Tenecteplase versus standard of care for minor ischaemic stroke with proven occlusion (TEMPO-2): a randomised, open label, phase 3 superiority trial

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Summary

Background Individuals with minor ischaemic stroke and intracranial occlusion are at increased risk of poor outcomes. Intravenous thrombolysis with tenecteplase might improve outcomes in this population. We aimed to test the superiority of intravenous tenecteplase over non-thrombolytic standard of care in patients with minor ischaemic stroke and intracranial occlusion or focal perfusion abnormality.

Methods In this multicentre, prospective, parallel group, open label with blinded outcome assessment, randomised controlled trial, adult patients (aged ≥18 years) were included at 48 hospitals in Australia, Austria, Brazil, Canada, Finland, Ireland, New Zealand, Singapore, Spain, and the UK. Eligible patients with minor acute ischaemic stroke (National Institutes of Health Stroke Scale score 0–5) and intracranial occlusion or focal perfusion abnormality were enrolled within 12 h from stroke onset. Participants were randomly assigned (1:1), using a minimal sufficient balance algorithm to intravenous tenecteplase (0·25 mg/kg) or non-thrombolytic standard of care (control). Primary outcome was a return to baseline functioning on pre-morbid modified Rankin Scale score in the intention-to-treat (ITT) population (all patients randomly assigned to a treatment group and who did not withdraw consent to participate) assessed at 90 days. Safety outcomes were reported in the ITT population and included symptomatic intracranial haemorrhage and death. This trial is registered with ClinicalTrials.gov, NCT02398656, and is closed to accrual.

Findings The trial was stopped early for futility. Between April 27, 2015, and Jan 19, 2024, 886 patients were enrolled; 369 (42%) were female and 517 (58%) were male. 454 (51%) were assigned to control and 432 (49%) to intravenous tenecteplase. The primary outcome occurred in 338 (75%) of 452 patients in the control group and 309 (72%) of 432 in the tenecteplase group (risk ratio [RR] 0·96, 95% CI 0·88–1·04, p=0·29). More patients died in the tenecteplase group (20 deaths [5%]) than in the control group (five deaths [1%]; adjusted hazard ratio 3·8; 95% CI 1·4–10·2, p=0·0085). There were eight (2%) symptomatic intracranial haemorrhages in the tenecteplase group versus two (<1%) in the control group (RR 4·2; 95% CI 0·9–19·7, p=0·059).

Interpretation There was no benefit and possible harm from treatment with intravenous tenecteplase. Patients with minor stroke and intracranial occlusion should not be routinely treated with intravenous thrombolysis.

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Introduction

Up to 50% of patients with ischaemic stroke initially present with minimal symptoms which are non-disabling.[1] Despite having low scores on the National Institutes of Health Stroke Scale (NIHSS), typically ranging from 0 to 5, a third of such patients are dead or disabled at 90-day follow-up if thrombolysis is withheld.[2–4] Patients with minor deficits and evidence of an intracranial occlusion are a subpopulation at high risk for early neurological deterioration,[5,6] which most often occurs within the first 24 h after presentation.[5] This is true even if the deficits have resolved.[7] Nevertheless, minor deficits are a common reason for withholding thrombolysis,[2] as many physicians have concerns regarding the potential harm from bleeding in the absence of major deficits. Most stroke thrombolysis trials

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Research in context

Evidence before this study

Intravenous thrombolysis with both alteplase and tenecteplase has been proven to improve clinical outcomes after ischaemic stroke. However, for patients with minor deficits, thrombolysis with either agent has not been shown to be superior to antiplatelet agents. The subgroup of patients with intracranial occlusion and minor stroke have an elevated risk of early deterioration and disability. We searched MEDLINE and PubMed for randomised trials published in English between Jan 1, 2000, and March 31, 2024, using the terms "stroke", "tenecteplase", and "alteplase", and "trial or study". We could not identify any phase 3 randomised trials comparing tenecteplase or alteplase with antiplatelet agents in patients with minor stroke and intracranial occlusion. Two phase 3 trials compared alteplase with antiplatelet agents in minor stroke, but none looked at the subset with intracranial occlusion and none looked at tenecteplase.

Added value of this study

This is the first phase 3 study to examine the efficacy of thrombolysis with tenecteplase in minor ischaemic stroke patients with intracranial occlusion within 12 h of onset. The trial showed that patients do not benefit from treatment with tenecteplase and that there is potential harm. This large, well conducted trial had a pragmatic control reflecting clinical practice.

Implications of all the available evidence

Findings from our study suggest that minor ischaemic stroke patients with intracranial occlusion should not be treated with intravenous thrombolysis with tenecteplase and that antiplatelet therapy is sufficient.

have excluded patients with minor stroke and thus high-quality data to guide thrombolytic treatment in these patients are scarce.

An individual patient data meta-analysis of the subset of patients with minor stroke included in randomised trials of thrombolysis with intravenous alteplase suggested that thrombolysis improved outcomes in these individuals (odds ratio [OR] 1·48, for good outcome [modified Rankin Scale, mRS score, 0–1] adjusted for age and time from onset; 95% CI 1·07–2·06).[8] However, randomised trials examining thrombolysis exclusively in individuals with minor stroke have not shown benefit over antiplatelet therapy. The PRISMS trial compared intravenous alteplase against aspirin monotherapy and showed no significant difference in 90-day functional outcomes between groups and higher rates of symptomatic intracerebral haemorrhage in the alteplase group.[9] The ARAMIS non-inferiority trial (–4·5% non-inferiority margin) found that dual antiplatelet therapy was non-inferior to intravenous alteplase for excellent functional outcome at 90 days with no significant difference in the risk of symptomatic intracranial haemorrhage between groups.[10] Both trials used intravenous alteplase as the comparative thrombolytic agent and restricted enrolment to either 3·0 or 4·5 h from symptom onset. Both trials also tried to exclude patients who could be at elevated risk for disability within this low NIHSS population by excluding patients scoring higher on certain subcategories of the NIHSS. Neither study focused specifically on the subpopulation with intracranial occlusion who seem to be at the highest risk for early deterioration and disability.[5,6]

Tenecteplase, a recombinant human tissue plasminogen activator similar to alteplase, has a longer half-life in part due to resistance to plasminogen activator inhibitor, it is more fibrin-specific, and results in less systemic depletion of circulating fibrinogen as compared with alteplase.[11] The AcT trial and others have shown that tenecteplase is non-inferior to alteplase,[12,13] which has led to guideline changes, with intravenous tenecteplase (0·25 mg/kg) now recommended for use in ischaemic stroke within 4·5 h of symptom onset.[14–16] The TIMELESS study[17] included patients with disabling stroke between 4·5 h and 24·0 h from onset with potentially salvageable tissue defined by CT perfusion imaging and randomly assigned patients to treatment with standard of care or intravenous tenecteplase. Although there was no observable difference in outcomes between groups in TIMELESS, there was no evidence of harm when tenecteplase was given in the 24 h time window. The TWIST study found similar safety in patients with stroke on awakening.[18] The TEMPO-1[19] study, which was a phase 2 dose escalation safety study assessing the feasibility of using tenecteplase in the treatment of minor stroke patients with intracranial occlusion, showed a low symptomatic intracranial haemorrhage rate (4%) and high recanalisation rates at the 0·25 mg/kg dose. All of these studies[12,17–19] have shown the safety of thrombolysis with tenecteplase in selected patients within 4·5 h and after 4·5 h from stroke onset.

The TEMPO-2 trial was designed to show superiority of intravenous tenecteplase (0·25 mg/kg) as compared with non-thrombolytic standard of care in patients with minor stroke with intracranial occlusion or focal perfusion lesion presenting within 12 h from symptom onset, on 90-day functional outcomes assessed with mRS.

Methods

Study design and participants

TEMPO-2 was an investigator-initiated, multicentre, prospective, randomised, open-label with blinded endpoint assessment (PROBE), parallel group, controlled trial, designed to test the superiority of intravenous tenecteplase (0·25 mg/kg) over non-thrombolytic standard of care in patients with minor ischaemic stroke deficits, defined as NIHSS 0–5, and intracranial occlusion

or focal perfusion lesion within 12 h from onset of symptoms. The trial was conducted at 48 hospitals in Australia, Austria, Brazil, Canada, Finland, Ireland, New Zealand, Singapore, Spain, and the UK. The methods of this trial have been previously published,[20] and the protocol and statistical analysis plan are available in the appendix. The trial was sponsored by the University of Calgary, AB, Canada. Data management and monitoring were conducted by the University of Calgary. The trial was monitored by an independent data and safety monitoring committee (DSMC) that conducted two planned unblinded interim safety analyses, one additional safety assessment and one planned interim analysis (DSMC members are listed in the appendix [p 2]). The trial was regulated by Health Canada and elsewhere as required in individual countries. The trial protocol was approved by local ethics boards. All patients or their representative provided written informed consent as approved by local ethics boards. Patients were eligible if they were 18 years or older; were functionally independent before the stroke (baseline pre-stroke mRS 0–2); had a minor stroke with NIHSS score of 0–5, presented within 12 h of last seen normal; had direct imaging evidence of an intracranial occlusion or indirect evidence of occlusion with a focal perfusion lesion relevant to the presenting symptoms; and had no region of well evolved infarction concordant with the acute presenting syndrome and an Alberta Stroke Program Early CT score (ASPECTS)[21] of 7 or greater. Perfusion imaging was not mandatory. Patients were not eligible if, in the judgement of the physician and the patient, routine intravenous thrombolysis treatment was warranted. The main exclusion criteria were standard contraindications to intravenous thrombolysis. Full inclusion and exclusion criteria are available in the study protocol. All patients were provided with standard stroke unit care, investigations for stroke mechanism, and stroke prevention care according to current guidelines. Patients with evidence of a vessel occlusion on baseline CT angiogram underwent a follow-up CT angiogram of the intracranial circulation at 4–8 h after randomisation in both groups to determine early recanalisation status of the occluded artery. All patients underwent routine follow-up brain imaging at 24 h with either CT or MR.

Randomisation and masking

Patients were randomly assigned (1:1) to intravenous tenecteplase versus non-thrombolytic standard of care (control). Randomisation was completed by a computer-generated minimisation algorithm, minimal sufficient balance randomisation, to ensure balance on key variables (age, sex assigned at birth, baseline NIHSS score, and time from symptom onset to randomisation).[22] These are the key variables known to influence outcome in minor stroke.[6,23,24] This algorithm was developed centrally and the details were not available to the treating sites. The first 40 patients were assigned using simple randomisation after which the minimal sufficient balance algorithm was activated. The standard distribution for randomisation was 50:50, but when an imbalance was detected, the distribution was biased to 65:35 in the direction against the imbalance; thus, there were no deterministic allocations. Randomisation was dynamic and generated in the moment via a web-based system such that the sequence of allocation was fully masked. Treatment allocation was open-label.

Procedures

Patients randomly assigned to tenecteplase received 0·25 mg/kg (maximum dose of 50 mg) as a single, intravenous bolus administered over 5–10 s immediately after randomisation. Patients assigned to control were treated with non-thrombolytic treatment. Per protocol, at minimum all patients received single agent antiplatelet therapy. Guideline-based care was recommended and this was implemented by the local investigator who chose which antithrombotic regimen should be used. Standard of care medications were given immediately after randomisation. Imaging was reviewed centrally at the University of Calgary core laboratory by a neuroradiologist (ZA) blinded to clinical information and treatment assignment. Imaging was assessed to confirm that patients met imaging entry criteria, recanalisation status was assessed on imaging done at 4–8 h from randomisation in patients with direct evidence of occlusion, and follow-up imaging was assessed for any intracranial haemorrhage, classified using the Heidelberg bleeding classification.[25]

Outcomes

The primary outcome was assessed at 90 days by an investigator blinded to the treatment allocation. The primary outcome was defined as return to baseline neurological functioning as measured by the mRS, using a sliding dichotomy approach. A responder was defined as follows: (1) if the pre-morbid mRS is 0–1, then mRS 0–1 at 90 days is a responder (good outcome) or (2) if the pre-morbid mRS is 2, then mRS 0–2 is a responder (good outcome). All raters were trained and certified in the use of the mRS.

Baseline pre-morbid mRS was assessed using the structured mRS before randomisation.[26] The 90-day mRS was rated using the structured mRS questionnaire.[26] The 90-day mRS was completed in person where possible and by telephone otherwise. The structured questionnaire has been shown to improve reliability in assessing the mRS both in person and by telephone.[26] Secondary outcomes included the absence of disability, defined as return to exact baseline mRS or better, functional independence defined as 90-day mRS 0–2, comparison of the mean 90-day mRS with linear regression using the mRS as a continuous variable, percent function on Lawton Instrumental Activities of Daily Living Scale at 90 days,[24] NIHSS at day 5 or on day of hospital discharge (whichever

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is earlier), quality of life measured at 90 days on the European Quality of Life score, five dimensions, five levels (EQ-5D-5L),[27] stroke progression and recurrent stroke,[7] all-cause mortality, and proportion of patients receiving rescue endovascular thrombectomy for the index stroke and recanalisation at 4–8 h.[28] Recanalisation was only assessed in patients with direct evidence of occlusion seen on baseline CT angiogram. Stroke progression was defined as a clear functional worsening where the imaging and clinical symptomology supported a worsening of the presenting event rather than a distinct new event.[7]

The main safety outcome was the proportion of patients with major bleeding within 48 h of randomisation. This included symptomatic intracranial haemorrhage alone as well as a composite of symptomatic intracranial haemorrhage and major extracranial haemorrhage. Symptomatic intracranial haemorrhage was defined as new intracranial haemorrhage (intracerebral, subarachnoid, intraventricular, or subdural haemorrhage) associated with clinical evidence of neurological worsening, in which the haemorrhage was judged to be the most important cause of the neurological worsening. Clinical worsening was defined by the NIHSS score worsening a minimum of 2 or more points different from baseline. The Heidelberg bleeding classification[25] was used for assessing intracranial haemorrhage on follow-up imaging. Major extracranial haemorrhage was defined as life-threatening bleeding, resulting in haemodynamic compromise or hypovolemic shock, requiring inotropic support or other means to maintain cardiac output, requiring blood transfusion of more than 2 units of packed red blood cells, or associated with a fall in haemoglobin greater than or equal to 5 g/L, temporally related to the treatment.

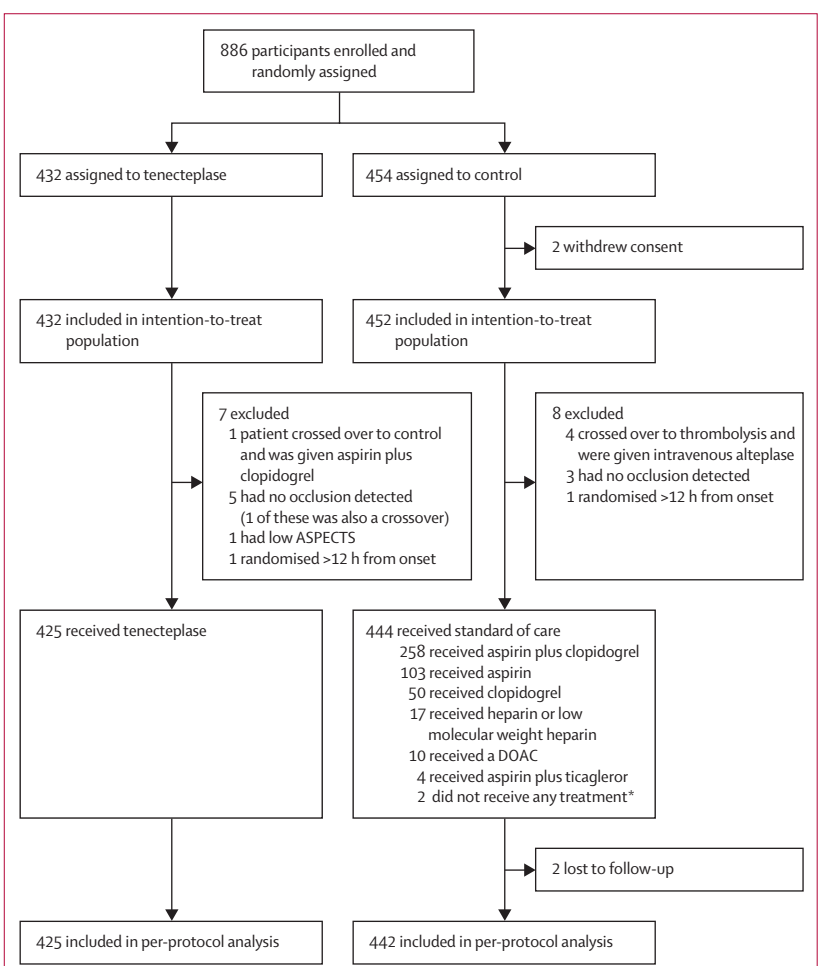

Figure 1: **Trial profile**
Exclusions data can overlap. ASPECTS=Alberta Stroke Program Early CT score. DOAC=direct oral anticoagulant. *Two patients were not given antiplatelet agents after randomisation as they had already taken antiplatelets at home.

Statistical analysis

The statistical analysis plan was finalised before database lock (on April 10, 2024). Past literature showed an effect size of 10% in the subset of minor stroke patients treated with thrombolysis.[8] Previous trials included in the meta-analysis of individuals with minor stroke did not require patients to have an intracranial occlusion. We expected that the effect size of thrombolysis would be greater in a population exclusively comprised of individuals with an intracranial occlusion. We estimated the sample based upon a predicted effective size of 9% absolute risk reduction. In TEMPO-1,[19] incidence of the primary outcome (mRS score 0–1) at 90 days was 66% in the combined 0·1 mg/kg and 0·25 mg/kg tenecteplase-treated groups. Based on this we estimated 60% good outcome in the control group and 69% in the tenecteplase-treated group for a sample size of 614 patients in each group (1228 total). Adding 4% loss to follow-up and adjusting for a single interim analysis for efficacy gave a sample size estimate of 1274 patients (637 in each treatment group).

An independent DSMC completed prespecified interim safety analyses after 100 patients and 450 patients were enrolled. There was a signal of excess deaths in the tenecteplase group at the second safety review and an additional safety analysis was completed after 650 patients were enrolled. There was one planned interim analysis after 850 patients had completed follow-up. At this interim analysis, the DSMC recommended stopping the trial.

