitivity analyses in the safety registry showed similar results (Supplemental Table S2).

Up to day 30, 38 serious adverse events were noted in 19/121 (15.7%) patients allocated to the intervention group and in 19/117 (16.2%) patients allocated to the control group (Table 4).

No clinically relevant differences in treatment effects were observed in pre-specified subgroup analysis (Supplemental Table S3).

Discussion

In patients with ischemic stroke meeting indications for treatment with intravenous thrombolytics and who were not eligible for treatment with endovascular therapy, dual thrombolytic therapy with small bolus IV alteplase followed by m-proUK was not superior to treatment with IV alteplase alone.

Although we found no significant differences in clinical and safety outcomes by treatment, patients in the intervention group had significantly higher fibrinogen levels at 1 hour, 3 hours, and 24 hours after treatment compared to patients treated in the control group. Previous studies have shown a strong association between fibrinogen depletion (<2.0 g/L) and occurrence of sICH.[14,15] This is also suggested in our data, which showed three sICHs in the control group versus none in the intervention group, although this association cannot be thoroughly assessed due to the low number of patients with sICH in our study population. Therefore, further evaluation of thrombolytic treatment with m-proUK in larger trials with patients with greater symptom severity is needed, especially because they are more at risk of sICH.

In Alteplase-Tenecteplase Trial Evaluation for Stroke Thrombolysis (ATTEST), alteplase caused significant disruption of the fibrinolytic system, whereas tenecteplase did not, consistent with the trend toward lower intracerebral hemorrhage incidence with tenecteplase.[29] Importantly, the first measurements were taken at 6 hours after treatment, and earlier measurements are not available. Our results indicate that the largest drop in fibrinogen level occur much earlier, already at 1 hour after treatment. This makes it difficult to compare the effect of tenecteplase on fibrinogen depletion with the results of our study.

Patients included in our trial had minor infarcts (median baseline NIHSS 3 [IQR 2-5]). The European Stroke Organisation (ESO) guidelines recommend treatment of patients with minor stroke (NIHSS 0-4) if deficits are disabling, which is defined as a deficit that, if unchanged, would prevent the patient from performing basic activities of daily living or returning to work.[30] The American Heart Association/American Stroke Association (AHA/ASA) guideline recommends treatment in patients with disabling mild stroke and deficit. The PRISMS trial of alteplase vs placebo in mild non-disabling stroke was stopped early with neutral results.[31] On the other hand, a meta-analysis of individual patient data from nine randomized trials showed that

alteplase significantly improves the overall odds of a good outcome irrespective of stroke severity.[32] Dutch neurologists are aware of this discussion and adhere to the Dutch guidelines, which are in line with the ESO guidelines. Large registries of patients treated with IV thrombolytics mostly included patients with higher NIHSS scores compared to our study.[27,33,34]

The inclusion of minor infarcts could explain the lower occurrence of any ICH than anticipated in the control group (13.7% instead of 20%), as baseline NIHSS is associated with ICH.[35] Moreover, not reaching the anticipated reduction in ICH could be explained by the lack of association between any ICH and thrombolytic treatment as we assumed based on earlier studies.[31,36] Recent trials evaluating the efficacy of EVT alone versus IV thrombolytics and EVT showed now almost similar rates of any ICH in both groups.[37,38]

We used an adaptive design for dose optimization, but no dose changes were advised by the Data Safety and Monitoring Board during the trial. Whether the dose used in DUMAS is the most optimal might depend on the number of tissue plasminogen activator (tPA) binding sites on fibrin, which depends on the size and the composition of the thrombus. Because we included patients with small infarcts and the dose was not adapted, this suggests that the dose is correct for patients included in our trial.

We excluded patients who were eligible for EVT (i.e., patients with a proximal intracranial large artery occlusion on CTA or MRA) because we reasoned that subsequent EVT might disturb the assessment of the intervention effect. However, although we allowed inclusion of all other types of ischemic stroke including lacunar infarcts, cortical infarcts, and posterior circulation strokes (i.e., different stroke etiology), it is known that site of occlusion interferes with the effect of thrombolytic treatment.[5] Our results may be generalizable to all patients with an indication for treatment with IV thrombolytics, but this needs further evaluation for patients with a large vessel occlusion. A limitation of the use of deferred consent is that selective withdrawal from the trial by patients with a poor outcome may have introduced selection bias. However, as the number of patients with a sICH was similar in the modified intention-to-treat population and in the safety registry, a bias associated with sICHs can be excluded.

