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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ABSTRACT

Background: Individuals with minor ischaemic stroke and intracranial occlusion are at increased risk of poor outcomes. Intravenous thrombolysis with tenecteplase may improve outcomes in this population.

Methods: In this multicentre, prospective, parallel group, open label with blinded outcome assessment, randomised controlled trial, patients were included at 48 hospitals in 10 North and South American, European and Australasian countries. Adult patients with minor acute ischaemic stroke defined as NIHSS ≤ 5, an intracranial occlusion or focal perfusion abnormality were enrolled within 12-hours from stroke onset. They were randomly assigned (1:1), using a minimal sufficient balance algorithm to intravenous tenecteplase (0·25 mg/kg) or non-thrombolytic standard of care. Primary outcome was a return to baseline functioning on pre-morbid modified Rankin Score (mRS). Safety outcomes included symptomatic intracranial haemorrhage (SICH) and death. The trial is registered with ClinicalTrials.gov, NCT02398656 and is closed to accrual.

Findings: The trial was stopped early for futility. Between 27th April 2015 and 19th January 2024, 886 patients were enrolled; 369 (41·6%) were female. 454 (51%) were randomised to non-thrombolytic standard of care and 432 (49%) to intravenous tenecteplase. The primary outcome occurred in 338 (74·8%) patients in the control group and 309 (71·5%) in the tenecteplase group (RR 0.96, 95% CI 0.88-1.04, p=0.2882). There were more deaths in the tenecteplase group, 5 deaths (1.1%) control, 20 (4.6%) tenecteplase (adjHR 3·8;95%CI 1·4-10·2, p=0.0085). There was a trend toward more SICH in the tenecteplase group, 2 control (0.4%), 8 tenecteplase (1.9%) (RR 4·2; 95%CI: 0·9-19·7, p=0·0588).

Interpretation: Patients with minor stroke and intracranial occlusion should not be routinely treated with intravenous thrombolysis.

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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 are at high risk of early deterioration and disability. We searched MEDLINE and PubMed for randomized trials published in English between Jan1, 2000 and March 31st 2024, using the terms “stroke”, “tenecteplase”, “alteplase”, and trial or study. We could not identify any phase 3 randomized trials comparing tenecteplase or alteplase with antiplatelet agents in patients with minor stroke and intracranial occlusion. There are two phase 3 trials comparing alteplase with antiplatelet agents in minor stroke, but none looking at the subset with intracranial occlusion and none looking 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 hours 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

Minor ischaemic stroke patients with intracranial occlusion should not be treated with intravenous thrombolysis with tenecteplase. Antiplatelet therapy is enough.

Up to 50% of ischaemic stroke patients initially present with minimal symptoms which are non-disabling. [1] Despite having low scores on the National Institutes of Health Stroke Scale (NIHSS) score, 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 sub-population at high risk for early neurological deterioration, [5,6] which most often occurs within the first 24 hours 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 have excluded minor stroke patients and thus high-quality data to guide thrombolytic treatment in these patients are lacking.

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 outcome in these individuals (OR 1.48, for good outcome (mRS 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 demonstrated benefit over antiplatelet therapy. The PRISMS (The effect of Alteplase vs Aspirin on Functional Outcome for Patients with Acute Ischaemic Stroke and Minor Nondisabling Neurological deficits) 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 (SICH) in the alteplase group[9]. The ARAMIS (Dual Antiplatelet Therapy vs Alteplase for Patients With Minor Nondisabling Acute Ischaemic Stroke) 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 SICH between groups. [10] Both trials used intravenous alteplase as the comparative thrombolytic agent and restricted enrolment to either 3 or 4.5 hours from symptom onset. Both trials also tried to exclude patients who may be at high 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 appear to be at the highest risk for early deterioration and disability. [5,6] Fundamentally the prognostic factors that accurately identify which patients will be disabled 3 months later is likely to vary greatly.

