SUPPLEMENTARY MATERIAL

e-Methods

Supplementary neuroimaging methods

A cerebral CT scan was required to rule out mainly the presence of intracranial hemorrhage. A second CT was performed 22 to 36 hours after starting the infusion of trial medication. Other scans (extra scans) were optional and were performed during admission within 7 days/discharge only in case of clinical deterioration; these scans were considered clinically relevant to the interpretation of the results as regard to the occurrence of symptomatic intracerebral hemorrhage (SICH).
Neuroimaging data included current infarct, cerebral edema, and dense artery sign; these variables were part of the list of variables collected in the eCRF (electronic Case Report Form) that was built on the basis of the international registry on thrombolysis in stroke, SITS-ISTR (Safe Implementation of Treatments in Stroke – International Stroke Thrombolysis register). This because all patients treated with IV alteplase in the TESPI trial had to be also entered into the SITS-ISTR registry. In fact, all participating center of TESPI trial were also members of the SITS-ISTR registry. Therefore, "current infarction" was used for indicating the presence of early ischemic signs on the baseline CT scan suggestive of the presence of a current infarction and for indicating the presence of an actual infarction on follow-up scans both those performed at 22-36 h and extra scans.
With regard to "cerebral oedema", although all investigators tended to use the score for cerebral edema used for SITS-ISTR for the above mentioned reasons (COED 1: Focal brain swelling up to one third of the hemisphere; COED 2: Focal brain swelling greater than one third of the hemisphere; COED 3: Brain swelling with midline shift), no definition for cerebral edema was pre-specified in the study protocol. In the TESPI trial, it was just asked the expert neuroradiologists/radiologists/stroke neurologists of each stroke center to indicate if cerebral oedema was present, absent or uncertain. Similarly, we did not give a pre-specified definition for "dense artery sign" in the study protocol, therefore its identification was left to the expert neuroradiologists/radiologists/stroke neurologists of each participating stroke center. In any case, the term generally refers to the hyperattenuation of the middle cerebral artery, and not only (e.g. basilar artery), on non-enhanced CT scan.
Different intracerebral hemorrhage types were defined according to the ECASS study, as follows: hemorrhagic infarction type 1 (HI1), small petechiae along the margins of the infarct; HI2, confluent petechiae within the infarcted area without space-occupying effect; parenchymal hemorrhage type 1 (PH1), local, or intra-ischemic confluent hematoma in ≤ 30% of the infarcted area with at the most some slight space-occupying effect; PH2, local, or intra-ischemic confluent hematoma >30% of the infarcted area with a substantial space-occupying effect. Remote hemorrhage indicated an hematoma of any size located remote from the index infarct.
Besides SICH per NINDS definition, pre-specified in the study protocol, SICH definitions according to ECASS II and SITS-MOST studies were also evaluated although they were not pre-specified in the study protocol. SICH according to the ECASS II study was defined as any hemorrhage plus a neurological deterioration of 4 points or more on the NIHSS from baseline, or from the lowest NIHSS value after baseline to 7 days or any hemorrhage leading to death. SICH according to SITS-MOST study was defined as local or remote parenchymal hemorrhage type 2 on the 22–36 h post-treatment imaging scan, combined with a neurological deterioration of 4 points or more on the NIHSS from baseline, or from the lowest NIHSS value between baseline and 24 h, or leading to death.
The definition of symptomatic cerebral edema was not pre-specified in the study protocol. It was intended post-hoc as brain edema detected at 22-36 h scans or extra scans as predominant cause of

clinical deterioration defined as an increase of 4 or more points of NIHSS at 24 h or 7 days from baseline or death.

Supplementary statistical methods

Handling of missing data
Regarding missing data, as pre-specified in the study protocol, the last observation carried-forward method (LOCF), i.e. data from the last available visit or measurement replacing the missing data, and the worst score principle were applied as appropriate as sensitivity analyses. In particular, for NIHSS and NIHSS-related assessments, in case of missing values LOCF was adopted. In one case only the baseline NIHSS value was available and, therefore, it was carried forward to the next assessments (2h, 24h, and 7d).
With regard to mRS, Barthel Index (BI), and Glasgow Outcome Scale (GOS), these measures were collected only at 90 days and no previous assessments were foreseen in the study protocol, therefore, in case of missing data on 90-day outcome among patients known to be alive, the worst possible outcome score was assigned for each of these measures as follows: 5 for mRS; 0 for BI; 4 for GOS. In case of missing data, including those on survival, the worst possible outcome scores were assigned.

e-Results

As to secondary endpoints, median NIHSS scores at 24 h and 7 days were lower in the alteplase group compared to control group with a borderline significant difference at 24 h and a statistically significant difference at 7 days (at 24 h, 5 [IQR 2-14] versus 9 [IQR 4-16], *P*=0.050; at 7 days, (2.5 [IQR 0-10] vs 4 [IQR 1-12.5], *P*=0.042) (Table e-2).

As regard the mean changes of NIHSS scores from baseline, patients treated with alteplase showed a statistically significant greater neurological improvement at both 24 h and 7 days compared with the control group (at 24 h: -3.6 [SD 5.6] vs -0.7 [SD 6.4], *P*=0.003; at 7 days, (-6.0 [SD 6.6] vs -3.5 [SD 5.9], *P*=0.005) (Table e-5). An improvement of ≥4 points at NIHSS or score 0-1 at 24 h and 7 days from baseline occurred in a significantly higher proportion of patients treated with alteplase compared with controls (43/86 [50.0%] vs 28/102 [27.5%], *P*=0.001 and 57/85 [67.1%] vs 52/102 [51.5%], *P*=0.032, respectively) (Table e-5).

Exploratory analyses suggested that a better benefit/risk profile from alteplase compared with standard care was observed up to 88 years (90-day mRS 0-2: 32.9% vs 27·8%; 90-day mRS 0-1: 27.1% vs 21.5%; death: 14.5% vs 22.2%; SICH/NINDS: 6.9% vs 6.3%; SICH/ECASS: 4.2% vs 6.3%; SICH/SITS-MOST: 1.4% vs 1.3%; fatal ICH: 1.4 vs 1.3). However, it should be taken into account that the number of patients in the over-88 year age category is small and therefore this data should be interpreted with caution.

Table e-1. Inclusion and exclusion criteria by treatment group

	All	Alteplase	Control
	N=191	N=88	N=103

Protocol violators (%)	23 (12.0)	6 (6.8)	17 (16.5)
Number of patients with more than one protocol violation (%)	1 (0.5)	0	1 (1.0)
Inclusion criteria			
Age >80 years	191 (100.0)	88 (100.0)	103 (100.0)
Clinical diagnosis of ischemic stroke	191 (100.0)	88 (100.0)	103 (100.0)
Onset of symptoms <3 hours	182 (95.3)	87 (98.8)	95 (92.2)
Patient is willing to participate voluntarily	191 (100.0)	88 (100.0)	103 (100.0)
Exclusion criteria			
Symptoms of ischemic attack began more than 3 hours	9 (4.7)	1 (1.4)	8 (7.8)
Severe stroke as assessed clinically (NIHSS >17)	7 (3.7)	2/88 (2.3)	5/103 (4.9)
Patients with any history of prior stroke and diabetes	5 (2.6)	1/88 (1.1)	4/103 (3.9)
Blood glucose <50 or >200 mg/dl	1 (0.5)	0	1 (1.0)
Known hemorrhagic diathesis	0	0	0
Patients receiving oral anticoagulants	1 (0.5)	0	1 (1.0)
Patients receiving oral anticoagulants	1 (0.5)	1 (1.1)	0
Documented arterial aneurysm	1 (0.5)	1 (1.1)	0

NIHSS indicates National Institutes of health Stroke Scale; rt-PA, recombinant tissue-plasminogen activator.

