Alteplase-Tenecteplase Trial Evaluation for Stroke Thrombolysis (ATTEST) – Phase 2 Randomised Clinical Trial

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Summary

Background: Alteplase given within 4·5 hours of onset is the only approved medical treatment in acute ischaemic stroke. Newer thrombolytic agents may be advantageous, with higher fibrin specificity, easier administration schedules, and longer half-life. Different doses of tenectplase have been investigated previously in two small randomised trials in acute stroke in 3 or 6 hour time windows, in one study using advanced imaging-based patient selection. We evaluated the efficacy and safety of tenecteplase versus alteplase in a less selected population than previous studies, using imaging biomarkers, in order to inform the appropriate design of a definitive clinical trial.

Methods: In a single-centre, phase 2, prospective randomised open-label blinded end-point evaluation (PROBE) trial, adult patients with supratentorial ischaemic stroke eligible for intravenous thrombolysis within 4·5 h of onset received tenecteplase 0·25 mg/kg (maximum 25 mg) or alteplase 0·9 mg/kg (maximum 90 mg). Treatment allocation used a mixed randomisation/minimisation algorithm including age and NIHSS score, generated by an independent statistician. Imaging comprised baseline computed tomography (CT), CT perfusion (CTP) and CT angiography (CTA), and CT+CTA at 24-48 hours. The primary end-point was penumbral salvage (CTP-defined penumbra volume minus follow-up CT infarct volume.

Results: We recruited 104 patients (52 in each group) over 20 months. In the protocol-defined analysis of 96 confirmed stroke patients, groups were balanced for clinical characteristics. Among 71 subjects contributing to the imaging primary endpoint, no significant differences were observed for penumbral salvage [68 (SD 28) % tenecteplase vs 68 (SD 23) % alteplase], mean difference 1% (95% confidence interval -10%, 12%, p=0·81) or for any secondary end-point. Neither SICH incidence (1/52, 2% vs 2/51, 4%, by SITS-MOST definition, p=0·55; by ECASS-2 definition, 3/52, 6% tenecteplase vs 4/51, 8% alteplase, p=0.59) nor total ICH events differed significantly (8/52 tenecteplase, 15% vs 14/51 alteplase, 29%, p=0·091). The incidence of

serious adverse events did not differ between groups (32 in the tenecteplase group, 3 considered probably or definitely related to drug treatment; 16 in the alteplase group, 5 considered drug-related).

Interpretation: Neurological and radiological outcomes did not differ between tenecteplase and alteplase. Further evaluation of tenecteplase in acute stroke is warranted.

Funding: The Stroke Association (TSA 2010/04).

The recombinant tissue plasminogen activator (rtPA) alteplase is the only medical treatment currently approved for acute ischaemic stroke, but while it significantly improves the likelihood of disability-free recovery, alteplase has limited fibrinolytic efficacy, achieving arterial recanalisation in fewer than 50% of patients [1]. Among those who recanalise, only about half do so within two hours of drug administration[2]. Tenecteplase (TNK) is a modified rtPA molecule engineered to improve efficacy[3] through higher affinity binding to fibrin, greater resistance to inactivation by Plasminogen Activator Inhibitor-1(PAI-1), lack of procoagulant effects, and longer free plasma half-life. In animal models, it achieved significantly shorter time to reperfusion, with greater recanalisation and reduction of thrombus burden compared to alteplase [4]. In acute myocardial infarction (MI), tenecteplase demonstrated equal therapeutic efficacy and lower bleeding risk than alteplase [5], with a trend towards better early reperfusion[6].