Outcomes for patients that were lost to follow-up were imputed as non-responders in the primary outcome but no imputation was done for two patients who withdrew consent. For individual secondary outcomes on the mRS scores, no missing data were imputed. We imputed the worst possible score for the EQ-5D-5L, Lawton Index, mRS, and NIHSS scores for patients who were known to be deceased at 90 days. Missing values on the NIHSS score at 5 days or discharge were imputed using the last score carried forward principle. Missing imaging variables were not imputed.

We analysed the primary outcome in the intention-to-treat (ITT) population, defined as all patients randomly assigned to a treatment group and who did not withdraw consent to participate. The primary outcome analysis was unadjusted. Secondary analysis included primary outcome analysis adjusted for age, sex at birth, baseline NIHSS, and time from symptom onset to randomisation and all secondary outcomes analysed unadjusted and adjusted for the same variables. These variables were chosen a priori because they are of prognostic or epidemiological importance and because these variables were used in the randomised minimisation algorithm. We used generalised linear modelling with a Poisson distribution and log link function in order to directly generate risk ratios. Robust (Huber–Sandwich) standard error estimation was used. We modelled death using survival analysis. A multivariable model adjusting for age, sex at birth, onset-to-randomisation time, and baseline NIHSS score was developed using a Cox model. The proportional hazards assumption was assessed graphically and statistically. Using multiplicative interaction terms, we assessed for heterogeneity of treatment effect across the prespecified subgroups of sex (male *vs* female), timing of treatment (≤4·5 h and >4·5 h from symptom onset), age (≤80 and >80 years), how occlusion was identified (directly observed on CT angiography *vs* inferred from CT perfusion or multiphase CT angiogram), occlusion location (large vessel occlusion [internal carotid artery or middle cerebral artery (MCA)-M1] *vs* medium vessel occlusion [MCA-M2 or distal, anterior cerebral artery or distal] *vs* vertebrobasilar [includes posterior cerebral artery]), recanalisation,[28] and baseline NIHSS score before randomisation. Analyses were completed using STATA (version 18). The trial is registered at ClinicalTrials.gov, NCT02398656, and is closed to accrual.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Between April 27, 2015, and Jan 19, 2024, 886 patients were enrolled at 48 sites (appendix pp 8–10); 369 (42%) were female and 516 (58%) were male at birth. The trial's enrolment was ended by the Steering Committee after a planned interim analysis resulted in the DSMC recommending that the trial be halted for futility. At the time of the interim analysis, the conditional power to show an effect favouring tenecteplase, assuming the outcomes rates remained the same for future patients as for currently observed, was less than 1%. Two (<1%) patients withdrew consent, leaving 884 patients in the ITT population. Of 886 patients, 432 (49%) were assigned to tenecteplase and 454 (51%) to control (figure 1). Four patients had missing mRS outcomes at 90 days, two (<1%) patients

	Control (n=452)	Tenecteplase (n=432)
Demographics		
Age, years	72 (61–79)	72 (62–80)
Sex at birth		
Male	272 (60%)	244 (56%)
Female	180 (40%)	188 (44%)
Race		
White	382 (85%)	371 (86%)
Asian	42 (9%)	40 (9%)
Black	7 (2%)	6 (1%)
First Nations	5 (1%)	4 (1%)
Pacific Islander	0	1 (<1%)
Other	16 (4%)	10 (2%)
Ethnicity		
Hispanic	9 (2%)	5 (1%)
Non-Hispanic	443 (98%)	427 (99%)
Medical history		
Hypertension	261 (58%)	265 (61%)
Past smoking	176 (39%)	172 (40%)
Hyperlipidaemia	172 (38%)	180 (42%)
Diabetes	86 (19%)	82 (19%)
Past stroke	85 (19%)	72 (17%)
Atrial fibrillation	78 (17%)	91 (21%)
Ischaemic heart disease	73 (16%)	69 (16%)
Congestive heart failure	18 (4%)	16 (4%)
Chronic renal failure	17 (4%)	22 (5%)
Peripheral vascular disease	15 (3%)	13 (3%)
Past intracranial haemorrhage	1 (<1%)	3 (1%)
Clinical presentation		
NIHSS score at baseline	2 (1–3)	2 (1–3)
Haemoglobin, g/L	141 (131–152)	140 (129–150)
Glucose, mM	6 (6–7)	6 (6–7)
Creatinine, μM	84 (70–97)	82 (70–98)
ASPECTS baseline	10 (9–10)	10 (9–10)
Onset to randomisation time, min	273 (162–448)	286 (161–440)
Onset to hospital arrival time, min	151 (72–337)	148 (76–332)
Onset to treatment time, min	311 (184–495)	293 (165–453)
Occlusion location at baseline		
Large vessel occlusion*	50 (11%)	53 (12%)
Medium vessel occlusion†	245 (54%)	235 (55%)
Vertebrobasilar circulation‡	25 (6%)	20 (5%)
Focal perfusion lesion	127 (28%)	118 (27%)
No occlusion detected	3 (1%)	5 (1%)

Data are n (%) or median (IQR). Race and ethnicity was self reported; ethnicity was binary with Hispanic or non-Hispanic being the options. ASPECTS=Alberta Stroke Program Early CT score. ITT=intention to treat. NIHSS=National Institutes of Health Stroke Scale score. *Intracranial internal carotid artery, M1 segment of the middle cerebral artery. †M2 segment of the middle cerebral artery or distal, A2 segment of the anterior cerebral artery or distal. ‡Intracranial vertebral artery, basilar artery or branches, posterior cerebral artery.

Table 1: **Baseline characteristics (ITT population)**

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See **Online** for appendix

were lost to follow-up, and two withdrew consent. There were no missing baseline data.

Baseline demographic and clinical characteristics of patients were similar between the tenecteplase group and the control group (table 1). The control medication was given at a median of 17 min later than tenecteplase. Median baseline NIHSS was 2 (IQR 1–3) overall (appendix p 11) and 149 (17%) of 884 participants reported complete symptom resolution at the time of randomisation. Median onset to randomisation was 4·6 h (IQR 2·7–7·5). Most patients in the control group were treated with dual antiplatelet therapy with aspirin and clopidogrel (259 [57%] of 452) or aspirin monotherapy (106 [23%] of 452). The control population treatments are shown in the appendix (p 13).

After a median follow-up time of 92 days (IQR 85–99), the primary outcome (mRS responder analysis) occurred in 338 (75%) of 452 patients in the control group and 309 (72%) of 432 in the tenecteplase group (risk ratio [RR] 0·96, 95% CI 0·88–1·04). Secondary outcomes are shown in table 2 and in the appendix (pp 14, 15). More patients had an NIHSS of 0 in the tenecteplase group versus control (247 [57%] of 432 *vs* 226 [50%] of 452, RR 1·16; 95% CI 1·02–1·31). In the subset of patients who had direct evidence of occlusion and underwent CT angiogram at 4–8 h (515 [58%] of 884), recanalisation rates overall were higher in the tenecteplase-treated patients than in the control group (122 [48%] of 256 *vs* 56 [22%] of 259, p<0·0001; appendix p 13). For patients with large vessel occlusion, recanalisation rates were 48% (22 of 46) in the tenecteplase group and 13% (five of 40; p=0·0005) for the control group. Recanalisation improved outcomes (appendix p 17).

In safety analysis, more symptomatic intracranial haemorrhages occurred in the tenecteplase group (eight [2%]) versus control (two [<1%], RR 4·2, 95% CI 0·9–19·6; table 3, appendix p 18). Four symptomatic intracranial haemorrhages occurred in the 0–4·5 h window and six in the 4·5–12 h window (p=0·75). 34 patients received a dose of tenecteplase greater than 25 mg, one of whom had a symptomatic intracranial haemorrhage. No extracranial haemorrhages were temporally related to treatment. There were 20 deaths in the tenecteplase group and five deaths in the control group (adjusted hazard ratio 3·8; 95% CI 1·4–10·2; table 2). Seven deaths (one in the control group and six in the tenecteplase group) were related to a symptomatic intracranial haemorrhage. Other than the deaths after symptomatic intracranial haemorrhage, most deaths occurred well after treatment and were not judged to be biologically related to tenecteplase (appendix pp 15–16).

In the subgroup analyses, female patients were more likely to do better with control than their male counterparts in whom there was no treatment effect ($p_{interaction}$=0·04) and patients older than 80 years were also more likely to do better with control as compared with younger patients in whom there was no treatment effect ($p_{interaction}$=0·04). No heterogeneity of treatment effect was observed across any other subgroups (figure 2, appendix p 12).

Discussion

Among patients with minor stroke symptoms (NIHSS 0–5) and intracranial occlusion presenting within 12 h from symptom onset, we found no benefit for

	Control (n=452)	Tenecteplase (n=432)	Risk difference (95% CI)	Unadjusted RR or HR (95% CI)	Adjusted RR, RD, or HR (95% CI)*
Primary outcome					
Responder	338 (75%)	309 (72%)	−3·3% (−9·1 to 2·6)	RR 0·96 (0·88 to 1·04)	RR 0·96 (0·89 to 1·04)
Secondary outcome					
mRS 0–1 at 90 days	321 (71%)	298 (69%)	−2·4% (−8·4 to 3·7)	RR 0·97 (0·89 to 1·05)	RR 0·97 (0·90 to 1·06)
mRS 0–2 at 90 days	391 (87%)	352 (81%)	−5·4% (−0·10 to −0·01)	RR 0·94 (0·89 to 0·99)	RR 0·94 (0·89 to 1·00)
NIHSS of 0 at 5 days or discharge	226 (50%)	247 (58%)†	7·8% (1·3 to 14·4)	RR 1·16 (1·02 to 1·31)	RR 1·15 (1·03 to 1·30)
mRS return to pre-morbid function	222 (49%)	212 (49%)	0 (−6·6 to 6·5)	RR 1·00 (0·87 to 1·14)	RR 1·00 (0·88 to 1·15)
Mean mRS score at 90 days	1·11	1·27	0·16 (−0·03 to 0·34)	..	0·13‡ (−0·05 to 0·31)
Median (IQR) mRS score at 90 days	1 (0–2)	1 (0–2)	..	..	..
Lawton IADL percent functioning score (n=850)	90·8	86·4	−4·5 (−7·9 to −1·1)	..	RR −4·0 (−7·3 to −0·7)
EQ-5D-5L index score (n=854; range 0 to 1)	0·84	0·81	−0·03 (−0·07 to −0·001)	..	RR −0·03 (−0·06 to −0·001)
EQ-5D-5L VAS score (n=839; range 0 to 100)	0·76	0·73	−3·4 (−6·3 to −0·5)	..	RR −3·2 (−6·1 to −0·3)
Death at 90 days	5 (1%)	20 (5%)	..	HR 3·9 (1·4 to 10·4)	HR 3·8 (1·4 to 10·2)

Data are n (%), unless otherwise indicated. EQ-5D-5L=European Quality of Life score, five dimensions, five levels. HR=hazard ratio. ITT=intention to treat. mRS=modified Rankin Scale. NIHSS=National Institutes of Health Stroke Scale score. Lawton IADL=Lawton–Brody Instrumental Activities of Daily Living Scale index. RD=risk difference. RR=risk ratio. VAS=visual analogue scale. *Adjusted for age, sex at birth, baseline NIHSS score, and onset to randomisation time; data violate the proportional odds assumptions and so an ordinal shift analysis is not presented. †There were 427 participants with available data. ‡Adjusted difference of means.

Table 2: **Outcomes (ITT population)**

the prevention of disability after treatment with 0·25 mg/kg of tenecteplase as compared with non-thrombolytic standard of care. There was a small increased risk of symptomatic haemorrhage in patients treated with tenecteplase and more deaths at 90 days in the tenecteplase group as compared with the control group.

Over the past decade, endovascular thrombectomy has become the standard of care for stroke due to large vessel occlusion. As a result, to define the angiographic occlusions, CT angiography of the circle of Willis and neck has become routinely used in addition to non-contrast CT brain for all patients with suspected ischaemic stroke, including those with milder deficits. With increased imaging, this approach has meant that many patients who present with relatively minor deficits are now identified to have evidence of an intracranial occlusion. Based upon prior work, the fundamental premise of TEMPO-2 was that presence of an intracranial occlusion defines the minor stroke population with the highest risk of poor outcome and that reperfusion in these patients would be beneficial.

Although there were significantly more patients with early recanalisation and an NIHSS score of 0 at day 5 or discharge after tenecteplase treatment, this did not translate into improved functional outcomes at 90 days. High recanalisation rates are concordant with a recently published meta-analysis that shows higher recanalisation rates with tenecteplase compared with alteplase.[29] All other secondary outcomes did not show any benefit for tenecteplase. There was a signal of an increased rate of symptomatic intracranial haemorrhage in the tenecteplase group, but at the relatively low absolute rate of 2%, which is lower than the 3% rate seen in the tenecteplase group of the AcT study. The PRISMS study[9] found that there was a low but increased risk of symptomatic haemorrhage (3%) in minor stroke patients treated with thrombolysis using intravenous alteplase. Like the PRISMS study, the low rate of harm from symptomatic haemorrhage in TEMPO-2 was not counteracted by a significant improvement in functional outcomes at 90 days. The symptomatic haemorrhage rate does not fully account for the absence of benefit at 90 days with tenecteplase. Similar to other trials[17,18] we allowed patients to be enrolled out to 12 h from symptom onset. We did not see any increase in the symptomatic intracranial haemorrhage rate in patients treated after 4·5 h versus before 4·5 h. The subgroup of patients treated at 4·5–12·0 h showed weak evidence of better outcomes with thrombolysis as compared with those treated before 4·5 h. This suggests that the 12 h window for TEMPO-2 did not explain the absence of benefit seen from tenecteplase.

Patients in the non-thrombolytic control group of the TEMPO-2 study did better than expected. This might be the result of chance, patient selection, greater penetrance of dual antiplatelet therapy in the standard-of-care group,

	Control (n=452)	Tenecteplase (n=432)	p value
Serious adverse event	80 (18%)	100 (23%)	0·045
Stroke progression	33 (7%)	35 (8%)	0·71
Stroke recurrence	15 (3%)	16 (4%)	0·86
Symptomatic intracranial haemorrhage	2 (<1%)	8 (2%)	0·059
Death after symptomatic intracranial haemorrhage within 90 days	1 (<1%)	6 (1%)	1
Any haemorrhage on follow-up imaging	40 (9%)	62 (14%)	0·02
Rescue endovascular thrombectomy for index stroke	10 (2%)	15 (3%)	0·31
Death within 5 days	1 (<1%)	8 (2%)	0·018
Death at 90 days	5 (1%)	20 (5%)	0·0018
Aspiration pneumonia	2 (<1%)	6 (1%)	0·17
Atrial fibrillation	3 (1%)	4 (1%)	0·72
Congestive heart failure	1 (<1%)	5 (1%)	0·12
Seizure	3 (1%)	3 (1%)	1
Urinary tract infection	4 (1%)	2 (<1%)	0·69

ITT=intention to treat.

Table 3: **Safety events (ITT population)**

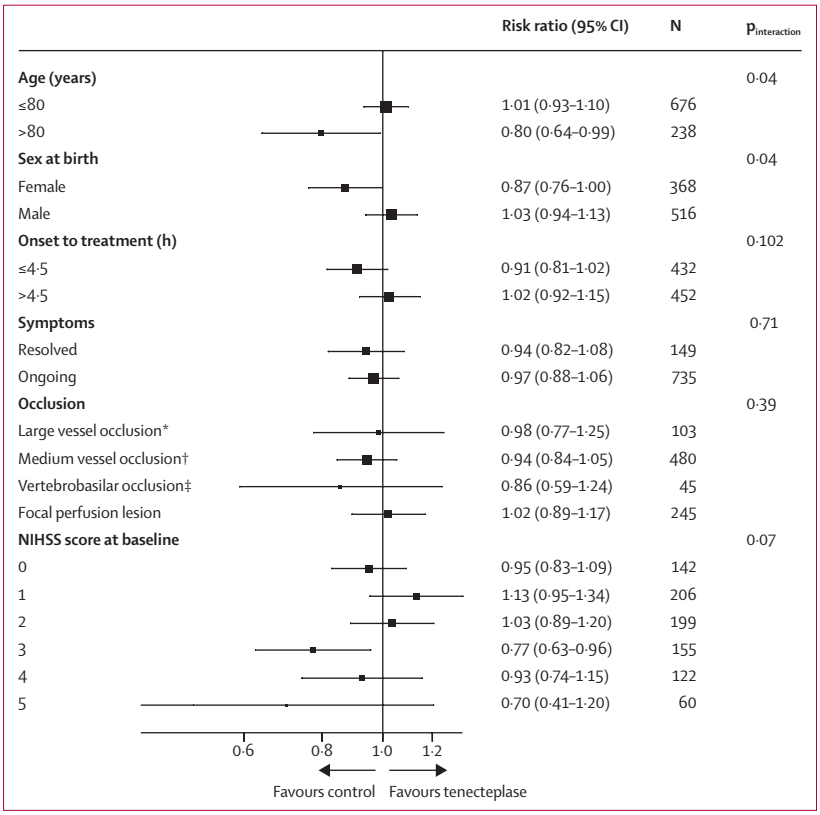

Figure 2: **Forest plot of effect size by subgroup**
Adjusted for sex at birth, age, time from onset and baseline NIHSS. NIHSS=National Institutes of Health Stroke Scale. *Intracranial internal carotid and M1 segment of the middle cerebral artery. †M2 segment of the middle cerebral artery or distal, A2 segment of the anterior cerebral artery or distal. ‡Including all branches of the posterior cerebral artery.

or better overall stroke care. The recanalisation rate in the control group was 22% overall in the study and in large vessel occlusion it was 13% in the non-thrombolytic control group, highlighting that antiplatelet treatment is still an active treatment. Despite the reported high recanalisation rates in the tenecteplase group (48%), there was no change in the rate of stroke progression between groups, with an 8% rate of progression seen overall in the study. We know from previous work that patients with minor stroke and with intracranial occlusion are at risk of both progression and disability.[23] It could be that medical care (eg, intravenous fluids and antiplatelet therapy) in this patient population reduced the rate of stroke progression in both groups. A rate of recanalisation of 48% might simply not be high enough to influence stroke progression and outcomes. Most of these patients are likely to have excellent collaterals and therefore good supportive care may have improved outcomes in both groups. It is also possible that the neurological deficit is so minimal that vessel recanalisation cannot make patients detectably much better using the outcome assessments we currently use. Consistent with the low rate of stroke progression, rescue endovascular thrombectomy rates were low in both treatment groups in the study. Studies examining the use of endovascular thrombectomy in the subset of patients with minor stroke and large vessel occlusion in trials such as the ENDOLOW trial (NCT04167527) are ongoing.