Table e-2. Demographics and baseline characteristics of patients (ITT population)

	Alteplase N=88	Control N=103
Demographics		
Race (%)		
- Caucasian	85 (96.6)	100 (97.1)
- Black	0	0
- Asiatic	3 (3.4)	3 (2.9)
- Other	0	0
Height (cm), mean (SD)	162·6 (13.6)	161·6 (20.4)
Risk factors		
Number of risk factors (%)		
- 0	4 (4.5)	9 (8.7)
- 1	31 (35.2)	24 (23.3)
- 2	27 (30.7)	33 (32.0)
- 3	15 (17.0)	27 (26.2)
- 4	8 (9.1)	7 (6.8)
- 5	3 (3.4)	3 (2.9)
Pre-stroke therapy		
Antiplatelets at stroke onset (%)		
- Dipyridamol	2 (2.3)	3 (2.9)
- Clopidogrel	2 (2.3)	3 (2.9)
- Other	8 (9.1)	11 (10.7)
Anticoagulants (IV) (%)		
- high dose (aPTT alterations)	0	0
- low dose (no aPTT alterations)	0	2 (1.9)
NIHSS, median (IQR)	12 (8-16)	11 (7-15)
- <5 (%)	2 (2.3)	6 (5.8)
- 5-10 (%)	35 (39.7)	37 (35.9)
- >10 (%)	51 (58.0)	60 (58.3)
Baseline Neuroimaging		
Local hemorrhage, CT (%)	0	0
Remote hemorrhage, CT (%)	0	0

PH2, CT Before (%)		
- no	87/88 (100.0)	103/104 (100.0)
- yes	0	0
- uncertain	0	0
Cerebral edema, CT (%)		
- no	86/87 (98.9)	102/103 (99.0)
- yes	0	0
- uncertain	1/87 (1.1)	1/103 (1.0)
Other Pathological Alterations (%)		
- no	78/87 (89.7)	91/103 (88.3)
- yes	9/87 (10.3)	12/103 (11.7)
Time intervals		
Onset-to-Randomization time (min), median (IQR)	152 (130-172)	161 (135-178)
- ≤60 (%)	0	0
- ≤90 (%)	2 (2.3)	6 (5.8)
- >90 (%)	86 (97.7)	97 (94.2)

a Variable with p value statistically significant.

aPTT indicates activated partial thromboplastin time; CT, computerized tomography; IQR, interquartile range; IV=intravenous; NIHSS, National Institutes of Health Stroke Scale; PH2, parenchymal hemorrhage 2; SD, standard deviation.

Table e-3. Post-randomization data (ITT population)

	Alteplase N=88	Control N=103	*P* value
Clinical assessment			
SBP, mean (SD), mmHg			
- 2h	148.7 (19.5)	148.9 (22.0)	0.948
- 24h	148.1 (21.6)	143.9 (23.3)	0.223
- 7d	138.5 (21.6)	142.0 (21.2)	0.348
DBP, mean (SD), mmHg			
- 2h	75.5 (13.3)	77.2 (12.9)	0.391
- 24h	75.2 (12.9)	74.3 (12.9)	0.646
- 7d	74.5 (13.1)	75.5 (9.8)	0.621
Total serum cholesterol levels at 24h, median (IQR)	170.5 (150.50-187.25)	176 (155-202)	0.315
Total serum cholesterol levels at 7d, median (IQR)	171 (152-195)	172.5 (157.25-195.5)	0.974
NIHSS, median (IQR)			
- After alteplase infusion			
- 2h	9 (5-15)	10 (6-15)	0.820[a]
- 24h	5 (2-14)	9 (4-16)	**0.050[a]**
- 7d	2.5 (0-10)	4 (1-12.5)	**0.042[a]**
Treatment since admission[c]			
Aspirin (%)[b]	44/81 (54.3)	64/91 (70.3)	**0.030**
Dypiridamol (%)	2/81 (2.5)	2/91 (2.2)	1.000[b]
Clopidogrel (%)	20/81 (24.7)	20/91 (22.0)	0.674
Other antiplatelets (%)	2/81 (2.5)	2/91 (2.2)	1.000[b]
Anticoagulants, high dose, (aPTT alterations) (%)	2/81 (2.5)	3/91 (3.3)	1.000[b]
Anticoagulants, low dose, (no aPTT alterations)(%)	47/81 (58.0)	59/91 (64.8)	0.359
Oral anticoagulants (%)	13/81 16.0)	14/91 (15.4)	0.905
Antihypertensive, IV(%)	13/81 (16.0)	12/91 (13.2)	0.595
Antihypertensive, oral (%)	61/81 (75.3)	67/91 (73.6)	0.801
Neuroimaging assessment			
Current infarct CT After (%)			0.170[b]
- no	22/83 (26.5)	19/96 (19.8)	
- yes	61/83 (73.5)	74/96 (77.1)	
- uncertain	0	3/96 (3.1)	

Current infarct CT Extra[d] (%)			1.000[b]
- no	2/22 (9.1)	2/23 (8.7)	
- yes	20/22 (90.9)	21/23 (91.3)	
- uncertain	0	0	
Dense artery sign CT After (%)			0.825[b]
- no	68/83 (81.9)	80/96 (83.3)	
- yes	12/83 (14.5)	14/96 (14.6)	
- uncertain	3/83 (3.6)	2/96 (2.1)	
Dense artery sign CT Extra (%)			0.511[b]
- no	15/22 (68.2)	18/23 (78.3)	
- yes	6/22 (27.3)	5/23 (21.7)	
- uncertain	1/22 (4.5)	0	
Local hemorrhage, CT (%)			
- After	7/83 (8.4)	11/96 (11.5)	0.502
- Extra	9/22 (40.9)	8/23 (34.8)	0.672
Remote hemorrhage, CT (%)			
- After	1/83 (1.2)	1/96 (1.0)	1.000[b]
- Extra	2/22 (9.1)	1/23 (4.3)	0.608[b]
PH2, CT After (%)	81/83 (97.6)	92/96 (95.8)	0.684[b]
- no	1/83 (1.2)	3/96 (3.1)	
- yes	1/83 (1.2)	1/96 (1.0)	
- uncertain			
PH2, CT Extra (%)			1.000[b]
- no	20/22 (90.9)	20/23 (87.0)	
- yes	2/22 (9.1)	3/23 (13.0)	
- uncertain	0	0	
Cerebral edema, CT After (%)			**0.004**
- no	74/83 (89.2)	69/96 (71.9)	
- yes	9/83 (10.8)	27/96 (28.1)	
- uncertain	0	0	
Cerebral edema, CT Extra (%)			0.140
- no	17/22 (77.3)	13/23 (56.5)	
- yes	5/22 (22.7)	10/23 (43.5)	
- uncertain	0	0	

a Mann-Withney U test.
b Fisher Exact
c "Treatment since admission" indicates both pre- and post-randomization treatments.
d Extra scans indicate other scans that were optional and were performed during admission within 7 days/discharge only in case of clinical deterioration.
CT indicates computerized tomography; DBP, diastolic blood pressure; IQR, interquartile range; NIHSS, National Institutes of Health Stroke Scale; PH2, parenchymal hemorrhage 2; SBP, systolic blood pressure; SD, standard deviation.