Investigation of new thrombolytic agents in acute stroke has, until recently, concentrated on time periods beyond the 4·5 hour time window from symptom onset established for alteplase. Dose-ranging studies of tenecteplase in acute stroke have been reported[7, 8]. Parsons and colleagues [9] reported superiority of tenecteplase over alteplase in terms of both imaging-defined reperfusion and clinical outcomes in a selected group of patients with large artery occlusion and favourable brain perfusion patterns defined on computed tomography (CT). However, it is unclear how the effect sizes observed in a highly selected population will translate into more general populations. We undertook a phase 2 randomised, controlled clinical trial comparing alteplase with tenecteplase in a thrombolysis-eligible acute ischaemic stroke population, using imaging criteria as exploratory outcome measures rather than selection criteria, in order to define possible effect sizes in a general stroke population eligible for intravenous thrombolysis.

Methods:

Study design, Setting and patients

The trial was a single centre phase 2 Prospective, Randomised, Open, Blinded End-point (PROBE) study comparing the efficacy and safety of alteplase and tenecteplase in thrombolysis-eligible patients with acute ischaemic stroke, using clinical and imaging biomarkers for outcome evaluation. The trial was conducted at a regional neurosciences centre providing a comprehensive stroke service to a population of approximately 450,000, with some additional secondary transfers for thombolysis assessment from seven regional stroke services. Total admissions are approximately 900 per annum, of whom approximately 600 are confirmed ischaemic strokes. Thrombolysis numbers average 100 per annum. Clinical research staff recruited to the trial and additionally supervised multimodal CT acquisitions, including out of hours.

Patients were eligible if they had clinically diagnosed supratentorial acute ischaemic stroke with measurable deficit on the NIH stroke scale (NIHSS),[10] were within 4·5 hours of symptom onset, were aged ≥18 years, were living independently pre-stroke, and were considered eligible for intravenous (IV) thrombolysis according to clinical guidelines[11]. Exclusion criteria reflected the current evidence regarding contraindications.[12, 13] We allowed inclusion of those aged >80 years, with minor neurological deficit, previous history of stroke and concomitant diabetes, or seizure at stroke onset, as per UK guidelines, and with the approval of the UK competent authority. Patients with other contraindications for alteplase[14, 15] were excluded, as were those with estimated Glomerular Filtration Rate (eGFR) < 30 ml/min or known iodinated contrast allergy. The protocol was approved by the ethical committee responsible for trials involving adults with incapacity (Scotland A REC, Reference: 11/SS/0039). This study is registered, NCT01472926.

Procedure, randomisation and masking

Written informed consent was given by the patient or their legal representative as defined by relevant Adults with Incapacity legislation[16]. Witnessed informed consent was permitted for those with capacity who could not sign due to physical impairment. Following non-contrast CT brain

(NCCT), patients were allocated to receive alteplase (0·9 mg per kilogram to a maximum 90 mg, with 10% of dose as initial bolus, followed by 90% over one hour infusion) or tenecteplase (0·25 mg per kilogram, to a maximum 25 mg as a single bolus) using a mixed minimisation/randomisation approach. Within each block of 8 patients, 2 were allocated at random (1 to alteplase, 1 to tenecteplase) and 6 were allocated using minimisation with respect to age (≤80, ≥81) and NIHSS score (1-9, 10-15, 16-42). When the minimisation algorithm did not favour allocation to either group, the allocation was random. The order with which patients were allocated by randomisation or minimisation (and the random allocation to be used, if required) was defined by a prespecified sequence generated using the method of randomised permuted blocks. This sequence was concealed from study researchers, and the allocation to treatment groups was obtained by telephone to an interactive voice response system (IVRS) developed by the Robertson Centre for Biostatistics, University of Glasgow. The computer program (and random seed) used to generate the minimisation/randomisation sequence was written by a statistician who was not involved with the final analysis, and was stored in a secure network location with access restricted to staff responsible for the development and maintenance of the study IVRS. Clinicians responsible for routine clinical care were aware of treatment assignment since drug administration schedules differ (additional infusion over 1 hour is required for alteplase administration). Patients were not informed of treatment allocation. The majority lacked capacity at the time of treatment, and would only have been aware of treatment allocation if they had prior knowledge of the different modes of drug administration.

Study imaging

Additional CT perfusion (CTP) and CT angiography (CTA) were performed at baseline. Follow-up imaging comprised NCCT and CTA at 24-48 hours post thrombolysis.