Tenecteplase, a recombinant human tissue plasminogen activator similar to alteplase, has a longer half-life in part due to resistance to plasminogen activator inhibitor, and is more fibrin-specific and results in less systemic depletion of circulating fibrinogen as compared to alteplase[11]. The ACT (Alteplase compared to Tenecteplase) 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 hours of symptoms onset. [14-16] The TIMELESS (Thrombolysis in Imaging Eligible, Late Window Patients to Assess the Efficacy and Safety of Tenecteplase) study[17] included patients with disabling stroke between 4.5 and 24 hours from onset with potentially salvageable tissue

defined by CT perfusion imaging and randomised patients to treatment with standard of care or intravenous tenecteplase. While there was no observable difference in outcomes between groups, there was no evidence of harm when tenecteplase was given in this later time window. The TWIST (Tenecteplase in Wake-up Ischaemic Stroke Trial) study found similar safety in patients with stroke-on-awakening. [18] We previously completed 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. This study showed a low SICH rate (4%) and high recanalization rates at the 0.25mg/kg dose. All of these studies[12,17-19] have shown the safety thrombolysis with tenecteplase in selected patients within 4.5 hours and after 4.5 hours from stroke onset.

The TEMPO-2 (Multicenter, prospective randomised open label, blinded-endpoint, controlled trial of thrombolysis with Tenecteplase versus standard of care in the prevention of disability at 3 months in minor ischaemic stroke with proven acute symptomatic occlusion) trial was designed to demonstrate superiority of intravenous tenecteplase (0.25mg/kg) as compared to non-thrombolytic standard of care in patients with minor stroke with intracranial occlusion or focal perfusion lesion presenting within 12 hours from symptom onset, on 90-day functional outcomes assessed with the modified Rankin Scale (mRS).

Methods

Study design and participants

The TEMPO-2 trial 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 (0.25mg/kg) over non-thrombolytic standard care in patients with minor ischaemic stroke deficits, defined as NIHSS 0-5, and intracranial occlusion or focal perfusion lesion within 12 hours from onset of symptoms. The trial was conducted at 48 hospitals in Canada, Australia, United Kingdom, Singapore, Brazil, New Zealand, Finland, Austria, Spain, and Ireland. The methods of this trial have been previously published, [20] and the protocol is available in the appendix. The trial was sponsored by the University of Calgary. 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). 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 surrogate 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 a NIHSS score $\leq$ 5, presented within 12 hours 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 ASPECTS (Alberta Stroke Program Early CT score) [21] score 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 appendix. 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 (CTA) underwent a follow-up CTA of the intracranial circulation between 4-8 hours after randomization in both groups to determine early recanalization status of the occluded artery. All patients underwent routine follow-up brain imaging at 24 hours with either CT or MR.

Randomization and masking

Patients were randomly assigned (1:1) to intravenous tenecteplase versus non-thrombolytic control. Randomization 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, 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 randomised 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 0.25mg/kg (maximum dose 50mg) as a single, intravenous bolus administered over 5-10 seconds immediately after randomisation. Patients randomised to control were treated with standard of care 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 medication(s) were given immediately after randomisation. Imaging was reviewed centrally at the University of Calgary core-lab 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 4-8 hour imaging in patients with direct evidence of occlusion and follow up imaging was assessed for any intracranial haemorrhage, and was 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: If the pre-morbid mRS is 0-1, then mRS 0-1 at 90 days is a responder (good outcome).

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 prior to 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 showed 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 using linear regression using the mRS as a continuous variable, percent function on Lawton Instrumental Activities of Daily Living Scale (IADL) at 90-days,[24] NIHSS at day 5 or on day of hospital discharge (whichever is earlier), quality of life measured at 90-days on EQ5D-5L[27], stroke progression and recurrent stroke, [7] all-cause mortality, proportion of patients getting rescue endovascular thrombectomy for the index stroke and recanalization at 4-8 hours. [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 hours of randomization. This included SICH alone as well as a composite of SICH and major extracranial haemorrhage. SICH 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.