Table e-4. Proportion of patients who completed the trial by treatment group in the ITT population

	All N=191	Alteplase N=88	Control N=103	*P* value
Patients completed the planned observation time until 90 days[a] (%)	137 (71.7)	68 (77.3)	69 (67.0)	0.117
If not please specify why (%)				0.170
- Death	41 (21.5)	15 (17.1)	26 (25.2)	0.911
- Lost to follow-up[a]	2 (1.0)	1 (1.1)	1 (1.0)	0.862
- Non compliant with protocol[b]	7 (3.7)	3 (3.4)	4 (3.9)	…
- Other[b]	1 (0.5)	1 (1.1)	0	
Patients completed the planned observation time until 90 days including patients who died[b] (%)	178 (93.2)	83 (94.3)	95 (92.2)	0.569

a Three more patients in the control group were considered lost to follow-up (it was impossible to contact them) because it was known that they were alive but data on mRS were missing; therefore they were not considered among the patients who had completed the planned observation time until 90 days.
b Patients that was declared by the investigators as not having completed the planned observation time until 90 days because non-compliant with protocol or for other reasons, were actually lost to follow-up because it was impossible to contact them otherwise these patients should have been considered at least "alive" by the investigators.

Table e-5. Secondary efficacy endpoints and other safety endpoints by treatment group (mITT population for all secondary analyses concerning efficacy and safety outcome measures at 90 days, otherwise ITT population)

	Alteplase N=88	Control N=103	*P* value
Other Safety endpoints			
- SICH (ECASS II) (%)	3/85 (3.5)	5/98 (5.1)	0.726[b]
Absolute difference (95% CI)	-1.6% (-6.2, 9.0)		
Odds ratio (95% CI)	0.68 (0.16-2.94)		
Relative risk (95% CI)[a]	0.69 (0.17-2.81)		
Relative difference	-31%		
- SICH (SITS-MOST) (%)	1/85 (1.2)	1/98 (1.0)	1.0[b]
Absolute difference (95% CI)	0.2% (-5.3, 6.3)		
Odds ratio (95% CI)	1.15 (0.07-18.75)		
Relative risk (95% CI)	1.15 (0.07-18.15)		
Relative difference	15%		
Secondary efficacy endpoints			
- NIHSS changes from baseline to 2 h			0.224[c]
mean (SD)	-2.0 (3.7)	-1.2 (4.8)	
median (IQR)	-1.0 (-4, 0)	0 (-2, 0)	
- NIHSS changes from baseline to 24h			**0.003**[c]
mean (SD)	-3.6 (5.6)	-0.7 (6.4)	
median (IQR)	-3.5 (-7, 1)	-1.0 (-3.25, 1.25)	
- NIHSS changes from baseline to 7d			**0.005**[c]
mean (SD)	-6.0 (6.6)	-3.5 (5.9)	
median (IQR)	-6.0 (-10, -2.25)	-4·0 (-7.5, -0.5)	
- NIHSS improvement (≥4 or score 0-1) at 7d (%)	57/85 (67.1)	52/102 (51.5)	**0.032**
- NIHSS <8 - mRS 0 response (%)	5/83 (6.0)	10/94 (10.6)	0.271
- NIHSS 8-14 - mRS 0-2 response (%)	11/83 (13.3)	8/94 (8.5)	0.309
- mRS at 90 days (0-1) (%)	20/83 (24.1)	17/95 (17.9)	0.309
- Barthel Index ≥95 at 90 days (%)	21/64 (32.8)	20/68 (29.4)	0.673
- Glasgow Outcome Scale 1-2 (good recovery/moderately disabled) at 90 days (%)	34/83 (41.0)	31/95 (32.6)	0.249
- Global Outcome (mRS 0-1; Barthel Index ≥95; Glasgow Outcome Scale 1-2) at 90 days (%)	19/64 (29.7%)	17/68 (25.0)	0.546
Other secondary efficacy outcomes (not pre-specified)			
- NIHSS improvement (≥4 or score 0-1) at 24 h (%)	43/86 (50.0)	28/102 (27·5)	**0.001**
- Barthel Index ≥90 at 90 days (%)	22/64 (34.4)	20/68 (29.4)	0.541
- Glasgow Outcome Scale 1 (good recovery) at 90 days (%)	21/83 (25.3)	17/95 (17.9)	0.229
Other safety endpoints			
- ICH (%)	12/85 (14.1)	15/98 (15.3)	0.821

- Asymptomatic ICH (%)	7/85 (8.2)	10/98 (10.2)	0.647
- Fatal SICH (%)	1/85 (1.2)	1/98 (1.0)	1.0[a]
- Symptomatic cerebral edema[e] (%)	5/85 (5.9)	9/97 (9.3)	0.478[b]

a RR is also presented for the main efficacy and safety outcome measures as it was pre-specified in the study protocol.
b Fisher's Exact Test
c Mann-Withney U test.
d Of note, the proportion of deaths in the control group (26.5%) is different from that reported in Figure 2 (27.4%) due to different denominators (95 in Figure 2), because there were 3 control patients alive but with a missing mRS.
e The denominator refers to the number of patients (182: 85 in the alteplase group and 97 in the control group) for whom data on the presence/absence of cerebral oedema on 22-36 h and extra scans (179: 83 in the alteplase group and 96 in the control group) or on extra scan alone (3: 2 in the alteplase group and 1 in the control group) were available.
CI indicates confidence intervals; ECASS, European Cooperative Acute Stroke Study; ICH, intracerebral haemorrhage; IQR, interquartile range; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; NINDS, National Institutes of Neurological Disorders and Stroke; SD, standard deviation; SICH, symptomatic intracerebral hemorrhage; SITS-MOST, Safe Implementation of Thrombolysis in Stroke - Monitoring Study.

Table e-6. Analyses on primary efficacy endpoints, secondary efficacy endpoints, and safety endpoints by treatment group including missing values[a] (ITT population)

	Alteplase N=88	Control N=103	*P* value
Primary efficacy endpoint			
- mRS at 90 days (0-2) (%)	24/88 (27.3)	22/103 (21.4)	0.341
Absolute difference (95% CI)	5.9% (-6.9, 18.8)		
Odds ratio (95% CI)	1.38 (0.71-2.68)		
Relative risk (95% CI)	1.28 (0.77-2.11)		
Relative difference	28%		
Safety endpoints			
- Death (with mRS 6 for missing values) (%)	20/88 (22.7)	31/103 (30.1)	0.251
Absolute difference (95% CI)	-7.4% (-6.0, 20.1)		
Odds ratio (95% CI)	0.68 (0.36-1.31)		
Relative risk (95% CI)	0.76 (0.47-1.23)		
Relative difference	24%		
Secondary efficacy endpoints			
- NIHSS changes from 0-2 h			0.068[b]
mean (SD)	-2.0 (3.7)	-1.2 (4.8)	
median (IQR)	-1.0 (-4, 0)	0 (-2, 0)	
- NIHSS changes from 0-24h			**0.003[b]**
mean (SD)	-3.6 (5.6)	-0.6 (6.6)	
median (IQR)	-3.0 (-7, 1)	-1.0 (-3, 2)	
- NIHSS changes from 0-7d			**0.010[b]**
mean (SD)	-5.7 (6.7)	-3.4 (5.9)	
median (IQR)	-6.0 (-10, -2.0)	-4.0 (-7.25, -0)	
- NIHSS improvement (≥4 or score 0-1) at 7d (%)	57/88 (64.8)	52/103 (50.5)	**0.047**
- NIHSS <8 - mRS 0 response (%)	5/88 (5,7)	10/103 (9.7)	0.302
- NIHSS 8-14 - mRS 0-2 response (%)	11/88 (12.5)	8/103 (7.8)	0.276
- mRS at 90 days (0-1) (%)	20/88 (22.7)	17/103 (16.5)	0.278
- Barthel Index ≥95 at 90 days (%)	21/88 (23.9)	20/103 (19.4)	0.456
- Glasgow Outcome Scale 1-2 (good recovery/moderately disabled) (%)	34/88 (38.6)	31/103 (30.1)	0.214
- Global Outcome (mRS 0-1; Barthel Index ≥95; Glasgow Outcome Scale 1-2) at 90 days (%)	19/88 (21.6%)	17/103 (16.5)	0.370
Other secondary efficacy outcomes(not pre-specified)			