Overall mortality was low with 25 (3%) deaths but mortality was higher in the tenecteplase group. Most of these deaths occurred late and were not temporally related to study drug, with seven of 25 deaths associated with a symptomatic intracranial haemorrhage (six in the tenecteplase group and one in the control group). The rates of stroke progression, stroke recurrence, and rescue endovascular thrombectomy were similar between groups. The increase in late deaths is unexplained and has not been seen in previous studies. Because of the low absolute numbers, this could be a chance finding.

Strengths of this study were that it was a large, well conducted, investigator-initiated, international, multicentre, randomised trial with near complete follow-up. Limitations were that the study took nearly 9 years to complete due to external factors including a global pandemic and drug supply issues. Although patients were eligible to be enrolled on the basis of a focal perfusion lesion on CT imaging, reflecting real-world practice, it is possible that these lesions are qualitatively different from those with an observable arterial occlusion. However, we did not see any treatment interaction based on overt evidence of an occlusion versus a focal perfusion lesion, suggesting that this was not the reason for the absence of benefit with tenecteplase. Patients in the non-thrombolytic control group principally received antiplatelet therapy, but treatment was not one single comparator. This pragmatic choice reflects clinical practice and might make the trial results more generalisable. Although we did not collect data in a parallel registry, guideline-based practice is to offer thrombolysis to patients who have disabling symptoms in the judgement of the treating physician and patient, and therefore we predict, but do not have data to support, that a majority of patients in the TEMPO-2 trial did not have disabling symptoms at the time of consent to the study. Because of the long duration of the study, there is the potential for selection bias in study inclusion and the possibility that secular changes in care affected outcomes.

In summary, we did not find any evidence of benefit in treating minor stroke patients with intracranial occlusion with tenecteplase as compared with non-thrombolytic control.

Contributors

SBC and MDH prepared the first draft of the report. SBC, CK, and MDH conceptualised the study design. SBC and MDH wrote the statistical analysis plan. MDH was the lead statistician with SBC, and CK providing additional data management and statistical support, and all had access to all the data. SBC, CK, NSi, and MDH had access to and verified the underlying study data. MH led the imaging core laboratory with ZA, MG, SBC, and BKM providing support. SBC and MDH participated in data analysis and interpretation. All authors participated in patient enrolment, trial execution and management, and critically reviewed the report and approved the final version before submission. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Declaration of interests

SBC received grant funding from the Canadian Institutes of Health Research (CIHR), Heart and Stroke Foundation of Canada (HSFC), and the British Heart Foundation (BHF) to complete the TEMPO-2 study. Boehringer Ingelheim provided off-the-shelf study drug (tenecteplase) for the study. JFA has received public research grants from the Spanish Ministry of Science, the regional health department, the European Commission, and a private research grant from AstraZeneca. He has received consultant or speaker fees from Pfizer-BMS, Medtronic, and Amgen, and travel support from Daiichi-Sankyo. KSB reports speaker fees from Boehringer Ingelheim and AstraZeneca. LC has received consulting or speakers fees from Roche. JF reports speaker fees from AstraZeneca, Bayer, BMS, Boehringer Ingelheim, Pfizer, Sankyo, and Daiichi. AG reports a grant from Microvention; speaker fees from Alexion, Biogen, and Servier Canada; and stock options for SnapDx and Collavidence. DG reports honoraria from Boehringer Ingelheim, Ipsen, and Eisai. MG reports grant funding from Medtronic and Cerenovus and consulting fees from Mentice, CSL Behring, MicroVention, and Medtronic. TSF participated in an advisory board for Roche. FL reports speaker fees and travel support from Boehringer Ingelheim. BKM has been paid honoraria from Boehringer Ingelheim and Roche. MM has received honoraria from Boehringer Ingelheim for participation at an advisory board on the approval of tenecteplase for treatment of acute ischaemic stroke. SOM has received speaker fees from Boehringer, Medtronic, Penumbra, Bayer, Pfizer, Novartis, Novo Nordisk, Servier, and Daiichi Sankyo. KWM reports grant funding from the BHF, and the Stroke Association; consultancy fees from Boehringer Ingelheim, Biogen, IschaemaView; lecture fees from Boehringer Ingelheim, IschaemaView, and Brainomix; and non-financial support (drug supply for ATTEST-2 trial) from Boehringer Ingelheim. MWP is on Boehringer Ingelheim Advisory Board for Metalyse in stroke. AP received speaker fees from Boehringer Ingelheim and travel support from AstraZeneca for attending a meeting. OMP-N has received speaker fees from Boehringer Ingelheim and AstraZeneca. RS is part funded by the UCLH Biomedical Research Centre. DS reports an unrestricted educational grant from Boehringer Ingelheim. MIW reports sponsorship or financial assistance to support attendance at scientific meetings from Boehringer Ingelheim, Bristol Myers Squibb, Daiichi Sankyo UK, and Bayer; honoraria for lectures given at education meetings from Boehringer Ingelheim, Bristol Myers Squibb, and AstraZeneca; and payment for participation in advisory panels from Boehringer Ingelheim and Daiichi Sankyo UK.

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Data sharing

Data collected for the study, including de-identified individual participant data and a data dictionary defining each field in the set, can be made available to others on reasonable request and after signing appropriate data sharing agreements. Please send data access requests to scoutts@ucalgary.ca. Such requests must be approved by all the respective ethics boards and appropriate data custodians.

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Advances and challenges in the acute treatment of minor ischaemic stroke

Although characterised by mild neurological symptoms, typically ranging from 0 to 5 on the National Institutes of Health Stroke Scale (NIHSS), minor ischaemic stroke is associated with frequent long-term disability and a significant risk of stroke recurrence.[1] Research on the treatment of minor stroke has progressed along two parallel paths: acute revascularisation[2–4] and early secondary prevention.[5] Navigating these pathways can sometimes lead to complex and confusing decision-making processes.

Acute revascularisation with intravenous thrombolysis may be a straightforward choice for patients with minor ischaemic stroke and measurable disabling deficits.[6,7] This approach is supported by an individual patient data meta-analysis of nine randomised trials of alteplase (a recombinant tissue plasminogen activator) versus placebo or open control.[3] The option to pursue revascularisation might be less straightforward in patients with non-disabling or resolved symptoms, but with evidence of vessel occlusion or perfusion lesions on neuroimaging. Although revascularisation might seem futile in the absence of significant deficits, it can still be considered to prevent potential clinical deterioration. In this regard, one trial showed no superiority of intravenous alteplase over aspirin in patients with minor non-disabling symptoms,[4] and another one demonstrated the non-inferiority of aspirin plus clopidogrel over intravenous alteplase in a similar population of patients with minor non-disabling symptoms.[2] Of note, none of these trials used tenecteplase, which holds some additional benefits compared with alteplase.[8] Additionally, even after revascularisation, patients can remain at risk of stroke progression and early recurrence. Thus, for those with non-cardioembolic events (who constitute most ischaemic stroke cases), early short-term dual antiplatelet treatment can be considered.[9] Unfortunately, patients who received urgent revascularisation were excluded from landmark trials on early dual antiplatelet treatment.[5]

In *The Lancet*, Shelagh Coutts and colleagues report the results of the TEMPO-2 trial involving 48 hospitals in Australia, Austria, Brazil, Canada, Finland, Ireland, New Zealand, Singapore, Spain, and the UK, providing further insight into the comparison of intravenous thrombolysis with standard care in acute minor ischaemic stroke.[10] 886 patients were enrolled (369 [42%] female, 517 [58%] male; approximately 85% of participants were White) and 454 (51%) were assigned to non-thrombolytic standard of care (control) and 432 (49%) to intravenous tenecteplase. TEMPO-2 was an investigator-initiated, multicentre, prospective, randomised, open-label with blinded endpoint assessment, controlled trial that tested the superiority of intravenous tenecteplase (0·25 mg/kg) over non-thrombolytic standard care in patients with minor ischaemic stroke, defined as NIHSS 0–5, and intracranial occlusion or focal perfusion lesion within 12 h of symptom onset. The trial was halted early due to futility after enrolling 886 patients (72% of the planned sample size). At 90 days, the number of patients meeting the primary outcome (ie, return to baseline functioning on premorbid modified Rankin Scale score) was 309 (72%) in the tenecteplase group and 338 (75%) in the control group (risk ratio 0·96, 95% CI 0·88–1·04, p=0·29).

TEMPO-2 included patients with minor stroke and disabling symptoms, non-disabling symptoms, or even completely resolved symptoms (149 [17%] of 884 participants). The trial's overall inconclusive findings might stem from the heterogeneity of the included population. Indeed, patients with disabling symptoms can experience improvement with revascularisation, whereas the other categories of patients might experience minimal or absent improvement. Additionally, the study's adoption of an extended 12 h treatment time window without the need to show persistent penumbral tissue might have contributed to the negative findings.

TEMPO-2 showed substantially higher rates of recanalisation at 4–8 h with tenecteplase as compared with standard of care (122 [48%] of 256 *vs* 56 [22%] of 259, p<0·0001). However, recanalisation was only assessed on a subpopulation of patients (ie, 515 [58%] of 886) and this encouraging result did not translate into improved functional outcomes. In

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addition, tenecteplase was associated with a four-fold increased risk of death at 90 days (20 deaths [5%] in the tenecteplase group *vs* five deaths [1%] in the control group; adjusted hazard ratio 3·8; 95% CI 1·4–10·2, p=0·0085), which could not be explained solely by the slight increase in the haemorrhagic risk—eight (2%) symptomatic intracranial haemorrhages in the tenecteplase group versus two (<1%) in the control group (relative risk 4·2; 95% CI 0·9–19·7, p=0·059)—that was low compared with literature data.[11]

It is noteworthy that TEMPO-2 took 9 years to be completed, which might suggest underlying challenges. Patients were not eligible if, in the judgement of the physician, routine intravenous thrombolysis was warranted. Thus, centres might have hesitated to recruit patients and withhold an established treatment from those with minor disabling strokes. Additionally, over the extended study period, there was a shift in the standard of care for minor ischaemic stroke, influenced by clinical trials and guidelines advocating for early dual antiplatelet therapy for non-cardioembolic minor ischaemic stroke.[5,9] A suboptimal proportion of patients in the standard care group (259 [57%] of 452) received dual antiplatelet treatment, whereas the proportion of patients receiving the same treatment in the tenecteplase group is unknown; this could have substantially influenced the 3-month outcomes.

TEMPO-2 presents clinically relevant information, but the study will not change our practice. Nevertheless, its inherent value lies in improving our understanding of the complex scenario of minor ischaemic stroke treatment, which could impact the design of future studies in the field. Different minor ischaemic stroke populations pose different therapeutic challenges. Thus, merging those populations in a single trial, although valuable for feasibility, might not yield conclusive results. Furthermore, observational data suggest a benefit of endovascular treatment for minor stroke with large vessel occlusion[12] and dedicated randomised controlled trials are ongoing (MOSTE, NCT03796468 and ENDO-LOW, NCT04167527). Additionally, as early dual antiplatelet treatment with appropriate loading doses is now the recommended treatment for minor stroke,[5,9] any study should use this treatment as active comparator for non-cardioembolic strokes. It is also worth noting that although revascularisation therapies are an acute treatment for minor disabling stroke and dual antiplatelet treatment is an early secondary prevention strategy, their combination has not been sufficiently explored. In fact, intravenous thrombolysis and early short-term dual antiplatelet treatment are not mutually exclusive and can potentially be combined to optimise outcomes for patients with minor ischaemic stroke.

In conclusion, TEMPO-2, despite not proving that tenecteplase is better than the standard of care for the acute treatment of minor stroke, confirms that tenecteplase is associated with a high rate of recanalisation. Fast recanalisation with intravenous thrombolysis, endovascular treatment, proper patient selection, and combination with dual antiplatelet treatment or early initiation of anticoagulants may translate into tangible clinical benefits for patients with minor ischaemic stroke, which should be tested in future studies.

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Supplementary appendix

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TEMPO-2

Supplementary Appendix

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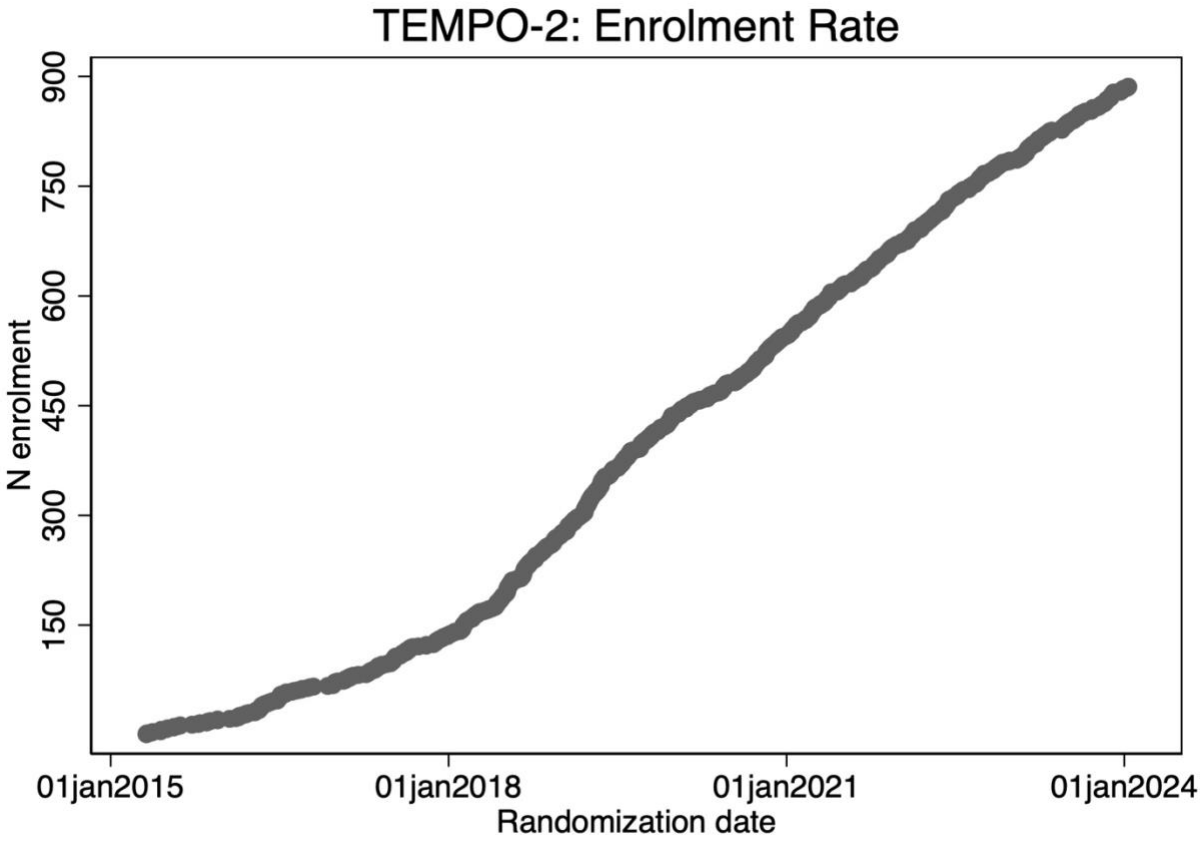
Supplementary table and figures

Figure S1: Enrolment rate



TEMP2

Table S1: Enrolment by Site

Site name	Freq.	
Calgary	253	**********
BoxHill	66	**********
VancouverVGH	60	**********
Edmonton	58	**********
Adelaide	42	********
Barcelona	38	*******
MelbourneRMH	35	*******
Kingston	32	******
TorontoSHSC	30	******
Glasgow	28	*****
Valladolid	23	****
Ottawa	21	****
Chester	19	****
Helsinki	13	***
GoldCoast	12	**
PortoAllegre	12	**
London	11	**
ViennaStJG	11	**
Saskatoon	11	**
Montreal_McGill	10	**
TorontoTWH	9	**
ViennaAKH	9	**
NewcastleNSW	8	**
LondonCCH	8	**
LondonUCL	7	*
VancouverRCH	6	*
Perth	5	*
LondonSGH	5	*
Hamilton	4	*
Stoke	4	*
LondonKCH	4	*
Fortaleza	4	*
QuebecCity	3	*
TorontoSMH	3	*
Girona	3	*
Victoria	2	
DublinBeaumont	2	
Oxford	2	
Belfast	2	
Cambridge	2	
Riberiao_Preto	2	
RedDeer	1	
DublinMater	1	
SingaporeTTSH	1	
Christchurch	1	
Birmingham	1	
Nottingham	1	
Botucatu	1	
Total	886	

Table S2: Enrolment by Country

	Control	Tenecteplase	Total
N	454 (51.2%)	432 (48.8%)	886 (100.0%)
Country			
Canada	263 (57.9%)	251 (58.1%)	514 (58.0%)
Australia	81 (17.8%)	87 (20.1%)	168 (19.0%)
United Kingdom	43 (9.5%)	40 (9.3%)	83 (9.4%)
Spain	33 (7.3%)	31 (7.2%)	64 (7.2%)
Austria	11 (2.4%)	9 (2.1%)	20 (2.3%)
Brazil	12 (2.6%)	7 (1.6%)	19 (2.1%)
Finland	8 (1.8%)	5 (1.2%)	13 (1.5%)
Ireland	2 (0.4%)	1 (0.2%)	3 (0.3%)
New Zealand	1 (0.2%)	0 (0.0%)	1 (0.1%)
Singapore	0 (0.0%)	1 (0.2%)	1 (0.1%)

There was no heterogeneity of treatment effect by country, either individually (χ^2 test heterogeneity, p = 0.8179) or when dichotomized as Canada vs. Other (χ^2 test heterogeneity, p = 0.9608).