In conclusion, dual thrombolytic treatment with a small bolus alteplase and m-proUK is safe and does not result in fibrinogen depletion. Further evaluation of thrombolytic treatment with m-proUK in larger trials

to improve outcome in patients with larger ischemic strokes is needed. Overall, in patients with minor ischemic stroke meeting indications for treatment with intravenous thrombolytics, but who are not eligible for treatment with EVT, we could not prove superiority of dual thrombolytic therapy with IV m-proUK is over treatment with IV alteplase alone.

List of abbreviations

DSMB: Data safety monitoring board; DUMAS: Dual thrombolytic therapy with mutant pro-urokinase and small bolus alteplase for ischemic stroke, ICH, intracranial hemorrhage; IV, Intravenous; m-proUK, mutant pro-urokinase; MRA, Magnetic resonance angiography; MRI, Magnetic resonance imaging; mRS, Modified Rankin Scale; NCCT, Non-contrast computed tomography; NIHSS, National Institutes of Health Stroke Scale; PROBE, Prospective randomized open-label blinded end-point; (S)AE, (Serious) adverse event; WMO, Medical Research Involving Human Subjects Act (in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen)

Other information

Registration

NL7409 (November 26, 2018)/NCT04256473 (February 5, 2020)

Protocol

The full protocol and previous versions is available on the DUMAS trial website (https://dumas-trial.nl/).

Funding

Funding of the trial is provided by Thrombolytic Science, LCC. The funder has contributed to and reviewed the study protocol. The study was conducted, analyzed, and interpreted by the investigators independently of the funder. The funder had no role in writing the report and decision to submit for publication. Funding of the study is based on milestones (numbers of patients included and written report).

Ethics approval and consent to participate

The medical ethical committee of the Erasmus MC University Medical Center in Rotterdam, The Netherlands, approved this study in 2019 (MEC number: 2019-0001). Written informed consent (deferred) for trial participation and their anonymised information to be published will be obtained from patient(s) or their legal representatives.

Availability of data and materials

Access to the trial dataset and statistical code will be made available upon request to the Principal Investigators. 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 was asked specifically for these purposes.

Competing interests

DD and AvdL report unrestricted grants from Stryker, Penumbra, Medtronic, Cerenovus, Thrombolytic Science, LLC, Dutch Heart Foundation, Brain Foundation Netherlands, The Netherlands Organization for Health Research and Development, and Health Holland Top Sector Life Sciences & Health for research, paid to institution. WM receives consulting fees from Berry Consultants, LLC which has a contract with and receives funds from Thrombolytic Science, LLC. AP reports institutional grants from Siemens healthineers and Bayer healthcare. All other authors report no competing interests.

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Figures, tables and additional information

Figure 1. Trial profile

EVT indicates endovascular therapy; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; ITT, intention-to-treat; IV, intravenous; and IVT, IV thrombolytics.

Table 1. Baseline characteristics and treatment process measures of the modified intention-to-treat population

	Intervention (n=121)	**Control (n=117)**
Age,median (IQR), years	68 (59-74)	69 (59-78)
Male sex, no. (%)	69 (57%)	78 (67%)
History of atrial fibrillation, no. (%)	5 (4%)	4 (3%)
History of hypertension, no. (%)	57 (47%)	61 (52%)
History of diabetes mellitus, no. (%)	20 (17%)	25 (21%)
Previous ischemic stroke, no. (%)	25 (21%)	27 (23%)
Antiplatelet use, no. (%)	44 (36%)	49 (42%)
Pre-stroke modified Rankin Scale score, no. (%)		
0	92 (76%)	85 (73%)
1	20 (17%)	19 (16%)
2	7 (6%)	10 (9%)
>2	2 (2%)	3 (3%)
Systolic blood pressure, median (IQR), mmHg[a]	162 (145-184)	161 (145-179)
NIHSS score, median (IQR)	3 (2-5)	3 (2-5)
Suspected location of stroke, no. (%)		
Left hemisphere	57 (47%)	50 (43%)
Right hemisphere	34 (28%)	29 (25%)
Posterior circulation	30 (25%)	38 (32%)
Lacunar syndrome, no. (%)[b]	15 (12%)	13 (11%)
Blood glucose, median (IQR), mmol/L[c]	6.8 (5.9-8.1)	6.6 (5.8-8.2)
Thrombocyte count (*10^9/L), median (IQR)[c]	243 (205-278)	234 (203-292)