- NIHSS improvement (≥4 or score 0-1) at 24 h (%)	43/88 (48.9)	28/103 (27.2)	**0.002**
- Barthel Index ≥90 at 90 days (%)	22/88 (25.0)	20/103 (19.4)	0.353
- Glasgow Outcome Scale 1 (good recovery) at 90 days (%)	21/88 (23.9)	17/103 (16.5)	0.204

a Regarding missing values, last observation carried-forward method, i.e. data from the last available visit or measurement replaced the missing data, and the worst score principle were applied as appropriate.
b Mann-Withney U test.
CI indicates confidence intervals; ECASS indicates European Cooperative Acute Stroke Study; ICH, intracerebral haemorrhage; IQR, interquartile range; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; NINDS, National Institutes of Neurological Disorders and Stroke; SD, standard deviation; SICH, symptomatic intracerebral hemorrhage; SITS-MOST, Safe Implementation of Thrombolysis in Stroke - Monitoring Study.

Table e-7. Causes of death by treatment group

	All N=191	Alteplase N=88	Control N=103	*P* value
Primary cause of death (%)				0.790
- Cerebral infarct	13/41 (31.7)	3/15 (20.0)	10/26 (38.5)	0.227
- Cerebral infarct and hemorrhage, without specification	4/41 (9.8)	2/15 (13.3)	2/26 (7.7)	0.563
- Pneumonia	7/41 (17.1)	3/15 (20.0)	4/26 (15.4)	0.709
- Other vascular	3/41 (7.3)	1/15 (6.7)	2/26 (7.7)	0.905
- Other	14/41 (34.1)	6/15 (40.0)	8/26 (30.8)	0.553
Cause of death reasonably related to alteplase treatment (%)	1/41 (2.4)	1/15 (6.7)	…	0.366[a]

a Fisher's Exact Test.

Table e-8. Adverse events[a] by treatment group in the ITT population

	All N=191	Alteplase N=88	Control N=103	*P* value
All adverse events (%)	69 (36.1)	34 (38.6)	35 (34.0)	0.506
- Mild	32 (16.8)	18 (20.5)	14 (13.6)	0.207
- Moderate	33 (17.3)	14 (15.9)	19 (18.4)	0.645
- Severe	33 (17.3)	18 (20.5)	15 (14.6)	0.284
Serious adverse events (%)	35 (18.3)	19 (21.6)	16 (15.5)	0.282
Patients with multiple serious adverse events (%)	9 (4.7)	5 (5.7)	4 (3.9)	0.560
Serious adverse events possibly related to study drug (%)	10 (5.2)	10 (11.4)	0	…
- SICH	2 (1.0)	2 (2.3)		…
- Allergic reaction	1 (0.5)	1 (1.1)		…
- Macroscopic haematuria	1 (0.5)	1 (1.1)		…
Serious adverse event for (%):				
- Death	11 (5.8)	4 (4.5)	7 (6.8)	0.507
- Life-threatening	6 (3.1)	4 (4.5)	2 (1.9)	0.305
- Disability	0	0	0	…
- Hospitalization or prolonged hospitalization	17 (8.9)	10 (11.4)	7 (6.8)	0.270
- Relevant medical event	9 (4.7)	8 (9.1)	1 (1.0)	**0.008**
- Other	0	0	0	…
Serious adverse event outcome (%)				

- Recovered	14 (7.3)	8 (9.1)	6 (5.8)	0.389
- Recovering/Resolving	7 (3.7)	6 (6.8)	1 (1.0)	**0.033**
- Not recovered	5 (2.6)	1 (1.1)	4 (3.9)	0.237
- Recovered with sequelae	1 (0.5)	1 (1.1)	0	…
- Fatal	16 (8.4)	9 (10.2)	7 (6.8)	0.395
- Unknown	1 (0.5)	0	1 (1.0)	…
Serious adverse event other than SICH and cerebral edema (%)	42 (22.0)	25 (28.4)	17 (16.5)	
- Pneumonia	11 (5.8)	6 (6.8)	5 (4.8)	0.563
- Pleuritis	1 (0.5)	1 (1.1)	0	…
- Cardiovascular	12 (6.3)	7 (7.9)	5 (4.8)	0.380
- Pulmonary edema	2 (1.0)	1 (1.1)	1 (1.0)	0.911
- Anemia	3 (1.6)	2 (2.3)	1 (1.0)	0.472
- Cerebrovascular ischemic event	4 (2.1)	3 (3.4)	1 (1.0)	0.242
- Dysphagia	1 (0.5)	0	1 (1.0)	…
- Agitation	2 (1.0)	0	2 (1.9)	…
- Acute cholecystitis	1 (0.5)	1 (1.1)	0	…
- Urinary sepsis	1 (0.5)	0	1 (1.0)	…
- Macroscopic hematuria	1 (0.5)	1 (1.1)	0	…
- Laterocervical hematoma (after TEA)	1 (0.5)	1 (1.1)	0	…
- Femoral fracture after fall	1 (0.5)	0	1 (1.0)	…
- Vocal string paralysis	1 (0.5)	1 (1.1)	0	…
- Allergic reaction	1 (0.5)	1 (1.1)	0	…
- Fever	1 (0.5)	1 (1.1)	0	…

a Analyses of adverse events were performed by comparing the proportion of patients with at least one event with patients randomized, all or by treatment group, as denominator.
SICH indicates symptomatic intracerebral hemorrhage; TEA, thromboendarterectomy.

Table e-9. Primary efficacy endpoint, secondary efficacy endpoints, and safety endpoints by treatment group in the Per-Protocol population

	Alteplase	Control	*P* value
	N=82	N=85	
Primary efficacy endpoint			
- mRS at 90 days (0-2) (%)	22/78 (28.2)	21/79 (26.6)	0.820
Absolute difference (95% CI)	1.6% (-13.0, 16.2)		
Odds ratio (95% CI)	1.09 (0.54-2.19)		
Relative risk (95% CI)	1.06 (0.64-1.77)		
Relative difference	6%		
Safety endpoints			
- Death (%)	15/77 (19.5)	16/82 (19.5)	0.996
- SICH (NINDS) (%)	5/80 (6.3)	4/82 (4.9)	0.744[a]
Absolute difference (95% CI)	1.4% (-7.4, 10.4)		
Relative risk (95% CI)	1.28 (0.36-4.6)		
Relative difference	28%		
Odds ratio (95% CI)	1.3 (0.34-5.03)		
- SICH (ECASS II) (%)	3/80 (3.8)	4/82 (4.9)	1.0[a]
Absolute difference (95% CI)	-1.1% (-7.1, 9.4)		
Relative risk (95% CI)	0.77 (0.18-3.33)		
Relative difference	-23%		
Odds ratio (95% CI)	0.76 (0.16-3.51)		