Table S3: Enrolment by NIHSS and mRS score at baseline

	Control	Tenecteplase	Total	P
N (%)	454 (51.2%)	432 (48.8%)	886 (100.0%)	
NIHSS score at baseline				
0	68 (15.0%)	74 (17.1%)	142 (16.0%)	0.7211
1	115 (25.3%)	92 (21.3%)	207 (23.4%)	
2	99 (21.8%)	100 (23.1%)	199 (22.5%)	
3	81 (17.8%)	74 (17.1%)	155 (17.5%)	
4	59 (13.0%)	63 (14.6%)	122 (13.8%)	
5	32 (7.0%)	29 (6.7%)	61 (6.9%)	
mRS score at baseline				
0	356 (78.4%)	336 (77.8%)	692 (78.1%)	0.8590
1	59 (13.0%)	61 (14.1%)	120 (13.5%)	
2	39 (8.6%)	35 (8.1%)	74 (8.4%)	

NIHSS = National Institutes of Health Stroke Scale; mRS = modified Rankin Scale

P-value refers to a Fisher's exact test

Table S4: Outcome Effect Size by NIHSS and mRS score at baseline

NIHSS score at baseline	Risk ratio (95% CI)	P
0	0.93 (0.81-1.08)	0.0544
1	1.13 (0.95-1.34)	
2	1.04 (0.89-1.22)	
3	0.76 (0.62-0.94)	
4	0.92 (0.73-1.15)	
5	0.71 (0.42-1.21)	
mRS score at baseline		
0	1.00 (0.92-1.09)	0.1098
1	0.79 (0.59-1.07)	
2	0.74 (0.51-1.09)	

NIHSS = National Institutes of Health Stroke Scale; mRS = modified Rankin Scale

P-value refers to a χ^2 test of heterogeneity.

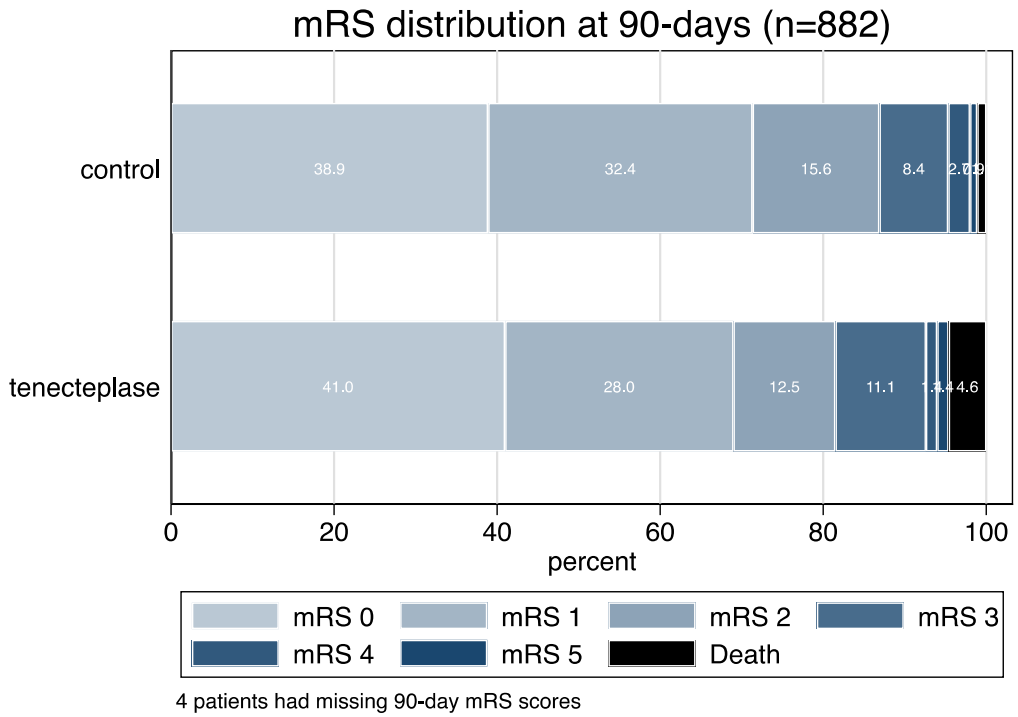
Effect size estimates are unadjusted.

Table S5: Control arm treatment

Control group non-thrombolytic treatment (n=452)	
ASA + clopidogrel	259 (57·3%)
ASA + ticagrelor	4 (0·9%)
ASA	106 (23·5%)
clopidogrel	51 (11·3%)
intravenous heparin or low-molecular weight heparin	17 (3·8%)
direct oral anticoagulant	11 (2·4%)
intravenous alteplase	4 (0·9%)

ASA = acetylsalicylic acid

Figure S2: Distribution of the modified Rankin Scale scores at 90 days, intention-to-treat population.



Scores range from 0 to 6, with 0 indicating no symptoms, 1 no clinically significant disability, 2 slight disability, 3 moderate disability, 4 moderately severe disability, 5 severe disability, and 6 death.

Table S6. Outcomes (Per Protocol population)

	Control (n=444)	Tenecteplase (n=425)	Risk Difference (CI_{95})	Risk Ratio (Unadjusted) (CI_{95})	Risk Ratio (Adjusted*) (CI_{95})
Primary outcome					
Responder	334 (75.2%)	305(71.8%)	-3.5% (-9.3 to +2.4)	0.95 (0.88-1.03)	0.96 (0.89-1.04)
Secondary outcome					
mRS 0-1 at 90 days	317 (71.7%)	294 (69.2%)	-2.5% (-8.6 to +3.5)	0.96 (0.88-1.05)	0.97 (0.90-1.06)
mRS 0-2 at 90 days	387 (87.7%)	347 (81.6%)	-5.9% (-0.11 to -0.01)	0.93 (0.88-0.99)	0.94 (0.89-0.99)
NIHSS = 0 at 5 days or discharge	221 (49.8%)	244 (58.1%)	8.3% (1.7 to 14.9)	1.17 (1.03-1.32)	1.16 (1.04-1.31)
mRS return to pre-morbid function	221 (49.8%)	209 (49.2%)	0.0% (-7.2 to +6.0)	0.98 (0.86-1.13)	0.99 (0.87-1.14)
					Risk Difference (adj)
mean mRS score at 90 days (n=882)	1.09	1.27	0.18 (-0.006 to +0.37)	---	0.15 (-0.03 to +0.33)
Lawton IADL percent functioning (n=850)	91.1	86.3	-4.8 (-8.2 to –1.4)	---	-4.2 (-7.5 to -0.88)
EQ5D-5L index (n=854)	0.84	0.81	-0.03 (-0.07 to -.001)	---	-0.03 (-0.06 to -0.002)
EQ5D-5L VAS (n=839)	0.76	0.73	-0.03 (-0.06 to -0.003)	---	-0.03 (-0.06 to -0.001)
				Hazard ratio	Hazard ratio (adj)
Death at 90 days	5 (1.1%)	20 (4.7%)	---	3.8 (1.4-10.4)	3.8 (1.4-10.2)

*Adjusted for age, sex, NIHSS score at baseline, onset to randomisation time.

The data violate the proportional odds assumptions and so an ordinal shift analysis (proportional odds model) is not presented. CI_{95} = 95% confidence interval; mRS = modified Rankin Scale score; NIHSS = National Institutes of Health Stroke Scale score; Lawton IADL = Lawton-Brody Instrumental Activities of Daily Living Scale index; EQ5D-5L index = European Quality of Life score index; EQ5D-5L VAS = European Quality of Life visual analog scale health score.

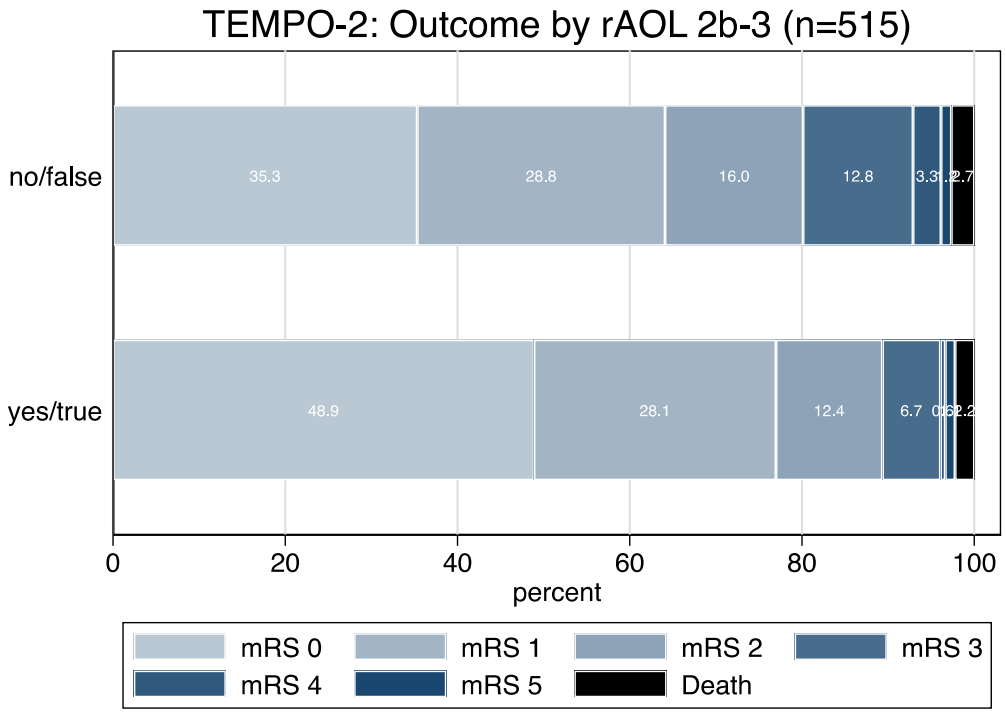
Table S7: Recanalization of occluded intracranial artery detected at baseline

Recanalization of direct occlusion	Control	Tenecteplase	p
Overall	56/259 (21·6%)	122/256 (47·7%)	<0·001
Large vessel occlusion (ICA and M1-MCA)	5/40 (12·5%)	22/46 (47·8%)	<0·001
Medium vessel occlusion (M2-MCA or distal, ACA or distal)	49/198 (24·7%)	96/192 (50·0%)	<0·001
Vertebrobasilar (includes PCA)	2/21 (9·5%)	4/18 (22·2%)	0·387

ICA = internal carotid artery, MCA – middle cerebral artery, ACA = Anterior Cerebral artery, PCA = posterior cerebral artery.

Recanalisation assessment was only completed in patients with direct evidence of occlusion. Scans were scored using the revised Arterial Occlusive Lesion score (rAOL) This is scored as follows: 0, primary occlusive thrombus remains same; 1, debulking of proximal part of the thrombus but without any recanalisation; 2a, partial or complete recanalisation of the primary thrombus with occlusion in major distal vascular branch; 2b, partial or complete recanalisation of the primary thrombus with occlusion in minor distal vascular branch, or partial recanalisation of the primary thrombus with no thrombus in the vascular tree at or beyond the primary occlusive thrombus; and 3, complete recanalisation of the primary occlusive thrombus with no clot in the vascular tree beyond. Recanalisation was scored as true if the assessment was rAOL 2b and 3.

Figure S3: Outcomes by recanalization status



Improved outcomes by recanalization (rAOL score 2b-3) among patients with proven baseline occlusions and follow-up CTA imaging. mRS 0-2 at 90 days = 89% (recan group) vs 80% (no recan group). RR = 1.11 (CI_{95} 1.03-1.20)

Table S8. Radiological and Clinically Significant Intracranial Hemorrhage

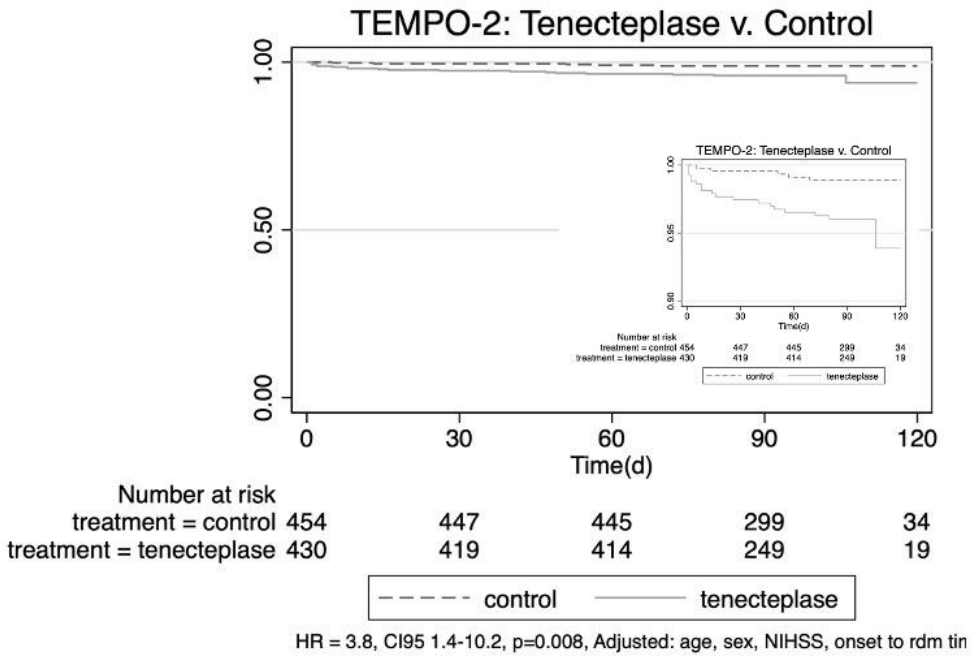
	Control	Tenecteplase
N	454 (51.2%)	432 (48.8%)
No available 24-hour scan	17	1
ECASS and Heidelberg radiological hemorrhage type		
None	397 (90.8%)	369 (85.6%)
HI type 1 (Heidelberg Class 1a)	19 (4.3%)	23 (5.3%)
HI type 2 (Heidelberg Class 1b)	11 (2.5%)	14 (3.2%)
PH type 1 (Heidelberg Class 1c)	1 (0.2%)	4 (0.9%)
PH type 2 (Heidelberg Class 2)	2 (0.5%)	11 (2.6%)
rPH (Heidelberg Class 3a)	3 (0.7%)	8 (1.9%)
SAH (Heidelberg Class 3c)	4 (0.9%)	2 (0.5%)
Clinically defined - Symptomatic ICH		
yes/true	2 (0.4%)	8 (1.9%)

HI = hemorrhagic infarction; PH = parenchymal hematoma; rPH = remote PH; SAH = subarachnoid hemorrhage; ICH = intracranial hemorrhage; ECASS = European Cooperative Acute Stroke Study

There were 4 cases of intraventricular hemorrhage (Heidelberg Class 3b) and 1 case of subdural hemorrhage (Heidelberg Class 3d). Each of these cases occurred concurrent with a PH type 2 hemorrhage (Heidelberg Class 2) and all 5 were considered to be clinically symptomatic ICH. In addition to the 6 instances of isolated SAH in the table above, there were 12 additional occurrences of SAH co-occurring with other hemorrhage types.

There were a total of 18 missing or uninterpretable scans at follow-up, 17 in the Control arm and 1 in the Tenecteplase arm.

Figure S4: Mortality Survival Analysis



Survival curves derived from a Cox proportional hazards model showing mortality by randomized treatment, adjusted for age, sex, baseline NIHSS score and time from stroke onset to randomization. The inset shows the same graph with a magnified Y-axis. The proportional hazards assumption was assessed and found to be valid.

TEMPO2

Table S9: Causes of death

Cause of Death	Group	Days to death
Day 0 to 5		
Symptomatic ICH	Tenecteplase	0
Symptomatic ICH (multiple remote ICH)	Tenecteplase	0
Symptomatic ICH	Tenecteplase	1
Symptomatic ICH	Tenecteplase	2
Symptomatic ICH. Subdural hemorrhage	Control	5
Progression of stroke treated with EVT. Palliated.	Tenecteplase	1
Progression of stroke treated with EVT. Palliated.	Tenecteplase	1
In-hospital sudden cardiac arrest. No clear cause.	Tenecteplase	2
Recurrent ischemic stroke	Tenecteplase	5
Day 6 and thereafter		
Symptomatic ICH	Tenecteplase	8
Recurrent ischemic stroke	Tenecteplase	8
Progression of stroke	Control	13
Upper GI bleed, aspiration pneumonia	Tenecteplase	14
Progression of stroke	Tenecteplase	16
Pancreatic adenocarcinoma	Tenecteplase	26
Leg fracture, dyspnea. Palliated.	Tenecteplase	40
Complications after Symptomatic ICH	Tenecteplase	47
Progression of stroke	Tenecteplase	49
Spindle cell malignancy, metastatic	Control	51
Sepsis due to bowel ischemia	Tenecteplase	55
Pancreatic adenocarcinoma	Control	57
Covid19 pneumonia	Control	69
Recurrent stroke, bladder cancer. Palliated.	Tenecteplase	72
Recurrent large ischemic stroke. Palliated.	Tenecteplase	80
Failure to thrive	Tenecteplase	106

Causes of death derived from serious adverse event narratives. In the first 5 days, 5 of 9 early deaths were associated with symptomatic intracranial hemorrhage. Later deaths, well after treatment, appear to have no clear pattern and we suspect that it is a chance finding that more deaths (12 of 16 events) occurred in the tenecteplase group.