All scans were performed on a Philips Brilliance 64 multidetector scanner. Whole brain NCCT was acquired first, (5 mm slice thickness FOV 218 x 218 mm, 120 kv, 171 mA or 0·9 mm slice thickness, FOV 250x250 mm, 120 kV, 404 mA) followed by CTP with 40 mm slab coverage from the basal ganglia (8x5 mm slices, FOV 25 cm, 80 kVp, 476 mA, 2 second cycle time, 30 cycles) using a 50 ml contrast bolus administered at 5 mls per second (350 Xenetix) via a large-gauge cannula. A CTA covering aortic arch to the top of the lateral ventricles (0·67 mm slice thickness, 120 kV, 475 mA) was acquired during the first arterial past of contrast (Xenetix 350, 60 mls, followed by 30 mls of saline bolus, both given at 5 ml per second). Follow-up CTA covered from base of skull to the top of lateral ventricles.

Outcomes

We defined the volumes of tissue fulfilling characteristics of the ischaemic penumbra (tissue that is hypoperfused but potentially salvageable) and core (hypoperfused tissue that will inevitably infarct) based on established probabilistic thresholds[17] for CT perfusion (CTP). The primary outcome measure was the percentage penumbral salvage at 24-48 hour post treatment[18, 19] using baseline CTP-defined penumbra volume minus final infarct volume on follow-up NCCT.

Secondary outcome measures were infarct volume on 24-48h NCCT (defined below, both whole brain infarct volume and co-registered infarct volume); the proportion of patients who exhibited recanalisation (Thrombolysis in Myocardial Infarction [TIMI] grade 2-3)[20] on follow-up CTA[21]; early clinical improvement defined by reduction of NIHSS score of eight points or more, or NIHSS score of zero or one at 24-48 hours post treatment, distribution of functional outcome on modified Rankin Scale (mRS) at 30 and 90 days, the proportion of excellent functional recovery (mRS 0-1) at 30 and 90 days[15, 22], average “home time” (number of nights spent in non-institutional private residence) by 90 days, and mortality at 90 days. Safety outcomes were the proportion of patients with Symptomatic Intracerebral Haemorrhage (SICH) at 24-48 hours post treatment defined by i) the Safe Implementation of

Thrombolysis in Stroke Monitoring Study (SITS-MOST)[23] criteria (Parenchymal haemorrhage (PH) type 2 or remote PH2 on 24-48h NCCT, plus neurological deterioration of ≥4 points NIHSS score); and ii) the European Cooperative Acute Stroke Study (ECASS) II definition[14] (any ICH on follow-up NCCT with clinical deterioration); and iii) any ICH.

NIHSS assessment was performed at baseline, 24-48 hours, 72 hours post treatment, and at seven days (or discharge if earlier). Modified Rankin scale assessment was performed at 30 days and 90 days via telephone interview. All clinical assessments were performed by trained observers. Whilst observers performing clinical assessments were not informed of treatment allocation at the time of follow-up, as a single-centre trial, blinding to treatment allocation for clinical end-points could not be guaranteed.

Imaging processing and analysis:

Imaging studies were anonymised, and analysed independently by two research fellows blinded to treatment allocation after the recruitment completion to minimise the odds of recognising scans with treatment allocation. CT perfusion was processed offline using MiStar (Apollo Medical Imaging Technology, Melbourne, Australia). Full technical details are available as an online supplement. In summary, deconvolution of tissue enhancement curves and arterial input function (AIF) selected from the anterior cerebral artery was performed using modified singular value decomposition (SVD) with compensation for the effects of arterial delay and dispersion. Delay time (DT) was determined as previously described. Cerebral Blood Flow (CBF) and Cerebral Blood Volume (CBV) were calculated from the peak height and area under tissue enhancement curves respectively, and Mean Transit Time (MTT) = CBV/CBF. Ischaemic core was defined as tissue with reduced CBF (relative CBF <40% of contralesional hemisphere) and prolonged delay time (relative DT >2 sec); penumbra volume was defined as tissue with relative DT >2 sec but relative CBF ≥ 40% of contralateral[17].