- SICH (SITS-MOST) (%)	1/80 (1.3)	1/82 (1.2)	1.0[a]
Secondary efficacy endpoints			
- NIHSS changes from 0-2 h			0.037[b]
mean (SD)	-2.0 (3.6)	-0.93 (4.1)	
median (IQR)	-1.0 (-4.25, 0)	0 (-1, 0)	
- NIHSS changes from 0-24h			**0.005[b]**
mean (SD)	-3.7 (5.6)	-0.9 (6.8)	
median (IQR)	-4.0 (-7, 1)	-1.0 (-4, 0.5)	
- NIHSS changes from 0-7d			**0.013[b]**
mean (SD)	-6.0 (6.6)	-4.3 (5.9)	
median (IQR)	-6.0 (-10, -3)	-4.0 (-8, -1)	
- NIHSS improvement (≥4 or score 0-1) at 7 d (%)	54/80 (67.5)	45/83 (54.2)	0.083
- NIHSS <8 - mRS 0 response (%)	5/78 (6.4)	9/78 (11.5)	0.262
- NIHSS 8-14 - mRS 0-2 response (%)	10/78 (12.8)	8/78 (10.3)	0.616
- mRS at 90 days (0-1) (%)	18/78 (23.1)	16/79 (20.3)	0.668
- Barthel Index ≥95 at 90 days (%)	19/59 (32.2)	19/62 (30.6)	0.854
- Glasgow Outcome Scale 1-2 at 90 days (%)	32/78 (41.0)	30/79 (38.0)	0.696
- Global Outcome (mRS 0-1; Barthel Index ≥95; Glasgow Outcome Scale 1-2) at 90 days (%)	17/59 (28.8%)	16/62 (25.8)	0.710
Other secondary efficacy outcomes (not pre-specified)			
- NIHSS improvement (≥4 or score 0-1) at 24 h (%)	42/81 (51.9)	24/84 (28.6)	**0.002**
- Barthel Index ≥90 at 90 days (%)	20/59 (33.9)	19/62 (30.6)	0.702
- Glasgow Outcome Scale =1 (good recovery) at 90 days (%)	19/78 (24.4)	16/79 (20.3)	0.537
Other safety endpoints			
- ICH (%)	11/80 (13.8)	11/82 (13.4)	0.950
- Asymptomatic ICH (%)	6/80 (7.5)	7/82 (8.5)	0.808
- Fatal SICH (%)	1/80 (1.3)	1/82 (1.2)	1.0[a]
- Symptomatic cerebral edema[c] (%)	5/80 (6.3)	7/81 (8.6)	0.565[a]

a Fisher's Exact Test.

b Mann-Withney U test.

c The denominator refers to the number of patients (161: 80 in the alteplase group and 81 in the control group) for whom data on the presence/absence of cerebral edema on 22-36 h and extra scans (158: 78 in the alteplase group and 80 in the control group) or on extra scan alone (3: 2 in the alteplase group and 1 in the control group) were available.

ECASS indicates European Cooperative Acute Stroke Study; IQR, interquartile range; mRS, modified Rankin Scale; NIHSS, National Institutes of Health Stroke Scale; NINDS, National Institutes of Neurological Disorders and Stroke; SD, standard deviation; SICH, symptomatic intracerebral hemorrhage; SITS-MOST, Safe Implementation of Thrombolysis in Stroke - Monitoring Study.

Figure e-1.

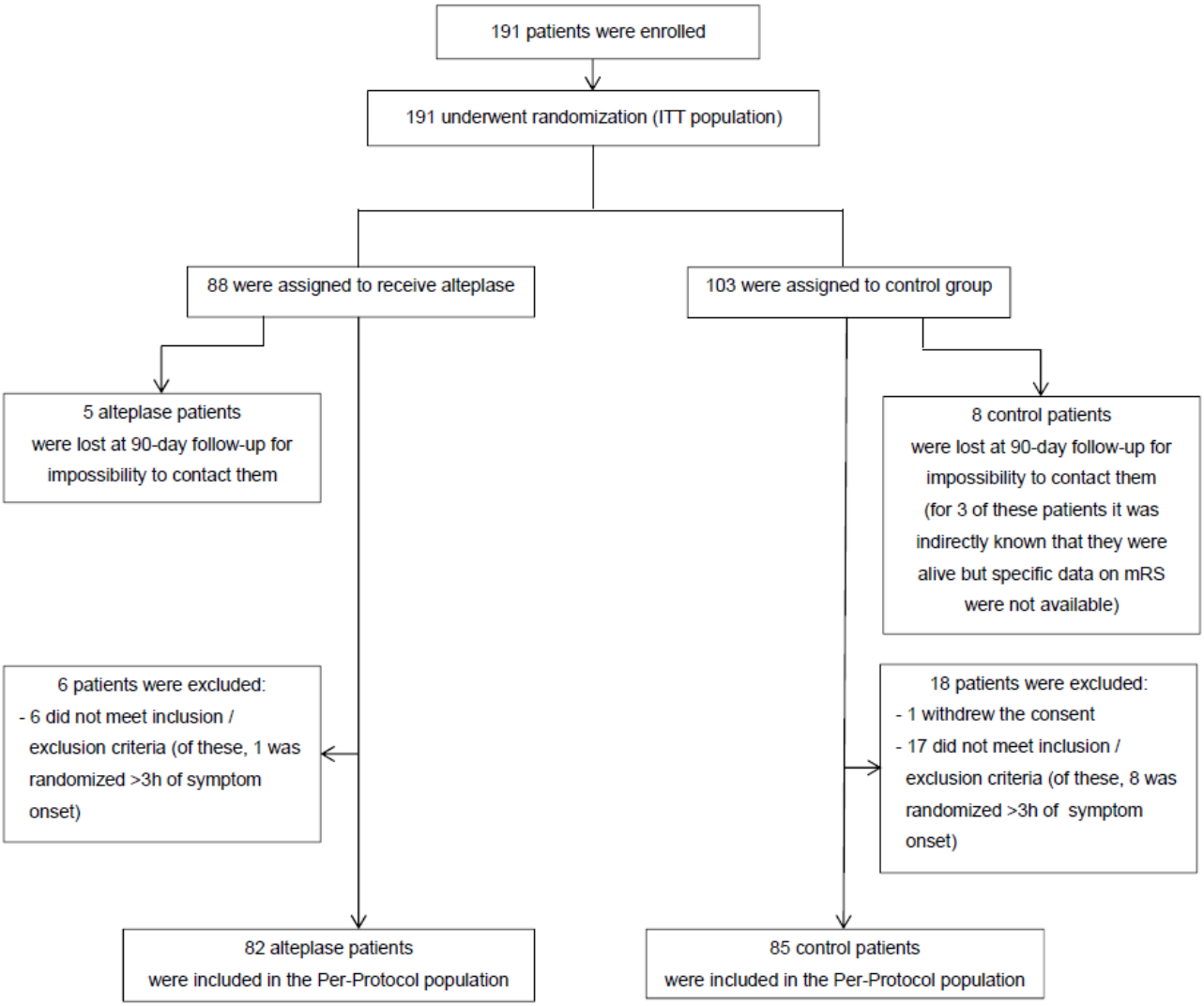

191 patients were enrolled
191 underwent randomization (ITT population)
88 were assigned to receive alteplase
103 were assigned to control group
5 alteplase patients were lost at 90-day follow-up for impossibility to contact them
8 control patients were lost at 90-day follow-up for impossibility to contact them (for 3 of these patients it was indirectly known that they were alive but specific data on mRS were not available)
6 patients were excluded:
- 6 did not meet inclusion / exclusion criteria (of these, 1 was randomized >3h of symptom onset)
18 patients were excluded:
- 1 withdrew the consent
- 17 did not meet inclusion / exclusion criteria (of these, 8 was randomized >3h of symptom onset)
82 alteplase patients were included in the Per-Protocol population
85 control patients were included in the Per-Protocol population

Figure e-2.