Statistical analysis

The statistical analysis plan was finalised before database lock (on April 10, 2024). Prior literature show 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 higher 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], the incidence of primary outcome (mRS score, 0–1) 90 days was 66% in the combined 0.1mg/kg and 0.25mg/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 and 450 patients were enrolled. There was a signal of excess death 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 and but not for the two that withdrew consent. For individual secondary outcomes on the mRS scores, no missing data were imputed. We imputed the worst possible score for the EQ5D-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 randomization and all secondary outcomes analysed unadjusted and adjusted for the same variables. There variables were chosen a priori because they are of prognostic or epidemiological importance and because these variables were used in the randomized minimization algorithm. We used generalized 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, onset-to-randomization 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 versus female), timing of treatment ($\leq$ 4·5 hours and >4·5 hours from symptom onset), age ($\leq$ 80 and >80 years), how occlusion was identified (directly observed on CT angiography versus inferred from CT perfusion or multiphase CTA), occlusion location (large vessel occlusion(internal carotid artery or middle cerebral artery(MCA)-M1) versus medium vessel occlusion (MCA-M2 or distal, Anterior Cerebral Artery or distal) versus vertebrobasilar (includes posterior cerebral artery)), recanalisation[28] and baseline NIHSS score prior to randomisation. Analyses were completed using STATA (VERSION 18). The trial was registered at ClinicalTrials.gov, NCT02398656.

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 27th April 2015 and 19th January 2024, 886 patients were enrolled at 48 sites (Figure S1, Table S1 and S2); 369 (42%) were female. 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 (0·2%) patients withdrew consent, leaving 884 patients in the ITT population. 432 (49%) were assigned to receive tenecteplase and 454 (51%) to receive non-thrombolytic standard of care (Figure 1). There were 4 patients with missing mRS outcomes at 90 days, 2 (0.2%) patients that were lost to follow-up and 2 that withdrew consent. There were no missing baseline data.

Baseline demographic and clinical characteristics of patients were similar between the tenecteplase and control groups (Table 1). The only difference between groups was that the control medications were given a median of 17 minutes later than tenecteplase. Median baseline NIHSS was 2 (1-3) overall in the study (Table S3, S4) and 149/884 (17%) had complete symptom resolution at the time of randomization. Median onset to randomisation was 4·6 hours (IQR 162- 447 minutes). The majority of the control group patients were treated with dual antiplatelet therapy with aspirin and clopidogrel (n=259; 57·3%) or aspirin monotherapy (n=106; 23·5%). The control population treatments are shown Table S5 in the Appendix.

After a median follow-up time of 92 days (IQR 85-99), the primary outcome (mRS responder analysis) occurred in 338 (74·8%) of patients in the control group and 309 (71·5%) in the tenecteplase group (RR 0.96, 95% CI 0.88-1.04). Secondary outcomes are shown in Table 2, Figure S2, and per protocol in table S6. More patients achieved a NIHSS of 0 in the tenecteplase group versus control (226 (50%) versus 247 (57.8%), RR 1·16; 95% CI: 1·02-1·31). In a subset of patients (515/884, 58%), recanalization at 4-8 hours assessed using a CT Angiogram was higher in the tenecteplase treated patients versus control (47·7% versus 21·6%, p<0·001). See Table S7 and Figure S3.

In safety analysis, there were more symptomatic intracranial haemorrhages in the tenecteplase group versus control (n=8; 1·9% versus n=2; 0.44%, RR 4·2; 95%CI: 0·9-19·6).(Table 3 and Table S8. Symptomatic intracranial hemorrhages were distributed 4 in the 0-4.5h window and 6 in the 4.5 to 12h window (p=0.7531). There were 34 patients who received a dose of tenecteplase greater than 25 mg and one of these patents suffered a symptomatic ICH. There were no extracranial haemorrhages temporally related to treatment. There were an excess of deaths in the tenecteplase group (adjHR 3·8; 95%CI 1·4-10·2). Seven deaths (1 in the control, 6 in tenecteplase groups) were related to a symptomatic ICH. Other adverse outcomes are shown for the ITT analysis in Table 3. Other than the deaths after SICH, most deaths occurred well after treatment and were not judged to be biologically related to tenecteplase. See Figure S4 and Table S9.