Title Page

TEMPO-2 – A randomized controlled trial of TNK-tPA versus standard of care for minor ischemic stroke with proven occlusion

Long title: Multicentre, prospective randomized open label, blinded-endpoint (PROBE) controlled trial of thrombolysis with low dose Tenecteplase (TNK-tPA) versus standard of care in the prevention of disability at 3 months in minor ischemic stroke with proven acute symptomatic occlusion

Protocol Version 3.3 24th March 2017

Table of Contents

Protocol synopsis

	TEMPO-2 trial
Objectives	The primary objective: to demonstrate the efficacy of using TNK-tPA to treat minor ischemic stroke with proven arterial occlusion.
Experimental Design	A Phase 3, prospective, randomized controlled, open-label with blinded outcome assessment (PROBE) controlled trial.
Population	Up to 1274 male and female adult patients Inclusion Criteria 1. Acute ischemic stroke in an adult patient (18 years of age or older) 2. Onset (last-seen-well) time to treatment time ≤ 12 hours. 3. TIA or minor stroke defined as a baseline NIHSS ≤ 5 at the time of randomization. Patients do not have to have persistent demonstrable neurological deficit on physical neurological examination. 4. Any acute intracranial occlusion or near occlusion (TICI 0 or 1) (MCA, ACA, PCA, VB territories) defined by non-invasive acute imaging (CT angiography or MR angiography) that is neurologically relevant to the presenting symptoms and signs. An acute occlusion is defined as TICI 0 or TICI 1 flow.[1] Practically this can include a small amount of forward flow in the presence of a near occlusion AND; Delayed washout of contrast with pial vessels on multiphase CTA in a region of brain concordant with clinical symptoms and signs OR, Any area of focal perfusion abnormality identified using CT or MR perfusion – e.g. transit delay (TTP, MTT or TMax), in a region of brain concordant with clinical symptoms and signs. 5. Pre-stroke independent functional status – mRS ≤ 2. 6. Informed consent. 7. Patients can be treated within 90 minutes of the first slice of CT (or MRI) Exclusion Criteria 1. Hyperdensity on NCCT consistent with intracranial hemorrhage. 2. Large acute stroke ASPECTS < 7 visible on baseline CT scan. 3. Core of established infarction. No large area (estimated >10 cc) of grey matter hypodensity at a similar density to

	white matter or in the judgment of the enrolling neurologist is consistent with a subacute ischemic stroke. 4. Patient has a severe or fatal or disabling illness that will prevent improvement or follow-up or such that the treatment would not likely benefit the patient. 5. Pregnancy. 6. Planned thrombolysis with intravenous tPA or endovascular acute treatment. 7. In-hospital stroke unless these patients are at their baseline prior to the stroke. 8. Commonly accepted exclusions for medical thrombolytic treatment that potentially put the patient at an increased risk of bleeding. Country specific product monographs and stroke thrombolysis guidelines should be consulted. These are commonly relative contraindications (i.e. the final decision is at the discretion of the treating physician) but for the purposes of TEMPO-2 include the following: a. Significant bleeding disorder either at present or within the past 6 months b. International normalized ratio > 1.7 or known full anticoagulation with use of any standard or direct oral anticoagulant therapy with full anticoagulant dosing. [DVT prophylaxis dosing shall not prohibit enrolment]. For low molecular weight heparins (LMWH) more than 48 hours off drug will be considered sufficient to allow trial enrollment. For direct oral anticoagulants; in patients with normal renal function more than 48 hours off drug will be considered sufficient to allow trial enrollment. Patients on direct oral anticoagulants who have any degree of renal impairment should not be enrolled in the trial unless they have not taken a dose of the drug in the last 5 days. c. Dual antiplatelet therapy does not prohibit enrolment. [For patients who are known not to be taking anticoagulant therapy it is not necessary to wait for coagulation lab results (e.g. PT, PTT) prior to treatment] d. Prolonged cardiopulmonary resuscitation (> 2 minutes) within the past 2 weeks e. Acute pericarditis and/or subacute bacterial endocarditis f. Acute pancreatitis

	g. Severe hepatic dysfunction, including hepatic failure, cirrhosis, portal hypertension (oesophageal varices) and active hepatitis h. Neoplasm with increased bleeding risk i. Arterial aneurysm and known arterial/venous malformation j. Patients who have been acutely treated with GP2b3a inhibitors. k. Arterial puncture at a non-compressible site in the previous seven days l. Clinical stroke or serious head or spinal trauma in the preceding three months that would normally preclude use of a thrombolytic agent. m. History of intracranial hemorrhage, subarachnoid hemorrhage or other brain hemorrhage that would normally preclude use of a thrombolytic agent. n. Major surgery within the last 3 months that the treating physician considers a contraindication to thrombolytic therapy. o. Severe hypo- (< 50 mg/dL or 2.8mmol/l)or hyperglycemia (>400 or 22.2mmol/l) p. Hypertension refractory to anti-hypertensive medication such that target blood pressure <185/110 cannot be achieved before treatment. q. Known platelet count below 100,000 per cubic millimeter. [Treatment should not be delayed to wait for platelet count unless thrombocytopenia is known or suspected] *r.* Gastrointestinal or genitourinary bleeding within the past 3 months that would normally preclude use of a thrombolytic agent.
Regions	North America, Europe, Asia, Australasia
Treatments	Patients will be randomized to TNK-tPA or standard of care. In the intervention group TNK-tPA is given as a single, intravenous bolus (0.25mg/Kg) immediately upon randomization. Maximum dose 50mg. The control group will receive antiplatelet agent(s) as decided by the treating physician. Antiplatelet agent(s) choice will be at the treating physician's discretion.
Duration of Treatment	One treatment delivered acutely with a 90-day follow-up period.
Evaluation Criteria	**Primary outcome:** Return to baseline neurological functioning as measured on the mRS. Analysis will be a responder analysis where return to baseline level of neurological functioning is defined as follows: If pre-morbid mRS is 0-1 then mRS 0-1 at 90 days is a good

	outcome. If pre-morbid mRS is 2 then mRS 0-2 is a good outcome. Pre-morbid mRS is assessed using the structured mRS prior to randomization. **Secondary outcomes:** Safety, recanalization, ordinal shift analysis of mRS, NIHSS 0 at day 5 (or discharge), Euroqol, everyday activities sub-question on Euroqol, Lawton Instrumental Activities of Daily Living Scale (IADL), all cause mortality, recurrent stroke or progression, mRS 0-1 at 90days, mRS 0-2 at 90 days, mean mRS using linear regression, composite of recanalization or mRS 0-1 at 90days and mortality.
Sample Size	We test the hypothesis that there is a 9% absolute risk benefit of TNK-tPA over standard of care in the treatment of minor stroke (NIHSS 0-5) with 90% power. The rate of good outcome in the standard of care group is assumed to be 60% and 69% in the TNK-tPA group and the predicted sample size is 1228. Sample size is inflated 4% to 1274 to account for loss to follow up.
Randomization	Randomization will be 1:1 to TNK-tPA or control. Randomization will be central, computer generated and utilize a minimization algorithm to ensure balance on key variables throughout the course of the trial.
Consent	Written informed consent is required.

Trial Organization

The trial will be coordinated and executed by a steering committee based in Calgary and involve approximately 80 sites in North America, Europe, Asia and Australasia. An independent DSMB will provide safety evaluation during the trial.

The trial will be lead by principal investigator: Shelagh B. Coutts.

Co-investigators – Michael D. Hill,
Mayank Goyal
Andrew M. Demchuk
Bijoy K. Menon

The trial will be lead in Europe by Peter Kelly, at the University College in Dublin, Ireland.

Study Objectives

To demonstrate the efficacy of using TNK-tPA (tenecteplase), a thrombolytic agent that is relatively novel to the treatment ischemic stroke but well-established in the treatment of myocardial infarction, to treat minor ischemic stroke patients with proven acute symptomatic occlusions or perfusion abnormalities.

Background

Bullet Point Rationale

(i) At least 50% of ischemic stroke is initially minor.
(ii) Minor or non-disabling ischemic stroke is frequently treated conservatively with antiplatelet agents only.
(iii) Up to a third of patients with TIA or minor stroke are dead or disabled at 90 days, implying that the initial severity of presenting symptoms can be misleading.
(iv) Arterial occlusion can be demonstrated non-invasively using CT angiography or MR angiography in 10-15% of patients with TIA or minor stroke.
(v) Arterial occlusion is strongly associated with a poor outcome (dead or disabled at 3 months).
(vi) Treatment to relieve arterial occlusion is expected to result in a greater proportion of patients achieving an excellent neurological outcome.
(vii) Advantages of TNK-tPA (tenecteplase) over tPA (alteplase)
   a. TNK-tPA has greater fibrin specificity and possibly a lower intracranial hemorrhage risk compared to tPA.
   b. Lower dose TNK-tPA may offer lower risk and higher recanalization rates due to a longer serum half-life.
   c. TNK-tPA is infused using a simple bolus injection, which reduces nursing needs compared to the 60-minute tPA infusion. This would, for example, facilitate further imaging.
(viii) Proof of efficacy and safety of thrombolytic therapy in the setting of minor stroke with proven occlusion would change clinical practice.

At least 50% of ischemic stroke is minor and initially non-disabling.[2] In the "get with the guidelines" registry in the United States 41% were not treated with thrombolysis due to mild or improving symptoms.[3] These patients present with a transient ischemic attack (TIA) or minor stroke. The treatment of minor stroke with thrombolysis has always been controversial with much variation in practice. Most physicians do not treat all patients with minor deficits presenting within the standard thrombolytic window due to concerns regarding balancing the risk of hemorrhage compared to any potential reduction in disability. However a number of studies have reported that this judgment of risk may be wrong. Several groups have reported that among patients considered too mild for thrombolysis, that up to a third are dead or disabled at the time of follow up.[4-7] Recent data, involving a small subset of patients in an individual patient data meta-analysis of randomised trials of tPA suggests that thrombolysis with IV tPA among patients with minor deficits may improve outcome (OR 1.48, adjusted for age and time from onset (95%CI:1.07 – 2.06).[8]

Association of vessel occlusion and outcome in minor stroke

Minor stroke patients with documented vessel occlusion are at the highest risk of early neurological deterioration and poor outcome when thrombolysis is withheld.[6,7,9,10] These studies all used MRI to assess arterial status, which has limited the number of patients assessed in these studies.

Multi-slice helical CT scanners with CT Angiography (CTA) capability are widely available in many emergency departments. CTA uses the administration of intravenous contrast media to assess the intracranial and extracranial vasculature with high spatial resolution. The addition of CTA adds less than 5 minutes to a standard CT brain and can be safely completed in most patients.[11] CTA is one potential way of increasing the number of patients that can have early vascular imaging among patients with minor stroke. Although we expect that most sites will use CTA to meet the inclusion criteria for this study, we will allow MRI/MRA in centres that have processes in place to manage patients in this way.

We recently completed a prospective cohort study of 510 TIA and minor stroke patients (NIHSS<4) who were not treated with thrombolysis – the CATCH study.[12] All of these patients had a CT and CTA completed with a median time to CTA of 5.5 hours (IQR: 6.4 hours) showing the feasibility of using CTA to screen these patients for large artery occlusion. 10% (52/510) of patients had an intracranial occlusion. 19% (10/52) of patients with intracranial occlusion had early neurological deterioration versus 2% (9/447) in patients without occlusion, p<0.0001. We found that stroke progression occurred in both proximal and distal occlusions with similar frequency.[13] Clinical outcomes were also worse with patients having an intracranial occlusion having more disability at the time of 90 day follow up (31% versus 13%, p=0.0016) than patients without an intracranial occlusion. This was true whether the patients clinically deteriorated or not.[14] Another group has found that large artery occlusion predicts disability even among patients who have completely symptomatically resolved at baseline (i.e. TIA patients).[15] In the setting of intracranial occlusion the proposed mechanism of neurological worsening is failure of collateral blood supply. [16,17]

In summary, minor stroke patients with a documented intracranial occlusion have a higher risk of neurological deterioration and disability than those without intracranial occlusion.

Thrombolysis in minor stroke patients: efficacy and safety

The biggest reason for physicians to withhold thrombolysis is a lack of evidence to counter their concerns regarding the potential risks of treatment. Most of the thrombolysis trials completed to date have included few or no minor stroke patients. The trial with the largest number of minor stroke patients treated to date is the third International Stroke Trial (IST-3).[18] In IST-3 minor stroke was defined as a baseline NIHSS of 0-5 inclusive. IST-3 included 612 patients with an NIHSS of 0-5 (304 with tPA, 308 to control). There was no evidence of a treatment benefit with good outcome (mRS 0-1) seen in 54% (164/304) of tPA treated patients vs. 48%

(147/308) of controls (RR 1.13, 95%CI: 0.97-1.32, p=0.12).[19] A recent analysis of the Virtual International Stroke Trial Archive (VISTA) database failed to demonstrate a benefit of thrombolysis in a NIHSS 1-4 population.[20]

Symptomatic intracranial hemorrhage (SICH) was seen in 3% of tPA patients (9/304, 95%CI: 1.3-5.5) in IST-3, but not in any control patients. There was no evidence of an interaction with time for SICH (less than 4.5 hours or greater than 4.5 hours). In a subgroup analysis of the CASES study there were 77 tPA treated patients with a NIHSS score < 6 and these patients had a 2.6% (95%CI: 0.8-9%) rate of symptomatic hemorrhage.[21] In the NINDS tPA study there was a similar hemorrhage rate of 2.3% (95% CI: 0.6-12%) among patients with NIHSS score < 6.[22]

We believe that the subgroup of patients with an intracranial occlusion are the population where the risk benefit swings towards benefit. The few patients with minor stroke (NIHSS<6) that have been included in thrombolysis studies have lower rates of intracranial hemorrhage (ICH) than in more severe strokes, however the confidence intervals are wide given how few patients have been enrolled in this group [NINDS tPA[22] 2.3% (95% CI: 0.6-12%), CASES[21] 2.6% (95%CI: 0.8-9%), IST-3[19] 3% (95%CI: 1.3-5.5)]. Overall rates of symptomatic ICH have also been falling as experience with stroke thrombolysis has grown worldwide.[23]

In general, symptomatic ICH among disabling stroke patients treated with intravenous tPA has been shown to be associated with the severity of infarction, the volume of infarction shown on imaging, leukoaraiosis, the time from stroke onset, anticoagulation use and elevated serum glucose.[24] However, these variables account for only a small proportion of the variance so that to a large extent, symptomatic ICH seems a random occurrence clinically. Thus, it is our expectation that the rates of symptomatic hemorrhage will be no more than 2% among patients treated in this study. We note that patient with established infarction observable on brain imaging are at greater risk of hemorrhage. We propose to exclude patients with evidence of large volumes of infarction or clearly subacute ischemia.

Most minor stroke patients are judged to have such a good prognosis that the risk of symptomatic ICH is not worth taking. However, the rate of poor outcome is much higher than previously assumed, particularly in patients with intracranial occlusion. And, with evolving knowledge and experience with stroke thrombolysis, the safety profile has improved substantially.

Tenecteplase (TNK-tPA, TNKase™)

Tenecteplase, a genetically engineered mutant tissue plasminogen activator, has a longer half-life, is more fibrin specific, produces less systemic depletion of circulating fibrinogen, and is more resistant to plasminogen activator inhibitor[25] than alteplase.[26] These pharmacodynamic differences result in more rapid reperfusion. Tenecteplase is now the first-line intravenous thrombolytic drug for

myocardial infarction[27,28] and has shown to cause complete reperfusion with reduced ICH in comparison to alteplase in animal stroke models.[29,30]

A dose escalation safety study of tenecteplase in patients with acute ischemic stroke observed no symptomatic intracranial hemorrhages (ICHs) among 88 patients treated with doses ranging from 0.1 mg/kg to 0.4 mg/kg.[31] At 0.5 mg/kg, the study was closed early in the dose tier due to an excess of symptomatic hemorrhage. 0.5 mg/kg, is the currently approved coronary thrombolysis dose. Asymptomatic hemorrhage began to appear at 0.1 mg/kg (8% of 25 patients) and was higher at 0.2 mg/kg (32% of 25 patients) and 0.4 mg/kg (28% of 25 patients), indicating that there may be some relationship with dose. This trial was stopped prematurely due to slow enrollment.

A more recent Phase IIb study comparing thrombolysis with tPA and low dose TNK (0.1mg/Kg or 0.25mg/Kg) in moderate to severe stroke was suggestive that TNK had higher recanalization rates than tPA.[32] The study was not powered to look at clinical differences between the groups, however there were clear differences in recanalization rates at 24 hours. Complete recanalization at 24 hours was seen in 36% of the tPA group, 35% of the 0.1mg/Kg TNK group and 80% of the 0.25/kg group (p=0.002). Partial or complete recanalization was seen in 68% of the tPA group, 78% of the 0.1mg/Kg TNK and 95% of 0.25mg/Kg TNK group (p=0.02). Not only was recanalization greater with TNK, the rate of symptomatic intracranial hemorrhage was lower in both the TNK treated groups (12% versus 4% and 4%). The investigators are currently running a phase 3 trial comparing tPA with 0.25mg/Kg TNK based on these results. [33]

We recently completed a dose-escalation safety study of TNK-tPA in the treatment of minor stroke with proven occlusion – TEMPO-1 study.[34] We prospectively enrolled 50 patients with minor stroke and proven intracranial occlusion, and treated them with TNK-tPA in a 12-hour window. The first tier of 25 patients was treated at a dose of 0.1 mg/kg. The second tier of 25 patients was treated at 0.25 mg/kg. The overall rate of sICH was 2% (1/50) CI_{95} 0.5%-10.6%. There were no drug related serious adverse events in tier 1. In tier 2 there was 1 symptomatic ICH (4%, 95%CI: 0.01-20.0). Stroke progression occurred in 6% of cases. Overall, 66% had excellent functional outcome (mRS 0-1) at 90-days. Recanalization rates were high; 0.1mg/Kg (39% complete, 17% partial), 0.25mg/Kg (52% complete, 9% partial). Complete recanalization was significantly related to excellent functional outcome (mRS 0-1) at 90-days (RR 1.65: CI_{95} 1.09-2.5, p=0.026, See Figure 1).