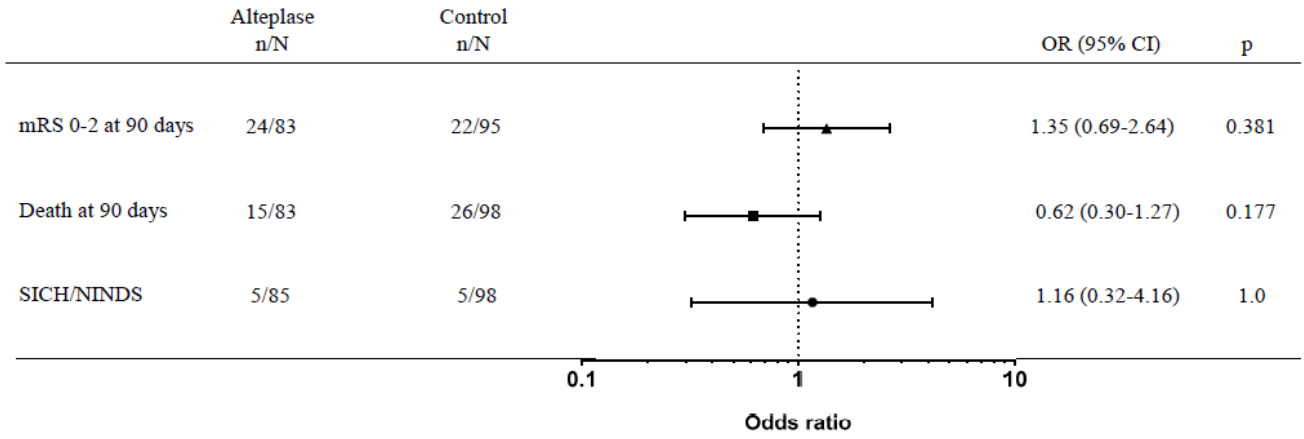


Figure e-3.

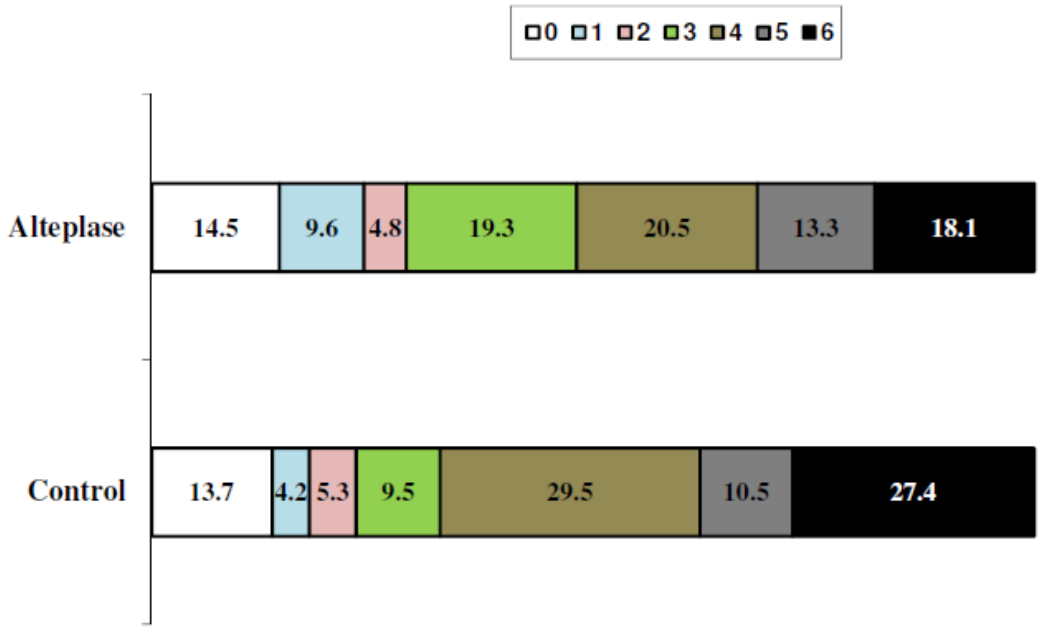


Figure e-4.

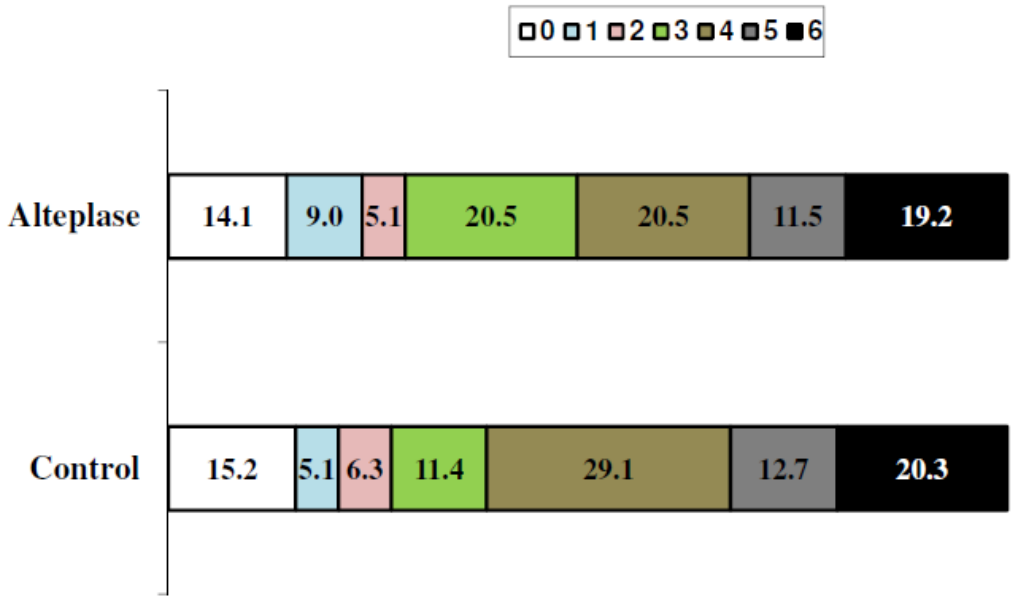
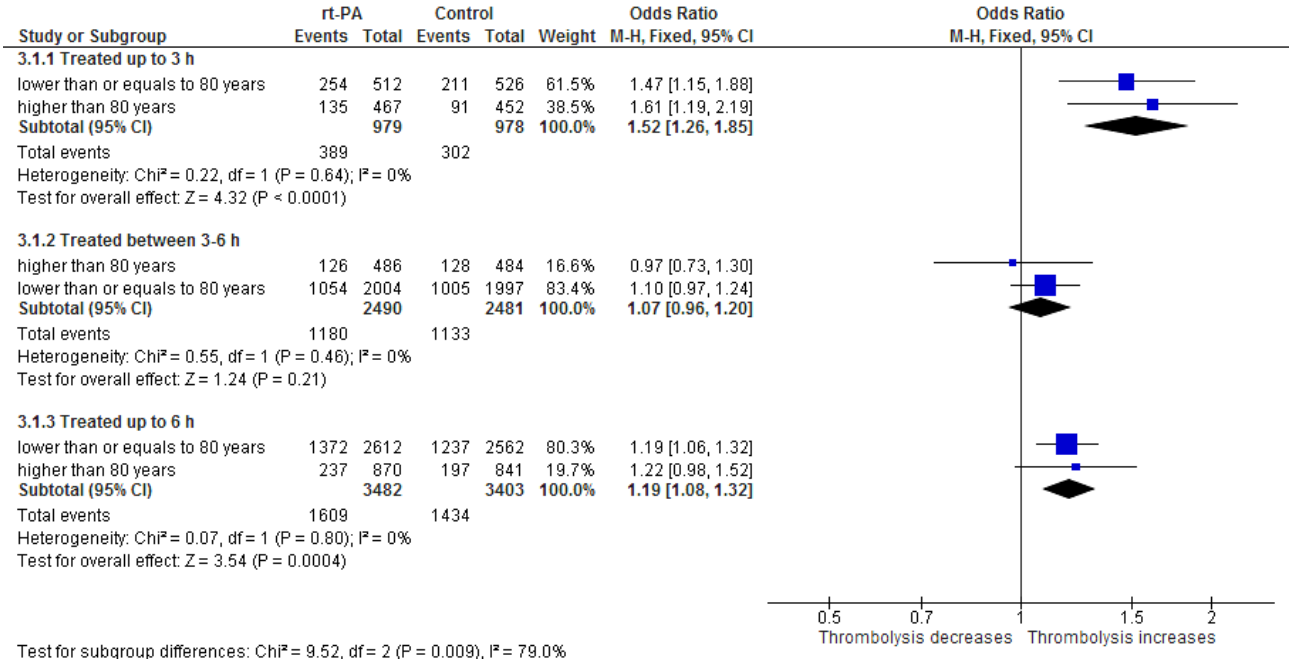