In the subgroup analyses, there was a suggestion of heterogeneity of treatment effect between males and females and by age. Females were more likely to do better with control (risk

difference 10·1%) as compared to males (no treatment effect) (p_int = 0.043) and patients older than 80 years of age were more likely to do better with control (risk difference 14.9%) as compared to younger patients (no treatment effect). (p_int = 0.038). There was no heterogeneity of treatment effect observed across any other subgroups (See Figure 2).

DISCUSSION

Among patients with minor stroke symptoms (NIHSS 0-5) and intracranial occlusion presenting within 12 hours from onset, we found no benefit for the prevention of disability after treatment with 0.25mg/kg of tenecteplase as compared to non-thrombolytic standard of care. There was a small increased risk of symptomatic haemorrhage in patients treated in the tenecteplase group and more deaths at 90-days in the tenecteplase group as compared to non-thrombolytic standard of care.

Over the last decade endovascular thrombectomy (EVT) 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 suspected ischaemic stroke patients including those with milder deficits. With increased imaging, this has meant that there are many patients presenting with relatively minor deficits that are now identified to have evidence of an intracranial occlusion. Based upon prior work, the fundamental premise of TEMPO-2 was that the intracranial occlusion defined the minor stroke population with the highest risk of poor outcome and that reperfusion would be beneficial.

While there were significantly more patients with early recanalization and a NIHSS=0 at day 5 or discharge after tenecteplase treatment, this did not translate into improved functional outcomes at 90 days. High recanalization rates are concordant with a recently published meta-analysis that shows high recanalization rates with tenecteplase as compared to alteplase. [29]All other secondary outcomes did not show any benefit for tenecteplase. There was a trend to an increased rate of symptomatic intracranial haemorrhage in the tenecteplase group, but at the relatively low absolute rate of 1.9%, which is lower than was seen in the tenecteplase group of the AcT study (3.4%). The PRISMS study[9] found that there was a low but increased risk of symptomatic haemorrhage (3.2%) 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 lack of benefit at 90 days with tenecteplase. Similar to other trials[17,18] we allowed patients to be enrolled out to 12 hours from symptom onset. We did not see any increase in the SICH rate in patients treated after 4.5 hours. The sub-group of patients treated 4.5-12 hours had a trend to better outcomes with thrombolysis as compared to under 4.5 hours. This suggests that the 12 hour window for TEMPO-2 did not explain the lack of benefit seen from tenecteplase.

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

in the standard-of-care group, or better overall stroke care. The recanalization rate in the non-thrombolytic group was 21.6% overall in the study and in large vessel occlusion was 12.5% in the non-thrombolytic group, highlighting that antiplatelet treatment is still an active treatment. Despite the reported high recanalisation rates in the tenecteplase group (47.7%), 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 minor stroke patients with intracranial occlusion are at risk of both progression and disability. [23] It may be that medical care (e.g. intravenous fluids, antiplatelet therapy) in this patient population reduced the rate of stroke progression in both groups. A rate recanalization of 47% may simply not be high enough to influence stroke progression and outcomes. It is likely that most of these patients have excellent collaterals and it is possible that good supportive care improved outcomes in both groups. It is also possible that the neurological deficit is so minimal that vessel recanalization cannot make patients detectably much better using the outcome assessments we currently use. Consistent with the low rate of stroke progression, rescue EVT happened at a low rate in both treatment groups in the study. Studies examining the use of EVT in the subset of these patients with LVO in trials such as the endovascular therapy for low NIHSS ischaemic stroke trial (ENDOLOW) are ongoing. [30]

Overall mortality was low at 25 (2.8%) patients but, there was an increased rate of death in the tenecteplase group. A majority of these deaths occurred late and were not temporally related to study drug, with only 7 of 25 deaths associated with a symptomatic intracranial haemorrhage (6 in tenecteplase group and 1 in control group). The rates of stroke progression, stroke recurrence and rescue EVT 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, it may be a chance finding.