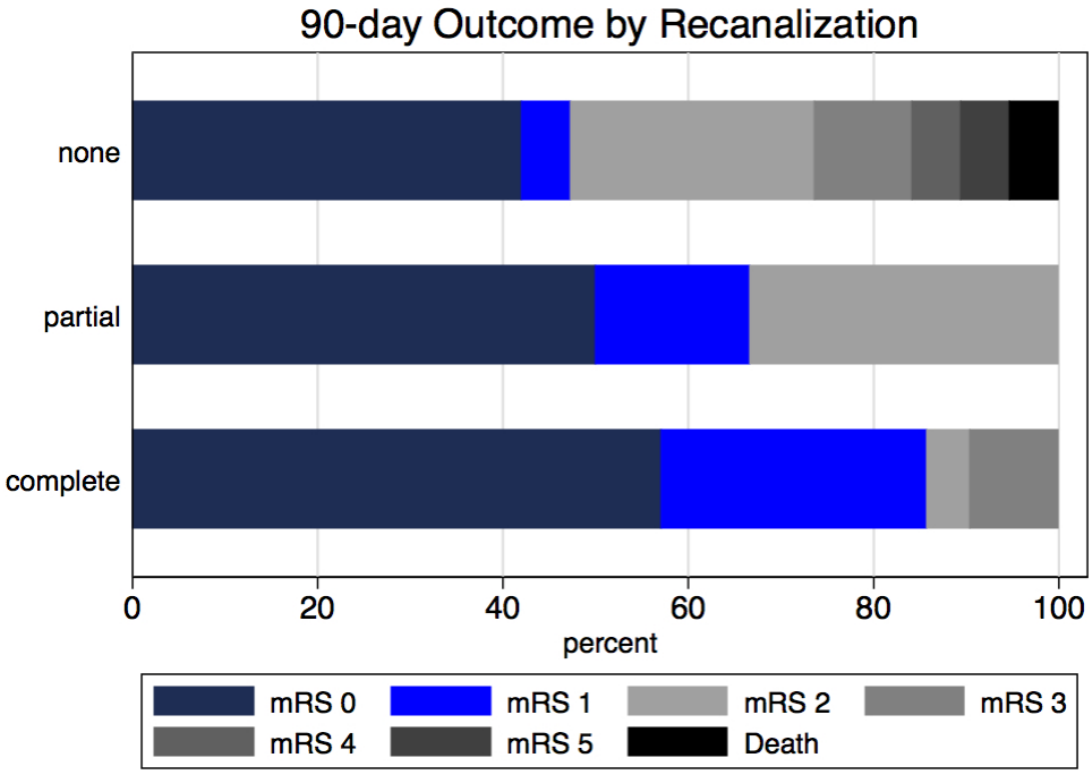


Figure 1: Figure shows the breakdown of functional outcomes at 90 days by recanalization status (complete, partial or no recanalization).

Based on the known pharmacological differences, the higher recanalization rate and an empiric dose-escalation safety study (TEMPO-1) we have chosen TNK at a dose of 0.25mg/kg.

Timing of treatment

IV tPA is in routine use in Canada up to 4.5 hours from symptom onset for treatment of disabling stroke. Patients with intracranial occlusion, but only mild symptoms are different than patients with more severe symptoms, likely due to collateral circulation.[17] These patients also have a tendency to present later than patients with more major symptoms. In the CATCH study[12] most patients deteriorated at a median time of 1 day (deterioration was mostly overnight the first night) suggesting that there may be an extended window in these patients. Many tertiary stroke centres, including the Calgary Stroke Program have been using the "small core, large area of brain at risk" paradigm to thrombolyse stroke patients outside of guideline-based care for a number of years. Different techniques have been used to identify this patient paradigm including MRI, CT perfusion (CTP) and CT Angiography (CTA). We have chosen a relatively simple approach, which is intracranial large artery occlusion (or a focal area of decreased perfusion and small area of infarcted brain). In TEMPO-1 we safely used 12 hours as our maximum potential treatment window. We showed that this was safe treatment paradigm. The reality is that most patients

present earlier rather than later. There is the occasional patient who wakes up with their deficits and their last time seen normal is close to 12 hours.

Study Design

The study will be a, prospective, randomized, open, blinded end-point (PROBE) study. Randomization will be 1:1 to 0.25mg/Kg TNK-tPA (experimental) or standard of care (control).

Primary Outcome

Primary outcome: Return to baseline neurological functioning as measured by the mRS.

Analysis will be a responder analysis where return to baseline level of neurological functioning is defined as follows:
If pre-morbid mRS is 0-1 then mRS 0-1 at 90 days is a good outcome.
If pre-morbid mRS is 2 then mRS 0-2 is a good outcome.

Pre-morbid mRS is assessed using the structured mRS prior to randomization. (see appendix 1).[35] Outcomes will be assessed by an individual blinded to the treatment assignment. The 90day mRS will be rated using the structured mRS questionnaire (see appendix 1). The 90 day mRS will be completed in person where possible and by telephone otherwise. The structured questionnaire has been showed to improve reliability in assessing the mRS both in person and by telephone.[35]

Secondary Outcomes

1) Proportion of patients with major bleeding: This will include an analysis of symptomatic intracranial hemorrhage alone and then combined with major extracranial hemorrhage. This is the main safety outcome.
   a) Symptomatic intracranial hemorrhage defined as new intracranial hemorrhage (ICH, SAH, IVH, SDH) associated with clinical evidence of neurological worsening, in which, the hemorrhage is judged to be the most important cause of the neurological worsening. Clinical worsening will be guided by the NIHSS score of a minimum of 2 or more points different from baseline.
   b) Major extracranial hemorrhage defined as life threatening, resulting in hemodynamic compromise or hypovolemic shock, requiring inotropic support or other means to maintain cardiac output, requiring blood transfusion of more than 2 units of packed red blood cells, or associated with a fall in hemoglobin greater than or equal to 5 g/L.
2) Proportion of patients with complete and partial recanalization (TICI 2b-3) post treatment. This will be assessed on CTA 4-8 hours post treatment. Recanalization will be assessed by the central core-imaging lab blinded to all clinical information.
3) Categorical shift analysis on the full range of the mRS (0-6).

4) Absence of disability defined as mRS 0-1.
5) Functional independence defined as mRS 0-2.
6) Comparison of the mean mRS using linear regression using the mRS as a continuous variable.
7) Lawton Instrumental Activities of Daily Living Scale (IADL)[36,37]
8) Proportion of patients with an NIHSS 0 at day 5 (or discharge from hospital if discharged before day 5)
9) Quality of life measured on EuroQol[38]
10) Quality of life as measured by the "problems with usual activities" question on the EuroQol.
11) Stroke progression and recurrent stroke.
12) All-cause mortality

Selection and Enrolment of Subjects

Inclusion criteria

1. Acute ischemic stroke in an adult patient (18 years of age or older)
2. Onset (last-seen-well) time to treatment time ≤ 12 hours.
3. TIA or minor stroke defined as a baseline NIHSS ≤ 5 at the time of randomization. Patients do not have to have persistent demonstrable neurological deficit on physical neurological examination.
4. Any acute intracranial occlusion or near occlusion (TICI 0 or 1) (MCA, ACA, PCA, VB territories) defined by non-invasive acute imaging (CT angiography or MR angiography) that is neurologically relevant to the presenting symptoms and signs. Multiphase CTA or CT perfusion are required for this study. An acute occlusion is defined as TICI 0 or TICI 1 flow.[1] Practically this can include a small amount of forward flow in the presence of a near occlusion
   AND,
   Delayed washout of contrast with pial vessels on multiphase CTA in a region of brain concordant with clinical symptoms and signs OR,
   Any area of focal perfusion abnormality identified using CT or MR perfusion – e.g. transit delay (TTP, MTT or T Max), in a region of brain concordant with clinical symptoms and signs.
5. Pre-stroke independent functional status – structured mRS ≤2.
6. Informed consent from the patient or surrogate. Surrogate consent is only allowed in countries/jurisdictions where this is approved.
7. Patients can be treated within 90 minutes of the first slice of CT or MRI. Scans can be repeated to meet this requirement; if there is no change neurologically then only a CT head need be repeated for assessment of extent and depth of ischemia.

Exclusion criteria

1. Hyperdensity on NCCT consistent with intracranial hemorrhage.
2. Large acute stroke ASPECTS < 7 visible on baseline CT scan.

3. Core of established infarction. No large area (estimated > 10 cc) of grey matter hypodensity at a similar density to white matter or in the judgment of the enrolling neurologist is consistent with a subacute ischemic stroke > 12 hours of age.
4. Patient has a severe or fatal or disabling illness that will prevent improvement or follow-up or such that the treatment would not likely benefit the patient.
5. Pregnancy. All women with the potential of being pregnant i.e. have not gone through menopause or have not undergone surgical sterilization, should have a pregnancy test prior to enrollment.
6. Planned thrombolysis with IV tPA or endovascular thrombolysis/thrombectomy treatment.
7. In-hospital stroke unless these patients are at their baseline prior to their stroke. E.g. a patient who had a stroke during a diagnostic coronary angiogram.
8. Commonly accepted exclusions for medical thrombolytic treatment that potentially put the patient at an increased risk of bleeding. Country specific product monographs and stroke thrombolysis guidelines should be consulted. These are commonly relative contraindications (i.e. the final decision is at the discretion of the treating physician) but for the purposes of TEMPO-2 include the following:
    a. Significant bleeding disorder either at present or within the past 6 months
    b. International normalized ratio > 1.7 or known full anticoagulation with use of any standard or direct oral anticoagulant therapy with full anticoagulant dosing. [DVT prophylaxis dosing shall not prohibit enrolment]. For low molecular weight heparins (LMWH) more than 48 hours off drug will be considered sufficient to allow trial enrollment. For direct oral anticoagulants; in patients with normal renal function more than 48 hours off drug will be considered sufficient to allow trial enrollment. Patients on direct oral anticoagulants who have any degree of renal impairment should not be enrolled in the trial unless they have not taken a dose of the drug in the last 5 days. Dual antiplatelet therapy does not prohibit enrolment. [For patients who are known not to be taking anticoagulant therapy it is not necessary to wait for coagulation lab results (e.g. PT, PTT) prior to treatment]
    c. Prolonged cardiopulmonary resuscitation (> 2 minutes) within the past 2 weeks
    d. Acute pericarditis and/or subacute bacterial endocarditis
    e. Acute pancreatitis
    f. Severe hepatic dysfunction, including hepatic failure, cirrhosis, portal hypertension (oesophageal varices) and active hepatitis
    g. Neoplasm with increased bleeding risk
    h. Arterial aneurysm and known arterial/venous malformation
    i. Patients who have been acutely treated with GP2b3a inhibitors.
    j. Arterial puncture at a non-compressible site in the previous seven days

k. Clinical stroke or serious head or spinal trauma in the preceding three months that would normally preclude use of a thrombolytic agent.
l. History of intracranial hemorrhage, subarachnoid hemorrhage or other brain hemorrhage that would normally preclude use of a thrombolytic agent.
m. Major surgery within the last 3 months that the treating physician considers a contraindication to thrombolytic therapy.
n. Severe hypo- (< 50 mg/dL or 2.8mmol/l) or hyperglycemia (>400 or 22.2mmol/l)
o. Hypertension refractory to anti-hypertensive medication such that target blood pressure <185/110 cannot be achieved before treatment.
p. Known platelet count below 100,000 per cubic millimeter. [Treatment should not be delayed to wait for platelet count unless thrombocytopenia is known or suspected]
q. Gastrointestinal or genitourinary bleeding within the past 3 months that would normally preclude use of a thrombolytic agent.

Selecting Patients

The principles of patient selection are based upon the broad criteria of:

a. TIA or minor stroke presentation with a diagnosis of an ischemic stroke syndrome
b. Imaging proof of an intracranial occlusion or a perfusion abnormality relevant to the presenting symptoms
c. No region of well-defined hypodensity on the NCCT consistent with the presenting symptoms or consistent with the suspected pathophysiology of the presenting symptoms that suggests well-evolved infarction, judged to be potentially prone to bleeding.

The most challenging of these principles is (c) since it requires judgment and imaging interpretation. We know from imaging studies using MR perfusion imaging that regions of very low CBV are prone to hemorrhage.[39] Yet, using MR diffusion imaging it can be shown that many patients with minor lesions who then present with subsequent major stroke and are treated with IV tPA do not suffer hemorrhage.[40] Clinically, it has been a maxim of stroke thrombolysis that among patients who present with a TIA-like presentation who neurologically resolve and then subsequently deteriorate, the clock can be reset to the time of deterioration. Yet, we know that 50% or more of patients with TIA/minor stroke presentations have MR defined small ischemic lesions.[41,42] Empirical clinical experience suggests that thrombolysis in presence of small lesion volumes is safe.

Patient who are at increased risk of hemorrhagic complications should not be enrolled in the trial. Generally, standard thrombolytic agent contraindications will be considered at the discretion of the treating physician as exclusion criteria. The use of tenecteplase or other thrombolytic agents in patients who are taking or have been recently taking direct oral anticoagulant medicine is uncertain. This is

particularly true for the medicines that are dependent upon normal renal function for excretion. There are 4 currently marketed direct oral anticoagulants: Dabigatran, rivaroxaban, apixaban, betrixaban. Therefore, patients with any degree of renal failure who have taken one or more doses of these medicines in the prior 5 days are excluded. Patients with normal renal function are excluded if they have taken one or more doses of these medicines in the prior 48 hours.

Enrolment

Patients will be screened using the usual stroke team process of care at the site. Candidates for enrolment will be approached for consent. Since all subjects are expected to be relatively mildly affected clinically at presentation, many/most will be able to provide consent themselves. In certain countries/jurisdictions an incompetent patient, who otherwise meets criteria, may still be enrolled with the consent of a surrogate or legally authorized representative. All patients or their surrogate must provide written informed consent.

All patients will be evaluated clinically and then undergo brain imaging using CT followed immediately by a CT angiogram. If they remain eligible, after review of clinical testing, imaging and laboratory testing, they will be immediately enrolled and treated. All patients will be treated within 90 minutes of the first slice of the baseline CT. In sites where MRI/MRA is routinely used this can be substituted for CT/CTA. In all parts of the protocol MRI/MRA can be substituted for CT/CTA.

A patient is considered enrolled into the trial at the point (date and time) of randomization. If randomized to active treatment they should immediately receive study drug. Randomization is considered time 0. A patient who provides consent but is not enrolled into the trial is considered a screen failure.

Study Interventions

Randomization will be 1:1 to TNK-tPA (experimental) or standard of care antiplatelet agents (control).

Experimental: TNK-tPA (0.25mg/kg) given as a single, intravenous bolus immediately upon randomization. Experimental treatment will be administered as a single intravenous bolus over 5-10 seconds as per the standard manufacturers' instructions for use. Please refer to current Product Monograph for details on reconstitution and infusion of the drug.

Control: Patients will be treated with standard of care based antiplatelet treatment – choice at the discretion of the investigator. Low dose aspirin (single agent) will be the choice of most physicians, however given the results of the FASTER trial[43] and the recently published CHANCE trial[44] some will chose to use the combination of aspirin and clopidogrel. As this is a multi-centre, international trial where local practices will vary, rather than mandating a specific antiplatelet agent, we will allow the local investigator to chose which antithrombotic regime should be used.

Standard of care medication(s) should be given immediately upon randomization.

Patients will undergo a study CT angiogram of the intracranial circulation between 4-8 hours after treatment to determine the biological effect of the drug - whether the occluded artery has recanalized or not. Any patient who has neurological worsening should have standard of care brain imaging completed to rule out intracranial hemorrhage.

All patients will have standard of care medical management on an acute stroke unit and undergo follow-up imaging at 24 hours with CT or MR. Use of MR will be encouraged.

Randomization: Concealment and Blinding

Randomization will be completed by a computer generated minimization algorithm – minimal sufficient balance randomization. This will ensure balance throughout the trial, based on key variables. This algorithm will be developed centrally and the details will not be available to the treating sites. The minimization algorithm preserves balance on pre-specified prognostic variables. Variables that will be included in the minimization algorithm are age, sex, baseline NIHSS score, pre-morbid mRS, and time of randomization (under 4.5 hours versus not). These are the key variables known to influence outcome in minor stroke.[10,14,45] Randomization will be dynamic and generated in the moment via a web-based system; thus a randomization list does not exist. The result will be random allocation that is fully concealed. Randomization will be biased coin that will vary from fully balanced (50:50) to biased (65:35) dependent on what characteristics been previously enrolled have. The system will be enabled for smart-phone, tablet, laptop or desktop computer use.

Study Drug

The trade name for tenecteplase is TNKase™ in North America and Metalyse™ in Europe and Australasia. Off the shelf tenecteplase will be used in this study. Staff will be trained in the mixing and administration of the drug.

STORAGE AND STABILITY

Store lyophilized TNKase™ (or Metalyse™) tenecteplase, TNK-tPA) at controlled room temperature (2-30°C) not to exceed 30°C or under refrigeration (2°C - 8°C). If standard hospital supplies are being used then temperature monitoring is not required. Do not use beyond the expiration date stamped on the vial. Unused reconstituted TNKase™ (in the vial) may be stored at 2°C - 8°C and used within 8 hours. After that time, any unused portion of the reconstituted material should be discarded.

DOSAGE FORMS, COMPOSITION AND PACKAGING

Dosage Forms:

There are different sized vials of TNKase™ (or Metalyse™) available in different countries. In Canada for example 50mg vials are available.
Each 50mg vial of TNKase™ (or Metalyse™) is packaged with one 10ml vial of Sterile Water for Injection for reconstitution. For other vial sizes follow the reconstitution instructions included with the drug. Reconstitution of 50mg of tenecteplase in 10 ml of sterile water results in a solution concentration of 5mg/ml. For other sizes of tenecteplase follow the reconstitution instructions included with the drug. The dose is 0.25 mg/kg or 0.05ml/kg.

Composition:

TNKase™ is a sterile, white to off-white, lyophilized powder for single intravenous (IV) bolus administration after reconstitution with Sterile Water for Injection, USP.

50 mg (10,000 units) / vial

Tenecteplase* 52.5 mg
L-Arginine 0.55 g
Phosphoric Acid 0.17 g
Polysorbate 20 4.3 mg
*This includes a 5% overfill so that each vial will deliver 50 mg of tenecteplase.

Packaging:

Each 50 mg vial of TNKase™ is packaged with one 10 mL vial of Sterile Water for Injection, USP
for reconstitution and one B-D
® 10 cc Syringe with TwinPak® Dual Cannula Device.