Figure e-5.

A.



B.

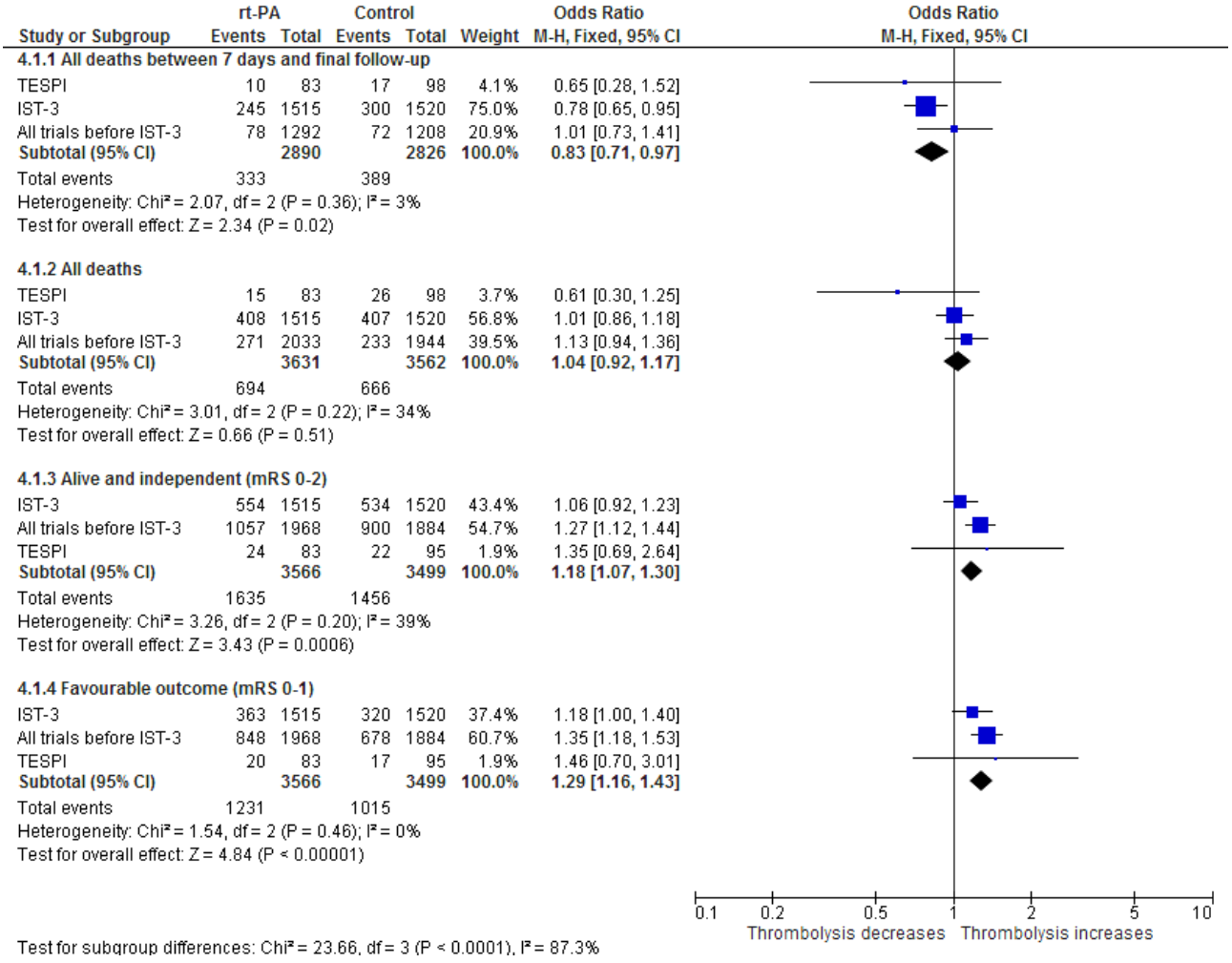


Figure e-1 legend
Patients enrolled and included in the Intention-to-Treat (ITT) and Per-Protocol (PP) population.

Figure e-2 legend
Effect of alteplase on favorable outcome and mortality at 90 days and on symptomatic intracerebral hemorrhage (SICH) (ITT population)

Figure e-3 legend
Distribution of modified Rankin Scale scores at 90 days in the mITT population. Ordinal logistic regression analysis showed non-statistically significant results (OR 1.49 95% CI 0.89-2.5; *P*=0.126).

Figure e-4 legend
Distribution of modified Rankin Scale scores at 90 days in the Per-Protocol population

Figure e-5 legend

Forest plots of the meta-analysis of previous randomized controlled trials on alteplase updated with TESPI data; IST-3 had the vast majority of the older 80 year olds in the totality of data (data are numbers, unless otherwise indicated; rt-PA indicates recombinant tissue plasminogen activator; IST-3, Third International Stroke Trial; mRS, modified Rankin Scale)
Panel A legend. Effects of alteplase (rt-PA) on alive and independent (mRS 0-2) at the end of follow-up, subgrouped by age and time to treatment (TESPI data are included in the subgroup of patients treated up to 3 h).
Panel B legend. Effects of alteplase on outcomes at final follow-up (treatment was administered up to 6 h after the stroke).