Strengths of this study are that it is a large, well-conducted investigator initiated, international, multi-centre study randomised trial with near complete follow-up. Weaknesses include 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 defined by a focal perfusion lesion on CT perfusion, 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 lack of benefit seen from tenecteplase. The control group patients principally received antiplatelet therapy, but treatment was not one single comparator. This pragmatic choice reflected real clinical practice and may make the trial result more generalizable. 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 prove, 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 to 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 SC, and CK providing additional data management and statistical support, and all had access to all the data. SBC, CK, NS, 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 CIHR, HSFC and the BHF to complete the TEMPO-2 study. SBC is Boehringer Ingelheim provides the 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 Astra-Zeneca. He has received consultant or speaker fees from Pfizer-BMS, Medtronic, Amgen, and travel support from Daiichi-Sankyo. KSB reports speaker's fees from Boehringer-Ingelheim and Astra-Zeneca. LC has received consulting/speakers fees for Roche. JF reports speaker fees from Astra-Zenica, Bayer, BMS, Boehringer-Ingelheim, BMS, Pfizer, Sankyo and Daiichi. AG reports a grant from Microvention and speaker fees from Alexion, Biogen and Servier Canada. AG holds stock options for SnapDx Inc and Collavidence Inc. DG reports honoraria from Boehringer Ingelheim, Ipsen, and Eisai. MG reports grant funding from Medtronic and Cerenovus. He has been paid consulting fees from Mentice Inc., CSL Behring LLC, MicroVention and Medtronic. TSF participated in an advisory board for Roche. FOL 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 TNK for treatment of acute ischaemic stroke. SM has received Speaker fees from: Boehringer, Medtronic, Penumbra, Bayer, Pfizer, Novartis, Novo Nordisk, Servier, Daiichi Sankyo. KM has Grant funding – British Heart Foundation, The Stroke Association; Consultancy – Boehringer Ingelheim, Biogen, IschaemaView; Lecture fees – Boehringer Ingelheim, IschaemaView, Brainomix; Non-financial support (drug supply for ATTEST-2) – Boehringer Ingelheim. MWP is on Boehringer-Ingelheim Advisory Board for Metalyse in stroke. AP received speaker fees from Boehringer Ingelheim and describes travel support from Astra Zeneca for attending a meeting. OMP has received speaker fees from Boehringer-Ingelheim, AstraZeneca. RS is part funded by the UCLH Biomedical Research Centre. DS reports an unrestricted educational grant from Boehringer Ingelheim. MIW has received sponsorship/financial assistance to support attendance at scientific meetings from pharmaceutical companies

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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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Table 1 – Baseline Characteristics (ITT population)

	Control (N=452, 51.1%)	Tenecteplase (N=432, 48.9%)
Demographics		
Age(y)	72 (61-79)	72 (62-80)
Female sex	180 (39·8%)	188 (43·5%)
Race		
Caucasian	382 (84·5%)	371 (85·9%)
Asian	42 (9·3%)	40 (9·3%)
Black	7 (1·5%)	6 (1·4%)
First nations	5 (1·1%)	4 (0·9%)
Pacific islander	0 (0·0%)	1 (0·2%)
Other	16 (3·5%)	10 (2·3%)
Ethnicity		
Hispanic	9 (2·0%)	5 (1·2%)
Medical History		
Hypertension	261 (57·7%)	265 (61·3%)
Past smoking	176 (38·9%)	172 (39·8%)
Hyperlipidemia	172 (38·1%)	180 (41·7%)
Diabetes mellitus	86 (19·0%)	82 (19·0%)
Past stroke	85 (18·8%)	72 (16·7%)
Atrial fibrillation	78 (17 ·3%)	91 (21·1%)
Ischaemic heart disease	73 (16·2%)	69 (16·0%)
Congestive heart failure	18 (4·0%)	16 (3·7%)
Chronic renal failure	17 (3·8%)	22 (5·1%)
Peripheral vascular disease	15 (3·3%)	13 (3·0%)
Past ICH	1 (0·2%)	3 (0·7%)