Schedule of Assessments

	Baseline	4-8 h	Day 1 (24 ±8 h from randomization)	Day 5 or discharge (±1 d)	Day 90 (±14 d)§
Informed consent	X				
Regained capacity consent (if needed)					X
History and examination	X				
Weight	X±	X±	X±	X±	
NIHSS	X		X	X	X
mRS					X
Pre-stroke mRS¶	X				
EuroQol					X
Lawton Instrumental Activities of Daily Living Scale (IADL)					X
NCCT head or MR	X*		X***		
CTA COW or MRA	X*	X**			
Full emergency stroke labs	X‡				
Creatinine	X		X		
ECG	X‡				
Adverse event assessment		X	X	X	
Serious adverse event assessment		X	X	X	X§
Prior medications	X‡				
Concomitant medications§	X	X	X	X	

* MRI/MRA can be substituted for baseline CT/CTA at the discretion of the local site.
**4-8 hours CTA Circle of Willis (COW). At the discretion of the local investigator the follow up CTA can be not completed if the eGFR is <40 ml/minute or there was an allergic reaction to the baseline scan.

***Day +1 NCCT head may be supplanted by an MR head including diffusion weighted imaging (DWI) and gradient echo (GRE) at the discretion of the local site.
¶ The pre-stroke mRS is an estimate of the pre-stroke score and is based on the history given by the patient/family.
‡ These tests are required at baseline. Blood should be drawn at baseline, but results are not required prior to randomization. In certain countries as recommended by national guidelines; blood work for group and hold (type and screen) should be collected. ECG should be done within 6 hours of hospital admission, but is not required prior to randomization. Prior medications should be collected but are not required prior to randomization.
§ Concomitant medications are collected out to Day 5 or in conjunction with any SAE. Collect concomitant medications at 90days only as part of the SAE narrative only on patients with SAEs.
All 90 evaluations should be performed by an evaluator blinded to the acute intervention. We are encouraging that this visit be completed in person, but if this is not possible a telephone follow up can be substituted.
d = days; h = hours
± Actual weight must be performed once by Day 5 or Discharge and recorded, not necessary to be done at all time points.

Laboratory Evaluations

Routine blood work will be taken in the emergency department. This will include PT/PTT, CBC, electrolytes, glucose and creatinine. The coagulation status must be known prior to treatment among patients who are known to be on any form anticoagulation therapy. ECG should be completed at baseline either prior to treatment or within 6 hours of treatment.

If the estimated GFR is subsequently found to be <40 ml/minute or there was an allergic reaction to the baseline CTA then the repeat CTA should not be completed. This is not a protocol deviation.

Clinical evaluations

All patients will have a stroke history and physical before treatment is commenced. All investigators will be trained in both the NIHSS and mRS. Patients will be assessed at 24 hours or at the time of any deterioration using the NIHSS. At 5 days (or at discharge from hospital if sooner) patients will have an NIHSS completed. At 90 days the NIHSS, Euroqol, Lawton Instrumental Activities of Daily Living Scale (IADL) and mRS will be completed by a blinded investigator. The mRS will be rated using the structured mRS questionnaire.[35] The investigator completing the 90d outcome assessment should be a blinded site trial investigator, sub-investigator or coordinator defined as absence of involvement in the first 48 hours of treatment of the patient. If not feasible to complete in person this interview can be completed by telephone.

Prohibited medications and procedures

In the experimental treatment group: no antiplatelet agent, other antithrombotic medicines should be given within the first 24 hours (+/- 8h) of the treatment. These can be started, if clinically indicated, once the 24-hour (+/- 8h) follow-up CT has been completed and shows no clinically significant intracranial hemorrhage. In practice, this means that if there is no hemorrhage on follow-up brain imaging, antithrombotic or antiplatelet medicines may be given without restriction. If there is hemorrhage, a judgment must be made about the relative safety of antiplatelet or antithrombotic medicine. For example, it is medically appropriate if the

hemorrhage is limited or small or simply petechial (hemorrhagic infarction type) and the benefit is judged to outweigh the risk.

It is expected that a majority of the control group will be treated with single (or dual) antiplatelet therapy. Given the presence of a large artery occlusion we would recommend not immediately using heparin or one of the direct oral anticoagulants even in the presence of atrial fibrillation. We would recommend that the use of anticoagulants is delayed for at least 24 hours in both groups of patients. However the final decision is left to the judgment of the treating physician.

Patients should not undergo endovascular thrombectomy or thrombolysis outside of the trial protocol. This is considered a protocol violation. However, in the event of a clinical deterioration and this type of protocol violation, the patients will be considered to have suffered an early recurrent stroke (which is a pre-specified secondary outcome), even if they are cured by endovascular therapy. Adverse events that occur related to such treatment will be recorded and adjudicated accordingly.

Guidelines for Clinical Care

It is expected that subjects will receive the best usual standard of stroke unit care. All subjects are expected to be admitted to hospital as part of routine standard of care. Most subjects will have mild symptoms and recover in 1-2 days and likely will be subsequently discharged home.

It is expected that all subjects will undergo a routine work-up for the mechanism of their stroke and be treated appropriately and definitively. This is critically important because subjects with mild stroke secondary to large artery disease are at the highest risk of early recurrent stroke. [46] We wish to prevent recurrent stroke from confounding the 90-day clinical outcome such that patients who are well at discharge remain that way for the duration of the 90-day follow-up period.

We expect that most patients with atrial fibrillation will be anti-coagulated. Patients with symptomatic carotid artery stenosis should undergo carotid revascularization early and definitely within 2 weeks of stroke onset.[47] Risk factors, including hypertension, elevated cholesterol, diabetes mellitus, tobacco smoking, should be treated appropriately and aggressively according to current standards of care.

We expect patients to receive adequate hydration to prevent renal complications of the use of radio-contrast media for diagnostic imaging. While this medication is generally extremely safe, simple hydration can prevent renal complications, particularly among patients with baseline borderline renal function and among those with diabetes mellitus. Further, patients with ischemic stroke are generally slightly hypovolemic at baseline. We recommend use of intravenous normal saline (0.9% saline) infusion at 1.5 – 2.0 cc/kg/h until the patient is eating and drinking

safely and well. Therefore, for the typical patient this will mean IV NS at 75-150 cc/h overnight only. We do not recommend the use of bicarbonate solutions or N-acetyl-cysteine solutions.

For patients that are disabled from their stroke and require a longer in-patient stay and/or rehabilitation, it is expected that they will receive standard stroke unit care to prevent complications. These include:

- DVT prophylaxis for patients who are bed-bound or primarily bed-bound
- Swallowing assessments and prevention of aspiration pneumonia
- Early mobilization and physiotherapy to prevent skin breakdown, pneumonia, DVT/PE
- Early diagnosis and treatment of fever

Imaging

All imaging completed of the brain, CT, CTA, and MRI in the first 48h will be rendered anonymous and sent to Calgary for central adjudication. Minimally the baseline, 4-8h CTA and the 24-hour imaging should be included.

Clinical Management of Adverse Experiences

An adverse event is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Adverse events can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporarily associated with the use of a drug, without any judgment about causality. Adverse events occur after enrolment and are defined as not being present prior to enrolment. For example, a patient with known episodic gouty arthritis of the great toe, who develops an attack of gout, is not considered to have suffered an adverse event; the event was known prior to enrolment. A patient who develops a new diagnosis of gout during the study period is judged have suffered an adverse event. This is reportable as an adverse event even though it is most likely entirely unrelated causally to the study drug, but is instead only associated with study drug use temporally. Adverse events should be managed according to the best current standard of care.

Serious adverse events (SAEs) are those adverse events that are life threatening, require a surgical or medical procedure to prevent disability or death, result in admission to hospital, prolongation of hospitalization or transfer to an ICU, or result in death. A SAE can also be an important medical event that may not result in death, be life-threatening, or require hospitalization, but may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Any new diagnosis of cancer (made after study enrollment) is considered an important medical event. A SAE is also an event that results in a congenital anomaly or birth defect, but this is an unlikely consideration for this trial since all or nearly all participants will not be of reproductive potential. Serious adverse events should be managed according to the best

current standard of care.

Adverse Event Reporting and Review

Adverse events will only be collected through the first 5 days of trial participation. Adverse events should be reported as they occur on the eCRF. There are no timelines for reporting simple adverse events. Documentation must be supported by an entry in the subject's file. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product as judged by the Investigator, action taken and outcome.

Serious adverse events (SAEs) will be collected for the full 90-day trial period. SAEs must be reported **immediately by the investigator within a very short period of time and under no circumstances should this exceed 24 hours following knowledge of the SAE**. SAEs will be reviewed by the trial medical monitor. SAEs will be reported to the appropriate regulatory authority in accordance to the relevant regulations and legislation in that region and state/country. Because the adverse event profile of TNK-tPA is well known due to the experience of its use for coronary thrombolysis, we do not predict that there will be unexpected adverse events.

Pregnancies occurring in study subjects will be treated procedurally as SAEs. Pregnancies occurring in study subjects after signing informed consent should be reported separately on Pregnancy Report Form.

Data Safety and Monitoring Board (DSMB)

Members of the DSMB will be acknowledged publically but will not be considered authors for any manuscripts that arise from this trial.

Expected Drug Reactions

For expected adverse drug reactions (ie. with relationship to Metalyse or TNKase) investigators are directed to the product monograph.

Criteria for Intervention Discontinuation

Because the study drug is a delivered by a single intravenous bolus injection, it will not be possible to discontinue the intervention.

In the event that a subject withdraws consent for follow-up in the study, that subject will be discontinued from the trial on the date of their withdrawal of consent. Data collected prior to this date will be included in the final study report.

Statistical Considerations

A sample of 1228 patients allows us to demonstrate a 9% absolute risk difference (60% → 69% primary outcome) with 90% power between intervention and control groups.

The recent pooled thrombolysis showed an effect size of 10% in the subset of minor stroke patients treated with thrombolysis.[8] Enrollment in the trials included in the meta-analysis did not require patients to have an intracranial occlusion, thus it is likely that the majority of these patients did not have an intracranial occlusion. Thus although we expect that the effect size is higher in a population that only includes patients with intracranial occlusion we will conservatively estimate an overall 9% effect size with a change in proportion with excellent neurological outcome from 60% to 69%. The sample size for each group is 614 (1228 total).

Adding 4% loss to follow up gives a sample size estimate of 1274 patients (637 in each treatment group). There will be ongoing monitoring for safety and full details will be available in a formal safety plan. A single interim analysis for futility and efficacy will be conducted at approximately two-thirds patient enrolment (n=840). Standard O'Brien Fleming boundaries will be used to establish the alpha spending function. Full details will be available in the DSMB charter.

It is possible that after central imaging review some patients will be enrolled in violation of the protocol or the treatment protocol may be breeched due to the dynamic nature of acute stroke. This may occur entirely in the best interests of patient care. In the primary analysis, all randomized patients will be included in the final analysis for safety and clinical outcome (ITT analysis). The safety population will be defined as all patients who receive any dose of study drug. The per-protocol population will be defined as all patients who received any dose of study drug and met all the inclusion and exclusion criteria.

Secondary analyses will include analysis of the pre-stated secondary outcomes and multivariable analyses of both the primary outcomes and pre-stated secondary outcomes. A formal Statistical Analysis Plan will be documented prior to breaking of the blind.

Data Collection and Management Overview

Data will be housed and managed in a custom database at the Hotchkiss Brain Institute Clinical Research Unit in Calgary, AB, Canada using regulatory compliant data systems.

Human Subjects

Local Regulations / Declaration of Helsinki

The Sponsor-Investigator (and any Participating Site Investigators) will ensure that this study is conducted in full conformance with the principles of the "Declaration of

Helsinki" or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual. The study must fully adhere to the principles outlined in "Guideline for Good Clinical Practice" ICH Tripartite Guideline or with local law if it affords greater protection to the patient.

Ethics approval

This protocol and the informed consent document and any subsequent modifications are reviewed and approved by the local ethics committee responsible for oversight of the study. Approval from the committee must be obtained before starting the study, and should be documented in a letter to the Sponsor-Investigator (and any Participating Site Investigators) specifying the date on which the committee met and granted the approval. A signed consent form must be obtained from the subject. In certain countries/jurisdictions where permitted, for subjects who cannot provide consent themselves, a legally authorized representative, or person with power of attorney, may sign the consent form. The consent form describes the purpose of the study, the procedures to be followed, and the risks and benefits of participation. A copy of the consent form must be given to the subject, the legally authorized representative, or the person with power of attorney; and this fact must be documented in the subject's record.

If new safety information results in significant changes in the risk/benefit assessment, the consent form should be reviewed and updated if necessary. All patients (including those already being treated) should be informed of the new information, given a copy of the revised form, and give their consent to continue in the study.

Conditions for Terminating the Study

The Sponsor-Investigator reserves the right to terminate the study at any time. Should this be necessary, the Sponsor-Investigator will work with any Participating Site Investigators to arrange the procedures on an individual study basis after review and consultation. In terminating the study, the Sponsor-Investigator (and any Participating Site Investigators) will assure that adequate consideration is given to the protection of the patients' interests.

Confidentiality

All imaging, evaluation forms, reports, and other records that leave the site are identified only by the site and subject number to maintain subject confidentiality. All records are kept in a locked file cabinet. Clinical information is not released without written permission of the subject, except as necessary for monitoring by ethics committees, regulatory bodies, the sponsor, or the sponsor's designee.

All study investigators at the clinical sites must ensure that the confidentiality of personal identity and all personal medical information of study participants is maintained at all times. Country specific privacy regulations where applicable, must be followed. On the CRFs and other study documents or image materials submitted to the CRU, the subjects are identified only by study identification codes.

Personal medical information may be reviewed for the purpose of verifying data recorded in the CRF by the site monitors. Other properly authorized persons, such as the regulatory authorities, may also have access to these records. Personal medical information is always treated as confidential.

Site Monitoring

All sites will have remote data monitoring conducted by the central trials staff. Data will be checked for completeness, logic, and validity. Queries will be sent to sites to verify data as required.

For on-site monitoring a variety of risk-based monitoring models will be used. This may include both trained employees and industry experienced independent contractor clinical research monitors. Details of monitoring will be in a separate site monitoring plan.

Study Documentation, CRFs and Record Keeping

Investigator's Files/Retention of Documents

The Sponsor-Investigator (and any Participating Site Investigators) must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should be classified into two different separate categories: (1) Investigator's Study File; and (2) patient clinical source documents.

The Investigator's Study File will contain the protocol/amendments, Case Report and Query Forms, ethics correspondence and governmental approval with correspondence, sample informed consent, drug records, staff curriculum vitae and authorization forms and other appropriate documents/correspondence, etc. Some or all of these files may be stored electronically.

Patient clinical source documents (usually defined by the project in advance to record key efficacy/safety parameters independent of the CRFs) would include patient hospital/clinic records, physician's and nurse's notes, appointment book, original laboratory reports, ECG, diagnostic imaging, pathology and special assessment reports, signed ICFs, consultant letters, and patient screening and enrollment logs. The Sponsor-Investigator (and any Participating Site Investigators) must keep these two categories of documents on file for 25 years after completion or discontinuation of the study. After that period of time the documents may be destroyed, subject to local regulations.

Source Documents and Background Data

Any Participating Site Investigators shall supply the Sponsor-Investigator on request with any required background data from the study documentation or clinic records. This is particularly important when CRFs are illegible or when errors in data transcription are suspected. In case of special problems and/or governmental

queries or requests for audit inspections, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

Audits and Inspections

The Sponsor-Investigator and any Participating Site Investigators should understand that source documents for this trial should be made available to appropriately qualified personnel from the Sponsor-Investigator or designee or to health authority inspectors after appropriate notification. The verification of the CRF data must be by direct inspection of source documents.

Case Report Forms

For each patient enrolled, a CRF must be completed and signed by the Sponsor-Investigator (and any Participating Site Investigator) or authorized delegate from the study staff. This also applies to records for those patients who fail to complete the study. If a patient withdraws from the study, the reason must be noted on the CRF.

All forms should be filled out clearly and legibly. Errors should be crossed out but not obliterated, the correction inserted, and the change initialed and dated by the Sponsor-Investigator (and any Participating Site Investigators) or his/her authorized delegate. The Sponsor-Investigator (and any Participating Site Investigators) should ensure the accuracy, completeness, legibility, and timeliness of the data reported to the Sponsor-Investigator in the CRFs and in all required reports.

Publication and Presentation Policy

The trial executive committee will be co-authors on all publications and presentations. The primary author list for the primary publication will consist of the executive committee and the site principal investigator at each of the sites. The results of this study may be published or presented at scientific meetings.

Ancillary Studies Policy

Ancillary or sub-studies may be considered by the trial executive committee. Important principles that guide the addition of ancillary studies are:

(1) no patient shall be enrolled in a concurrent investigational drug/device trial during the study period.

(2) concurrent enrollment of a TEMPO-2 study patient in a site specific observational cohort study is allowable, where the following conditions are met:

a. the executive committee is notified
b. the concurrent study does not interfere with any study follow-up procedures or potentially confound the outcome of the TEMPO-2 trial

c. the site PI of the concurrent study explicitly acknowledges that the treatment given in the TEMPO-2 trial may confound the outcome of the site-specific concurrent study
d. the patient may not be included in any publication or report until the TEMPO-2 study has been concluded and published.

(3) Ancillary or sub-studies shall be vetted and approved by the trial executive committee.

Data-sharing plan

The Executive Committee will follow the spirit of the NIH policy on data-sharing [http://grants2.nih.gov/grants/policy/data_sharing/data_sharing_guidance.htm]. In addition, the Executive Committee will follow the CIHR guidelines on public access to trial results and make the results available as free-access using PubMed. Upon completion of the TEMPO-2 Trial, a public use database will be prepared by stripping any and all personal identifiers. The public use database, consisting of several data files, should contain: (1) baseline and demographic characteristics; (2) outcomes assessments; (3) CT/MRI data; (4) concomitant medications and procedures; and (5) adverse events. Each data file is made available as a formatted SAS dataset or other electronic format. The data files are distributed along with the data dictionary and a brief instruction ("Readme") file. These data files will be made available to the public only after all major manuscripts (including secondary analysis papers) of the Trial are accepted for publication in peer-reviewed journals.