Fibrinogen, median (IQR), g/L[d]	2.9 (2.5-3.5)	3.0 (2.4-3.5)
Ischemia on baseline NCCT, no. (%)[e]		
None	109 (90%)	103 (89%)
Right anterior circulation	6 (5%)	3 (3%)
Right posterior circulation (ACP territory)	0 (0%)	0 (0%)
Left anterior circulation	3 (2%)	4 (3%)
Left posterior circulation (ACP territory)	1 (1%)	0 (0%)
Infratentorial	2 (2%)	6 (5%)
Perfusion deficit volume, median (IQR), mL[f]	0 (0-4.0)	0 (0-0.4)
Intracranial occlusion on CT angiography, no. (%)[g]		
None	92 (77%)	94 (82%)
ICA	1 (1%)	1 (1%)
M1	2 (2%)	4 (3%)
M2	8 (6%)	4 (3%)
BA	1 (1%)	3 (3%)
P1	1 (1%)	1 (1%)
Other	14 (12%)	8 (7%)
Endovascular therapy, no. (%)	5 (4%)	9 (8%)
Time from stroke onset to door, median (IQR), minutes[a]	82 (51-126)	80 (50-119)
Time from stroke onset to randomization, median (IQR), minutes[e]	115 (79-156)	109 (84-146)
Time from stroke onset to bolus alteplase, median (IQR), minutes[h]	118 (83-170)	121 (89-171)

Final diagnosis, no. (%)		
Ischemic stroke	114 (94%)	104 (89%)
No ischemic stroke	7 (6%)	13 (11%)

[a] Data was missing in 1 patient in the intervention group and 1 patient in the control group.

[b] Lacunar syndrome was a clinical diagnosis.[39]

[c] Data was missing in 1 patient in the intervention group.

[d] Only available for 44 patients in the intervention group and for 18 patients in the control group.

[e] Data was missing in 1 patient in the control group.

[f] A perfusion deficit was defined as the presence of brain areas with a Tmax >6 seconds on baseline CT perfusion. The number of patients with no perfusion deficit on baseline CT perfusion was 62 in the intervention group and 69 in the control group. Data was missing for 22 patients in the intervention group and 20 patients in the control group.

[g] Data was missing for 2 patients in the intervention group and 2 patients in the control group.

[h] Data was missing for 6 patients in the intervention group and 3 patients in the control group.

Table 2. Primary outcome (any ICH) and treatment effects in the primary and secondary analyses.

	Intervention	**Control**	**Unadjusted OR (95% CI)**	**Adjusted OR (95% CI)[a]**
Primary analysis				
Simple modified intention-to-treat	16/121 (13.2%)	16/117 (13.7%)	0.96 (0.45 to 2.03)	0.98 (0.46 to 2.12)
Secondary analyses				
Targeted modified intention-to-treat	16/114 (14.0%)	16/104 (15.4%)	0.90 (0.42 to 1.91)	0.94 (0.43 to 2.04)
Targeted modified on-treatment	12/102 (11.8%)	15/102 (14.7%)	0.77 (0.34 to 1.75)	0.82 (0.35 to 1.91)
Per protocol	12/95 (12.6%)	11/91 (12.1%)	1.04 (0.43 to 2.50)	1.26 (0.50 to 3.18)

Data are n/N (%). OR indicates odds ratio.

[a] Treatment effects were adjusted for age, time from symptom onset or last seen well to randomization, and baseline NIHSS score.

Table 3. Secondary outcomes and treatment effects in the simple modified intention-to-treat population

	Intervention (n=121)	**Control (n=117)**	**Unadjusted effect estimate (95% CI)**	**Adjusted effect estimate (95% CI)[a]**
Clinical				
NIHSS				
At 24 hours	1 (0-2)	1 (0-2)	β -0.20 (-1.10 to 0.71)[b]	β -0.19 (-1.10 to 0.72)
Improvement at 24 hours[d]	77 (63.6%)	83 (70.9%)	OR 0.72 (0.42 to 1.25) [b]	OR 0.68 (0.39 to 1.20)
At 5-7 days or discharge	0 (0-2)	0 (0-2)	β -0.76 (-1.79 to 0.27) [b]	β -0.76 (-1.79 to 0.27)
mRS score at 30 days				
Ordinal	2 (1-3)	2 (1-3)	cOR 1.09 (0.69 to 1.72)	cOR 1.16 (0.74 to 1.84)
0 vs 1-6	21 (17.4%)	18 (15.4%)	OR 1.16 (0.58 to 2.31)	OR 1.21 (0.60 to 2.45)
0-1 vs 2-6	49 (40.5%)	45 (38.5%)	OR 1.09 (0.65 to 1.84)	OR 1.18 (0.68 to 2.06)
0-2 vs 3-6	89 (73.6%)	87 (74.4%)	OR 0.96 (0.54 to 1.72)	OR 0.94 (0.52 to 1.70)
0-3 vs 4-6	110 (90.9%)	101 (86.3%)	OR 1.58 (0.70 to 3.59)	OR 1.61 (0.70 to 3.70)
0-4 vs 5-6	118 (97.5%)	112 (95.7%)	OR 1.76 (0.41 to 7.58)	OR 2.04 (0.44 to 9.39)
Neuroimaging				
Infarct volume at 24 hours, mean [range], mL[e]	5.1 [0-113]	7.0 [0-263]	β -1.81 (-7.11 to 3.50)	β -2.13 (-7.33 to 3.07)
Perfusion deficit volume at 24 hours, mean [range], mL[f]	2.0 [0-62]	1.6 [0-39]	β -0.85 (-2.70 to 0.99) [c]	β -0.80 (-2.62 to 1.01)
Blood biomarker				
Fibrinogen, g/L[g]				
At 1 hour[h]	3.0 (2.5-3.5)	1.8 (1.6-3.5)	β 0.69 (0.29 to 1.10)	β 0.65 (0.26 to 1.05)
At 3 hours[i]	2.9 (2.4-3.4)	2.2 (1.7-2.6)	β 0.52 (0.10 to 0.65)	β 0.47 (0.02 to 0.93)
At 24 hours[j]	3.0 (2.5-3.6)	2.5 (2.0-2.7)	β 0.54 (0.14 to 0.95)	β 0.51 (0.10 to 0.92)
Safety				