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 randomization 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 -- core lab		
Large vessel occlusion (intracranial ICA, M1-MCA)	50 (11·1%)	53 (12·3%)
Medium Vessel Occlusion (M2-MCA or distal, A2-ACA or distal)	245 (54·4%)	235 (54·5%)
Vertebrobasilar circulation (VA, BA or branches, PCA)	25 (5·6%)	20 (4·6%)
Focal perfusion lesion	127 (28·2%)	118 (27·4%)
No occlusion detected	3 (0·7%)	5 (1·2%)

NIHSS = National Institutes of Health Stroke Scale score; ASPECTS = Alberta Stroke Program Early CT score; ICA = internal carotid artery; M1-MCA = M1 segment of the middle cerebral artery; M2-MCA = M2 segment of the middle cerebral artery; A2-ACA = A2 segment of the anterior cerebral artery; VA = intracranial vertebral artery; BA = basilar artery; PCA = posterior cerebral artery). The table represents the intention to treat population: 886 were randomised and 2 withdrew consent leaving 884 patients. The 8 patients with no occlusion detected were protocol violations and were excluded in the per protocol analysis. All imaging interpretation was from central review.

Table 2. Outcomes (ITT population)

	Control (n=452)	Tenecteplase (n=432)	Risk Difference (CI_{95})	Risk Ratio (Unadjusted) (CI_{95})	Risk Ratio (Adjusted*) (CI_{95})
Primary outcome					
Responder	338 (74·8%)	309 (71·5%)	-3·3% (-9·1 to +2·6)	0·96 (0·88-1.04)	0·96 (0·89-1·04)
Secondary outcome					
mRS 0-1 at 90 days	321 (71·3%)	298 (69·0%)	-2·4% (-8·4 to +3·7)	0·97 (0·89-1·05)	0·97 (0·90-1·06)
mRS 0-2 at 90 days	391 (86·9%)	352 (81·5%)	-5.4% (-0.10 to -0.006)	0·94 (0·89-0·99)	0·94 (0·89-1·00)
NIHSS = 0 at 5 days or discharge	226 (50·0%)	247 (57·8%)	7·8% (1·3 to 14·4)	1·16 (1·02-1·31)	1·15 (1·03-1·30)
mRS return to pre-morbid function	222 (49·1%)	212 (49·1%)	0·0% (-6·6 to +6·5)	1·00 (0·87-1·14)	1·00 (0·88-1·15)
					Risk Difference (adj)
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 at 90 days	1 (0-2)	1 (0-2)	---	---	---
Lawton IADL percent functioning (n=850)	90·8	86·4	-4·50 (-7.91 to – 1·09)	---	-4·02 (-7·30 to -0·74)
EQ5D-5L index (n=854)	0·84	0·81	-0·03 (-0·07 to -0·001)	---	-0·03 (-0·06 to -0·001)
EQ5D-5L VAS (n=839)	0·76	0·73	-3·38 (-6·29 to -0·48)	---	-3·24 (-6·13 to -0·35)
				Hazard ratio	Hazard ratio (adj)
Death at 90 days	5 (1·1%)	20 (4·6%)	---	3·9 (1·4-10·4)	3·8 (1·4-10·2)

*Adjusted for age, sex at birth, baseline NIHSS score, onset to randomisation time.
The data violate the proportional odds assumptions and so an ordinal shift analysis is not presented. CI_{95} = 95% confidence interval; mRS = modified Rankin Scale score; NIHSS = National Institutes of Health Stroke Scale score; IQR = interquartile range

Lawton IADL = Lawton-Brody Instrumental Activities of Daily Living Scale index with units %
EQ5D-5L index = European Quality of Life score index with no units, scaled from 0 to 1.
EQ5D-5L VAS = European Quality of Life visual analog scale health score with no units, scaled from 0 to 100.