Appendix e-1. List of TESPI trial Investigators

Principal Investigator and National Coordinator: Danilo Toni
Steering Committee: Cesare Fieschi (Chairman); Danilo Toni, Domenico Inzitari, Roberto Sterzi, Antonio Carolei (Members)
Data Monitoring Safety Board (DSMB): Gianluigi Lenzi (Chairman); Marco Fiorelli, Annarita Vestri (Members)
CRO: Roberto Verna, Moira Cordisco, Ugo Lancia (CRISC - Centro di Ricerca per la Sperimentazione Clinica (Centre for Clinical Research), Sapienza University, Rome, Italy); CRO Scientific Board and Technical Coordinator: Paolo Primiero; Administrative and monitoring Practices: Antonella Noy di Lannoy
Statistical analyses: Svetlana Lorenzano; Annarita Vestri (statistician)
Investigators: *Roma, Policlinico Umberto I* - Danilo Toni (PI), Svetlana Lorenzano, Agata Correnti; *Verona, University Hospital* - Paolo Bovi, Manuel Cappellari; *Roma, Policlinico Tor Vergata Hospital* - Paolo Stanzione, Domenico Samà; *Firenze, Santa Maria Annunziata* - Maddalena Bruscoli, Germana Ruggiano; *Modena, Ospedale Civile S.Agostino-Estense* - Milena Cavazzuti, Andrea Zini; *Roma, S. Andrea Hospital* - Maurizia Rasura, Mario Beccia; *Milano, Scientific Institute S. Raffaele* - Giancarlo Comi, Maria Sessa; *Genova, University Hospital* - Carlo Gandolfo, Maurizio Balestrino; *Perugia, S. Maria della Misericordia Hospital* - Giancarlo Agnelli, Valeria Caso, Monica Acciaresi, Maurizio Paciaroni; *Cuneo, Santa Croce e Carle Hospital* - Piercarlo Gerbino Promis; *Roma, San Camillo Hospital* - Claudio Pozzessere, Sabrina Anticoli; *Vicenza, Bortolo Hospital* - Francesco Perini, Michela Marcon; *Prato, Prato Hospital* - Annalisa Vinattieri, Alba Caruso; *Brescia, Spedali Civili di Brescia* - Mauro Magoni; *Imperia, Ospedale Civile di Imperia* - Mauro Furlan; *Bari, Azienda Ospedaliera Ospedale Policlinico Consorziale* - Francesco Federico, Domenico Mezzapesa; *Pisa Azienda Ospedaliera Universitaria Policlinico S. Chiara* - Giovanni Orlandi; *Roma, Policlinico "A. Gemelli"* - Vincenzo Di Lazzaro; *Cremona, Azienda Istituti Ospedalieri di Cremona* - Luigi Bettoni, Gianstefano Baietti; *Udine, University Hospital* - Maria Rosaria Valente; *Firenze, Azienda Ospedaliera Universitaria Careggi* - Patrizia Nencini.

Appendix e-2. Author contribution

Svetlana Lorenzano: study concept/design, study supervision, data acquisition, statistical analysis/interpretation of data, drafting/revising the manuscript for intellectual content

Annarita Vestri: study design, statistical analysis/interpretation of data, revising the manuscript for intellectual content
Paolo Bovi, Manuel Cappellari, Paolo Stanzione, Domenico Samà, Maddalena Bruscoli, Milena Cavazzuti, Andrea Zini, Maurizia Rasura, Mario Beccia, Giancarlo Comi, Maria Sessa, Carlo Gandolfo, Maurizio Balestrino, Giancarlo Agnelli, Valeria Caso, Piercarlo Gerbino Promis, Claudio Pozzessere, Sabrina Anticoli, Francesco Perini, Michela Marcon, Annalisa Vinattieri, Alba Caruso, Mauro Magoni, Mauro Furlan, Giovanni Orlandi, Vincenzo Di Lazzaro, Mariarosaria Valente, Patrizia Nencini: data acquisition, revising the manuscript for intellectual content
Danilo Toni: study concept/design, study supervision, data acquisition, interpretation of data, drafting/revising the manuscript for intellectual content.

CONSORT 2010 checklist of information to include when reporting a randomised trial*

Section/Topic	Item No	Checklist item	Reported on page No
Title and abstract			
	1a	Identification as a randomised trial in the title	1
	1b	Structured summary of trial design, methods, results, and conclusions (for specific guidance see CONSORT for abstracts)	3
Introduction			
Background and objectives	2a	Scientific background and explanation of rationale	4,5
	2b	Specific objectives or hypotheses	4,5
Methods			
Trial design	3a	Description of trial design (such as parallel, factorial) including allocation ratio	5
	3b	Important changes to methods after trial commencement (such as eligibility criteria), with reasons	5-9 Supplementary material: 1,2
Participants	4a	Eligibility criteria for participants	5
	4b	Settings and locations where the data were collected	5
Interventions	5	The interventions for each group with sufficient details to allow replication, including how and when they were actually administered	5
Outcomes	6a	Completely defined pre-specified primary and secondary outcome measures, including how and when they were assessed	5,6
	6b	Any changes to trial outcomes after the trial commenced, with reasons	5,6
Sample size	7a	How sample size was determined	7
	7b	When applicable, explanation of any interim analyses and stopping guidelines	NA
Randomisation:			
Sequence generation	8a	Method used to generate the random allocation sequence	5
	8b	Type of randomisation; details of any restriction (such as blocking and block size)	5
Allocation concealment mechanism	9	Mechanism used to implement the random allocation sequence (such as sequentially numbered containers), describing any steps taken to conceal the sequence until interventions were assigned	NA
Implementation	10	Who generated the random allocation sequence, who enrolled participants, and who assigned participants to interventions	5
Blinding	11a	If done, who was blinded after assignment to interventions (for example, participants, care providers, those assessing outcomes) and how	5,6

	11b	If relevant, description of the similarity of interventions	NA
Statistical methods	12a	Statistical methods used to compare groups for primary and secondary outcomes	7-9
	12b	Methods for additional analyses, such as subgroup analyses and adjusted analyses	7-9; Supplementary material: 1,2
Results			
Participant flow (a diagram is strongly recommended)	13a	For each group, the numbers of participants who were randomly assigned, received intended treatment, and were analysed for the primary outcome	10; Supplementary material: 2,3,11,14
	13b	For each group, losses and exclusions after randomisation, together with reasons	10; Supplementary material: 11,14
Recruitment	14a	Dates defining the periods of recruitment and follow-up	10
	14b	Why the trial ended or was stopped	4,5
Baseline data	15	A table showing baseline demographic and clinical characteristics for each group	23,24; Supplementary material: 3,4
Numbers analysed	16	For each group, number of participants (denominator) included in each analysis and whether the analysis was by original assigned groups	10
Outcomes and estimation	17a	For each primary and secondary outcome, results for each group, and the estimated effect size and its precision (such as 95% confidence interval)	10-13,25; Supplementary material: 2,4-6,12,14
	17b	For binary outcomes, presentation of both absolute and relative effect sizes is recommended	10-13,25; Supplementary material: 2,4-6,12
Ancillary analyses	18	Results of any other analyses performed, including subgroup analyses and adjusted analyses, distinguishing pre-specified from exploratory	11-13,22,26,27; Supplementary material: 2,4-6,8-10,13,14
Harms	19	All important harms or unintended effects in each group (for specific guidance see CONSORT for harms)	11,12; Supplementary material: 8,9

Discussion			
Limitations	20	Trial limitations, addressing sources of potential bias, imprecision, and, if relevant, multiplicity of analyses	16
Generalisability	21	Generalisability (external validity, applicability) of the trial findings	16
Interpretation	22	Interpretation consistent with results, balancing benefits and harms, and considering other relevant evidence	13-17
Other information			
Registration	23	Registration number and name of trial registry	6
Protocol	24	Where the full trial protocol can be accessed, if available	7
Funding	25	Sources of funding and other support (such as supply of drugs), role of funders	9