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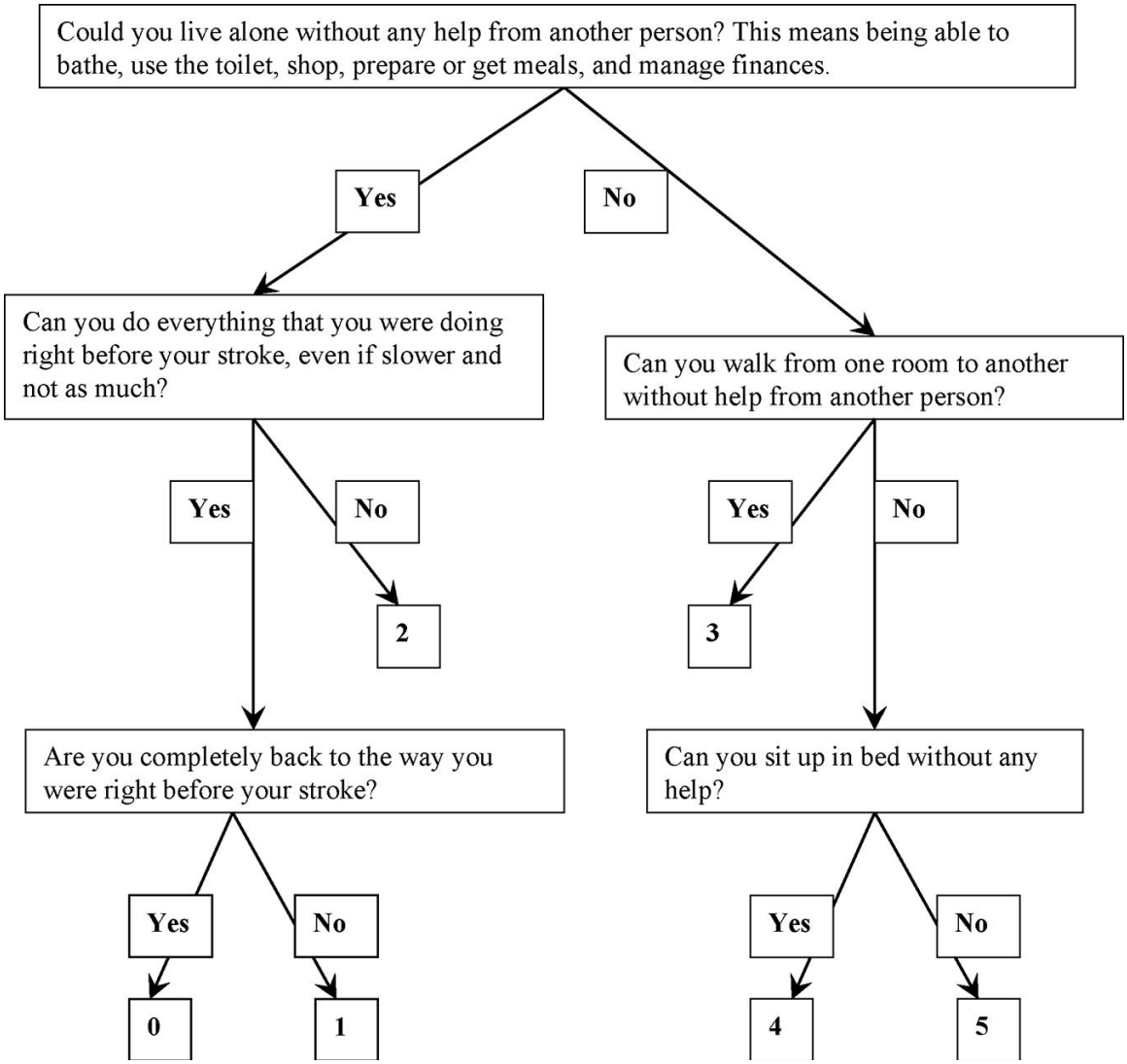
Appendix 1: Structured mRS - Taken from Bruno et al.[35]



TEMPO-2 trial: Statistical Analysis Plan (SAP)

1. Introduction

This SAP is for the TEMPO-2 study (TNK-tPA Evaluation for Minor ischemic stroke with Proven Occlusion-2).

The trial is an academic trial comparing two approved treatment approaches for acute ischemic stroke therapy. The trial is not a registration trial for the purposes of licensing a new or novel endovascular device. The trial sponsor is the "Governors of the University of Calgary". The trial is registered at Clinicaltrials.gov (NCT02398656).

Although the trial is not a registration trial, it will be conducted in Canada under a Health Canada CTA investigational drug license. Similar investigational drug licenses will be sought from international drug regulatory authorities.

2. Trial Objectives

The trial is designed to evaluate if treatment with intravenous tenecteplase (TNKase™, MetaLyse™, TNK-tPA) is superior to best standard of care in a population of minor ischemic stroke patients with proven intracranial occlusion. Patients must be treated within 12 hours of symptom onset and must not be eligible for routine treatment with intravenous alteplase (Activase™, Actilyse™).

3. Primary Outcome

Primary outcome: Return to baseline neurological functioning as measured by the mRS.

Analysis will be a responder analysis where return to baseline level of neurological functioning using a variation of the sliding dichotomy modified Rankin Scale score outcome, defined as follows:
If pre-morbid mRS is 0-1 then mRS 0-1 at 90 days is a good outcome.
If pre-morbid mRS is 2 then mRS 0-2 is a good outcome.

Pre-morbid mRS is assessed using the structured mRS prior to randomization.(1) Outcomes will be assessed by an individual blinded to the treatment assignment. The 90day mRS will be rated using the structured mRS questionnaire (see appendix 1). The 90 day mRS will be completed in person where possible and by telephone otherwise. The structured questionnaire has been showed to improve reliability in assessing the mRS both in person and by telephone.(1)

4. Sample size

A sample of 1228 patients allows us to demonstrate a 9% absolute risk difference (60% → 69% primary outcome) with 90% power between intervention and control groups.

The recent pooled thrombolysis individual patient meta-analysis showed an effect size of 10% in the subset of minor stroke patients treated with thrombolysis.(2) Enrollment in the trials included in the meta-analysis did not require patients to have an intracranial occlusion; thus it is likely that the majority of these patients did not have an intracranial occlusion. Although we expect that the effect size is higher in a population that only includes patients with intracranial occlusion we will conservatively estimate an overall 9% effect size with a change in proportion with excellent neurological outcome from 60% to 69%. The sample size for each group is 614 (1228 total).

Adjusting for alpha-spending for a single interim analysis and adding 4% loss to follow up gives a sample size estimate of 1274 patients (637 in each treatment group). There will be ongoing monitoring for safety and full details will be available in a formal safety plan. A single interim analysis for futility and efficacy will be conducted at approximately two-thirds patient enrolment (n=850). O'Brien Fleming boundaries will be used to establish the alpha spending function. Full details will be available in the DSMB charter.

It is possible that after central imaging review some patients will be enrolled in violation of the protocol or the treatment protocol may be breeched due to the dynamic nature of acute stroke. This may occur entirely in the best interests of patient care. The primary analysis population will be all patients randomized in their as-randomized assignments regardless of actual treatment – the intention to treat (ITT) population. The safety population will be defined as all patients who receive any dose of study drug. The per-protocol population will be defined as all patients who received any dose of study intervention (treatment or control) met all the inclusion and exclusion criteria and were appropriately consented.

Secondary analyses will be considered exploratory and include analysis of the pre-stated secondary outcomes and multivariable analyses of both the primary outcomes and pre-stated secondary outcomes. This Statistical Analysis Plan will be reviewed and finalized prior to breaking of the blind.

5. Interim Analyses

We will plan for a single interim analysis after two thirds patient enrolment is complete and 90-day follow-up is completed on those patients. There will be a safety analysis after 400 patients have been enrolled.

We will use O'Brien-Fleming boundaries at the interim analysis as follows:(3, 4) We will use a simple dichotomous analysis of the responder proportion (based on the mRS at 90 days from randomization). The Z-statistic for this analysis shall be derived from the normal approximation of the binomial distribution as an unadjusted two-sample test of proportions. This risk of stopping a trial early will be mitigated by having stringent futility and efficacy boundaries using O'Brien-Fleming methods (which are known to be conservative at the interim analysis stage).

O'Brien Fleming Boundary for a Binary Primary Endpoint

For a RCT comparing two treatment arms with respect to a binary outcome and one interim analysis, the binary test statistic is given as

$$Z_k = \frac{(p_{Ak} - p_{Bk})}{\left\{\sqrt{\overline{p_k}(1-\overline{p_k})/\left(\frac{1}{n_A}+\frac{1}{n_B}\right)}\right\}}, k = 1, \ldots, K = 2$$

where $\bar{p}_k = \frac{p_{Ak}+p_{Bk}}{2}$, *where* P_{Ak} *and* P_{Bk} *are the estimated response proportions in treatment arms A and B at stage k, respectively. The two-sided sequential test based on O'Brien & Fleming boundary is given as*

1. At stage 1 (interim analysis, n=850: Reject H_0 *and stop the trial at stage k if:* $|Z_k| \geq C_B(2_z0.05)\sqrt{2} = 2.834$

Else if $|Z_k| < C_B(2_z0.05)\sqrt{2} = 2.834$, *continue to stage 2.*

2. At stage 2 (final analysis): Reject H_0 *at stage k if:* $|Z_k| \geq C_B(2_z0.05) = 2.004$

Therefore, for a RCT with one interim analysis and a final analysis (i.e., K = 2), the critical boundaries at Stage 1 and Stage 2 (final analyses) are 2.834 and 2.004, respectively.

Instructions to DSMB: Stopping Rules/Guidance

- **Thus, if the Z statistic is greater than 2.834 at the interim analysis, the committee will then consider that there is statistical evidence for overwhelming efficacy.**

The committee is then entrusted with a decision to make recommendations about the continuation of the trial in the context of the data and the context of the current and known evidence about stroke treatment using their best judgment.

6. Definition of the target populations

6.1. Efficacy population

All patients enrolled in the trial randomized on an intent-to-treat basis (as randomized).

6.2. Safety population

All patients enrolled in the trial who received the intervention, any dose of study drug. All patients in the control group who received best standard of care.

6.3. Per-protocol population

All patients enrolled in the trial who received any dose of study drug and met all the inclusion and exclusion criteria and were appropriately consented.

7. Randomization

Randomization will be managed using a custom SQL server-based database that will instantly and dynamically assign treatment using the minimal sufficient balance algorithm. Randomization will therefore be conducted over the internet via a desktop computer or a web-enabled smart phone.

Randomization will be 1:1. Allocation will be 1:1 set at p(0.5) for the first 40 patients. Thereafter, a randomization minimization algorithm (minimal sufficient balance) will be utilized to ensure ongoing balance in the trial on the following 4 factors:

Age
Sex
Baseline NIHSS score
Time of onset (or last seen well) to randomization

The minimal sufficient balance (MSB) randomization is a minimization procedure that preserves balance in smaller trials, such as this one, where imbalances in important baseline prognostic variables may occur by chance and confound the primary outcome. In addition, it preserves a greater degree of randomness in patient allocation compared to permuted block designs.(5) Because of the MSB process, randomization assignments will be stochastically derived in real time using a interactive web-site and therefore concealment can never be breached. Randomization will be biased coin that will vary from fully balanced (50:50) to biased (65:35) dependent on what characteristics been previously enrolled have. The system will be enabled for smart-phone, tablet, laptop or desktop computer use. The allocation sequence will therefore be fully masked, but treatment is open-label.

Reliance on a process that requires real-time data entry makes the process susceptible to error. For example, incorrect information (eg. wrong sex or age) could be mistakenly entered into the randomization process and affect the minimization algorithm. Post-hoc, when such errors become known, the quality-controlled database entry will be considered the source of truth and the randomization database will be updated to ensure that ongoing randomized minimization utilizes the most correct data to determine balance in an ongoing way.

The randomization process is neither blocked nor stratified by site. Therefore, the number of patients enrolled into each arm of the study may not be exactly even at the time of interim analysis or when the study is completed. The proportion of patients enrolled into each arm at each site may also vary and not be equally distributed. These decisions were taken explicitly with the knowledge and belief that balance on 4 key patient characteristics in the trial overall are more important than balance by site.

8. Blinding

Treatment assignment is open-label. Blinding of the outcome assessment at 90 days will be ensured at the site by having a person who was blinded to treatment allocation and not involved in the acute treatment period conduct the assessment.

9. Missing data and imputation rules

Under the ITT principle, all patients who are randomized are included in the analysis. Therefore, missing data, especially in the outcome measures, can be problematic. Every effort will be made to keep all missing data to a minimum. We will follow a data-informed imputation process.

If a patient is known to be deceased, they will be assigned a score of 6 on the mRS, 42 on the NIHSS and 0 on the Barthel Index for all outcome time points at or following the date of death, and therefore a non-responder.

If patient is known to be alive at day 5, but has missing day 90 status, the patient will be imputed to be alive. If the patient has unknown vital status from day 5/discharge onward, the patient will be imputed to be deceased, and therefore a non-responder.

If the assessment of the primary outcome (mRS) was conducted outside of the protocol-specified time window, data obtained are still included in the analysis, with the rationale that it is a more accurate measure than those obtained by imputation. At a minimum 90-day outcome assessments will be accepted within a +/- 30-day window.

If the primary outcome (mRS at 90 days) is missing but the patient is known to be alive, the patient will be imputed to a non-responder

If the rate of missing primary outcome data is <5% no further imputation will be done. In the event that there are more than 5% missing primary outcome data, we will perform the following sensitivity analyses:

To assess the impact of those missing data by using imputation with the following methods:

- If 5-day/discharge outcome scores are available, carry forward those values to determine responder status; else, impute the patient as a non-responder.
- Assign non-responder status to all subjects with missing 3-month outcome data.
- Hot-deck or nearest neighbor method, using clinical site, age, sex, baseline NIHSS, baseline serum glucose, baseline ASPECTS, , treatment group as classification variables.

- Regression method, with age, sex, baseline NIHSS, baseline serum glucose, and treatment group as covariates.

Similar imputation methods will be employed for secondary categorical outcomes. For the raw NIHSS score, multiple imputation, regression, and mean substitution methods will be used in the sensitivity analyses. Missing covariate data, if any, will be imputed using either multiple imputation or regression method, if needed.

Finally, we will conduct a "Tipping Point" analysis to assess the influence of missing data on the primary effect size estimate and direction of effect.

10. Efficacy Analysis

10.1. Primary analysis

The primary analysis will be conducted using a two-sample test of proportions (Fisher's exact test). This will be supported by a secondary analysis will use an additive multivariable model (generalized Poisson mixed-effects model with log link) adjusting for all the minimization variables included as co-variables. Site will be considered a random effect and not pooled. Only main effects will therefore be considered in this model. The results will be expressed as a risk ratio with 95% confidence limits. Additional analyses will include a safety population analysis defined to include only those patients who received tenecteplase, a per-protocol analysis including those patients who were treated according to protocol.

The primary analysis will be unadjusted. Because the randomization is being balanced a priori according to key prognostic variables (age, sex, NIHSS, and time to treatment), we expect that the unadjusted analysis will be similar to the adjusted analysis.

A revised statistical analysis plan may be modified according to the statistical distribution of variables and finalized prior to breaking the blind.

10.2. Secondary analyses

Pre-specified secondary outcome and safety analyses of proportions will be conducted in a similar way to the primary analysis using logistic regression or using a multivariable generalized linear model with log link to derive risk ratios directly. Pre-specified secondary analyses will include the following:

10.2.1. Proportion of patients with major bleeding: This will include an analysis of symptomatic intracranial hemorrhage alone and then combined with major extracranial hemorrhage. This is the main safety outcome.

10.2.1.1. Symptomatic intracranial hemorrhage defined as new intracranial hemorrhage (ICH, SAH, IVH, SDH) associated with clinical evidence of

neurological worsening, in which, the hemorrhage is judged to be the most important cause of the neurological worsening. Clinical worsening will be guided by the NIHSS score of a minimum of 2 or more points different from baseline.

10.2.1.2. Major extracranial hemorrhage defined as life threatening, resulting in hemodynamic compromise or hypovolemic shock, requiring inotropic support or other means to maintain cardiac output, requiring blood transfusion of more than 2 units of packed red blood cells, or associated with a fall in hemoglobin greater than or equal to 5 g/L.

10.2.2. Proportion of patients with complete and partial recanalization (mAOL 2-3) post treatment. This will be assessed on CTA 4-8 hours post treatment. Recanalization will be assessed by the central core-imaging lab blinded to all clinical information.

10.2.3. Categorical shift analysis on the full range of the mRS (0-6).

10.2.4. Absence of disability defined as mRS 0-1.

10.2.5. Functional independence defined as mRS 0-2.

10.2.6. Return to exact baseline function or better. If pre-morbid mRS is 0 then mRS 0 at 90 days is a good outcome. If pre-morbid mRS is 1 then mRS 1 is a good outcome. If pre-morbid mRS is 2 then mRS 0-2 is a good outcome.

10.2.7. Comparison of the mean mRS using linear regression using the mRS as a continuous variable.

10.2.8. Lawton Instrumental Activities of Daily Living Scale (IADL)(6, 7)

10.2.9. Proportion of patients with an NIHSS 0 at day 5 (or discharge from hospital if discharged before day 5)

10.2.10. Quality of life measured on EQ5D-5L (EuroQol)(8)

10.2.11. Quality of life as measured by the “problems with usual activities” question on the EuroQol.

10.2.12. Stroke progression and recurrent stroke (separately and together).

10.2.13. All-cause mortality

10.2.14. Discharge location – home, rehab facility, long term care etc.

10.2.15. Proportion of patients getting rescue EVT

10.2.16. Economic analysis will be conducted using Canadian hospital data and quality of life measure to estimate treatment utility.

10.3. Pre-specifed subgroups of interest:

Sex
Patients treated <4.5 hours and after 4.5 hours
Outcomes in patients with recanalization vs. partial vs. no recanalization
Patients with direct evidence of occlusion on CTA vs. indirect evidence of occlusion on CTP or multiphase CTA

Occlusion location
Over age 80 vs. 80 years of age or less
Complete resolution of symptoms at randomization versus not.

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