Table 3 – Safety Events (ITT population)

	Control (N=452, 51·1%)	Tenecteplase (N=432, 48·9%)	P
SAE occurred	80 (17.7%)	100 (23.1%)	0·0454
Stroke progression	33 (7·3%)	35 (8·1%)	0·7056
Stroke recurrence	15 (3·3%)	16 (3·7%)	0·8554
Symptomatic ICH	2 (0·4%)	8 (1·9%)	0·0588
Death after symptomatic ICH (n=10) within 90 days	1 (0.2%)	6 (1.4%)	1·0000
Any hemorrhage on follow-up imaging	40 (9.2%)	62 (14.4%)	0.0202
Rescue EVT for index stroke	10 (2·2%)	15 (3·5%)	0·3120
Death within 5 days	1 (0·2%)	8 (1·9%)	0·0184
Death at 90 days	5 (1·1%)	20 (4·6%)	0·0018
Aspiration pneumonia	2 (0·4%)	6 (1·4%)	0·1688
Atrial fibrillation	3 (0·7%)	4 (0·9%)	0·7198
Congestive Heart Failure	1 (0·2%)	5 (1·2%)	0·1159
Seizure	3 (0·7%)	3 (0·7%)	1·0000
Urinary Tract Infection	4 (0·9%)	2 (0·5%)	0·6869

SAE = serious adverse event; ICH = intracranial haemorrhage; EVT = endovascular thrombectomy