Symptomatic intracranial hemorrhage	0 (0%)	3 (2.6%)	NT	NT
Death from any cause at 30 days	2 (1.7%)	4 (3.4%)	OR 0.47 (0.08 to 2.67)	OR 0.37 (0.06 to 2.38)
Major extracranial hemorrhage according to the ISTH criteria within 24 hours of study drug administration	1 (0.8%)	1 (0.9%)	OR 0.97 (0.06 to 15.9)	OR 0.83 (0.05 to 14.9)

Data are n (%) or median (IQR).

β indicates regression coefficient beta; cOR, common odds ratio; OR, odds ratio; NIHSS, National Institutes of Health Stroke Scale; and NT, not tested.

[a] Adjusted for age, time from symptom onset or last seen well to randomization, and baseline NIHSS score.

[b] Adjusted for baseline NIHSS score.

[c] Adjusted for baseline perfusion deficit volume.

[d] Improvement of at least 4 points or NIHSS 0 or 1 at 24 hours

[e] Missing for 2 patients in the control group.

[f] Missing for 45 patients in the intervention group and 48 patients in the control group.

[g] Blood samples were only drawn in 2 centers. For the regression models, only patients randomized in those 2 centers were included (n=128). Those regression models were adjusted for baseline fibrinogen.

[h] Available for 42 patients in the intervention group and 31 patients in the control group.

[i] Available for 39 patients in the intervention group and 44 patients in the control group.

[j] Available for 35 patients in the intervention group and 44 patients in the control group.

Table 4. Serious adverse events in the modified intention-to-treat population

	Intervention (n=121)	**Control (n=117)**
Patients with at least one serious adverse event at 30 days	19 (15.7%)	19 (16.2%)
Intracranial hemorrhage	0 (0%)	3 (2.6%)
Extracranial hemorrhage	1 (0.8%)	1 (0.9%)
Stroke progression	2 (1.7%)	2 (1.7%)
New ischemic stroke	2 (1.7%)	2 (1.7%)
Allergic reaction	0 (0%)	0 (0%)
Pneumonia	1 (0.8%)	3 (2.6%)
Other infection	1 (0.8%)	2 (1.7%)
Other serious adverse event	12 (9.9%)	9 (7.7%)

Data are no. (%).

SUPPLEMENTAL MATERIAL ON

Safety and efficacy of dual thrombolytic therapy with mutant pro-urokinase and small bolus alteplase for ischemic stroke (DUMAS): a randomized clinical trial