Figure 1: Trial profile. ITT = Intention-to-treat

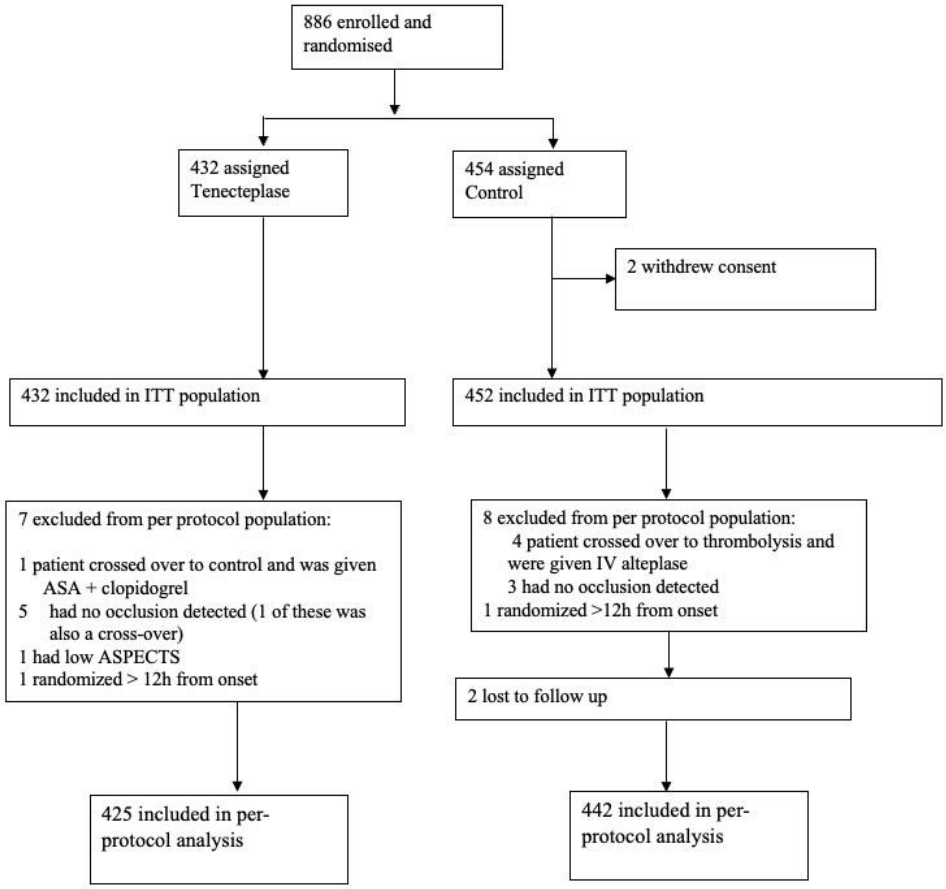
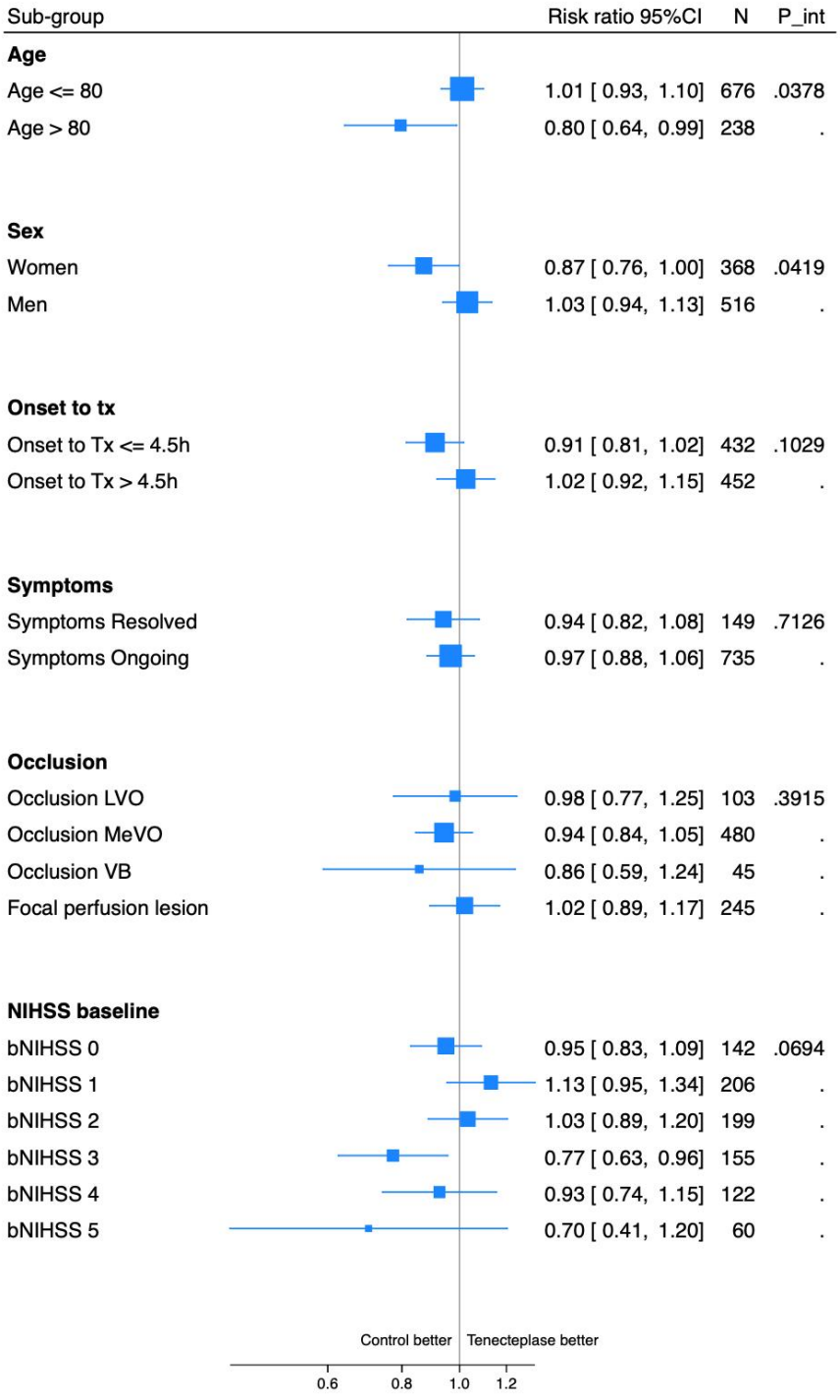


Figure 2 Forest Plot of effect size by sub-groups



Adjusted for sex, age, time from onset and baseline NIHSS. Tx = treatment. h= hours. LVO=Large Vessel occlusion (intracranial internal carotid and M1-middle cerebral artery), MeVO = medium vessel occlusion (M2-middle cerebral artery or distal, A2-anterior cerebral artery or distal). VB=vertebrobasilar occlusion including all branches of the posterior cerebral artery).