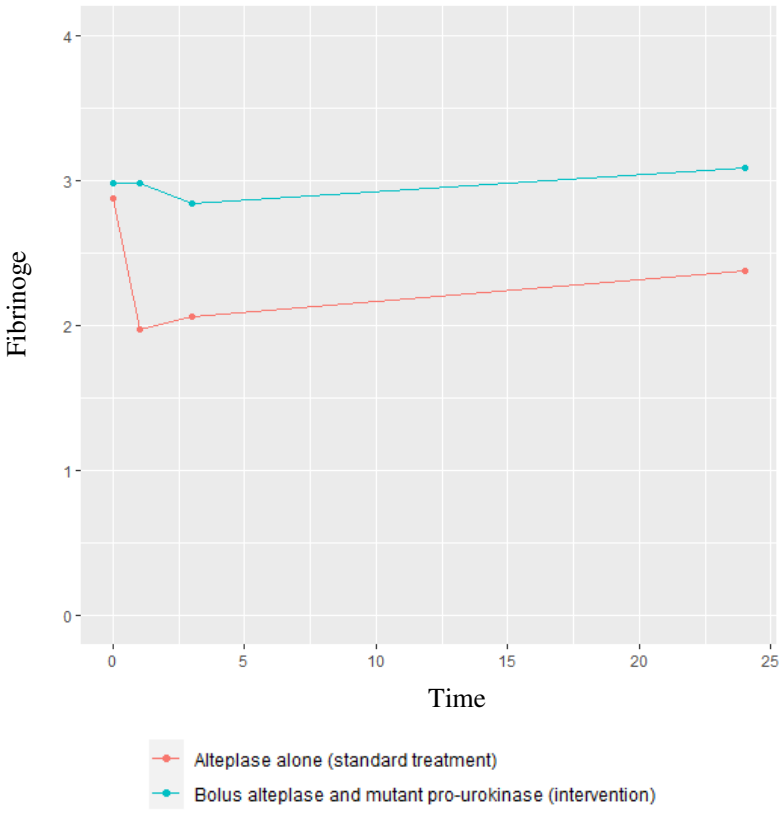
Supplemental Table S1. Any ICH subtypes according to the Heidelberg Bleeding Classification in the modified intention-to-treat population.

Class	Type	Description	Intervention (n=121)	Control (n=117)
1	Hemorrhagic transformation of infarcted brain tissue			
1a	HI1	Scattered small petechiae, no mass effect	6 (5.0%)	6 (5.1%)
1b	HI2	Confluent petechiae, no mass effect	8 (6.6%)	3 (2.6%)
1c	PH1	Hematoma within infarcted tissue, occupying <30%, no substantive mass effect	0 (0%)	1 (0.9%)
2	Infarcted hemorrhage within and beyond infarcted brain tissue			
	PH2	Hematoma within infarcted brain tissue, occupying >30%, with obvious mass effect	2 (1.7)	4 (3.4%)
3	Intracerebral hemorrhage outside the infarcted brain tissue or intracranial-extracerebral hemorrhage			
		Parenchymal hematoma remote from infarcted brain tissue, intraventricular hemorrhage, subarachnoid hemorrhage, subdural hemorrhage	0 (0%)	2 (1.7%)

Data are n (%).

HI indicates hemorrhagic infarction; and PH, parenchymal hematoma.

Supplemental Figure S1. Fibrinogen levels from baseline to 24-hour post thrombolytic treatment in the modified intention-to-treat population.



Supplemental Table S2. Safety outcomes with treatment effects in the safety registry.

	Intervention (n=135)	**Control (n=133)**
Symptomatic intracranial hemorrhage	0 (0%)	3 (2.3%)
In-hospital death from all causes	0 (0%)	5 (3.8%)

Supplemental Table S3. Treatment effect estimates on any post-intervention intracranial hemorrhage in pre-specified subgroups.

	OR (95% CI)	**P-value for interaction**
Age		0.84
<70 years (n=125)	1.29 (0.35 to 4.75)	
⩾70 years (n=113)	0.77 (0.29 to 2.06)	
Sex		0.58
Male (n=147)	0.79 (0.27 to 2.30)	
Female (n=91)	1.19 (0.37 to 3.88)	
Baseline systolic blood pressure		0.62
<162 mmHg (n=118)	0.47 (0.13 to 1.68)	
⩾162 mmHg (n=119)	1.84 (0.63 to 5.36)	
Time from symptom onset to IV thrombolytics		0.78
<121 min (n=121)	0.83 (0.28 to 2.40)	
⩾121 min (n=116)	1.18 (0.38 to 3.66)	
Baseline NIHSS		0.49
<4 (n=127)	1.02 (0.33 to 3.21)	

⩾4 (n=111)	0.98 (0.35 to 2.77)	
Pre-treatment antiplatelet use		0.41
No (n=145)	1.16 (0.46 to 2.96)	
Yes (n=93)	0.63 (0.13 to 2.98)	
DWI lesion on follow-up imaging		0.99
No (n=100)	5314 (0.00 to inf)	
Yes (n=138)	0.79 (0.35 to 1.81)	
Lacunar syndrome		0.94
No (n=210)	1.00 (0.45 to 2.21)	
Yes (n=28)	0.95 (0.02 to 51.3)	

Subgroup analyses were not performed for ASPECTS and extracranial occlusion, because the number of patients with ASPECTS<10 and an extracranial occlusion were very low.