Total infarct volumes were calculated from follow-up NCCT covering the entire brain. Follow-up NCCT co-registered with baseline CTP (thus restricted to the 4 cm width covered by CTP) was used to obtain "coregistered final infarct volume".

We used the following definitions:

- Penumbra volume salvaged = penumbra volume on baseline CTP – penumbra volume that infarcted on 24h NCCT;

- Percentage of penumbra salvaged = (penumbra salvage/penumbra Volume) x 100

Successful recanalisation was defined as TIMI 2-3 on 24h CTA[20, 21] Discrepancies were resolved by the consensus of experienced neurologists or neuroradiologists who were also blinded to treatment allocation.

Scans with ICH of any type were adjudicated independently and categorised according to the ECASS II radiological definition[14].

Statistical analysis

The trial was primarily intended to inform the design of a larger definitive study by yielding information on potential recruitment rates, incidence of relevant imaging abnormalities, and distribution of outcome events; however, a sample size based on imaging parameters was determined, assuming that tenecteplase would exhibit a 15% absolute superior recanalisation rate compared to alteplase[8, 24] with quicker recanalisation. We estimated a potential 25% reduction in mean infarct volume (38 ml vs 49 ml, standard deviation 20ml),[25] equating to 52 subjects per group for 80% power at a 5% level of significance. The findings were expected to assist in deciding whether a definitive trial based on imaging selection criteria or clinical features alone would be preferable.

The primary outcome and other continuous variables were analysed with a linear regression model adjusted for the stratification variables of age and baseline NIHSS scale. Binary variables were tested using logistic regression models, adjusted for the same stratification variables. P-values for difference between treatment groups have been extracted from these models. The protocol-defined analysis population comprised all randomised patients who had a final diagnosis of stroke, selected as appropriate for a phase 2 study using an explanatory imaging primary end-point. Normally distributed variables are described as mean and standard deviation (SD), ordinal variables as median and interquartile range (IQR) and categorical variables as number and percentage per category.

Role of the funding source

The funding body had no role in study design, data collection, analysis, interpretation or writing of the report. All authors had access to full study data. The responsibility for submission was that of the corresponding author, agreed by the Trial Steering Committee.

Results

Between January 2012 and September 2013, 355 patients were screened (Figure 1), of whom 157 were eligible for IV thrombolysis and 104 patients were enrolled, 52 assigned to each treatment group. Reasons for exclusion are detailed in Figure 1. Eight patients ultimately had a diagnosis of non-stroke conditions and were excluded from the protocol-defined analysis. Groups were well-balanced for clinical baseline characteristics and co-morbidities (Table 1), had moderate stroke severity (median NIHSS 11 to 12), and similar onset-to-treatment time at just over three hours. Participants randomised to tenecteplase had a larger median core volume (20 ml [IQR 2-55] vs 15 ml [IQR 3-40]), and a higher proportion with large artery occlusion (internal carotid artery or proximal middle cerebral artery occlusion in 26/35 [75%] tenecteplase-treated vs 23/38 [61%] alteplase

treated) on baseline CTA, although these potential differences were not statistically significant.

One patient assigned to alteplase did not receive study drug after randomisation, following clinician review of baseline CT querying minor ICH; all other participants received the full dose of study treatment. Excluding those with no baseline perfusion lesion or vessel occlusion, 71 had technically satisfactory imaging for the primary outcome analysis, and 67 were suitable for recanalisation assessment. Patients who did not contribute to the primary imaging analysis (n=33) were younger (mean age 66 years, SD 13) and had less severe strokes (median NIHSS score 9, IQR 6-12) than those with analysable imaging.

All patients with confirmed stroke were followed –up for three months after study entry.

Efficacy

There were no significant differences in the primary endpoint of percentage penumbra salvaged (mean difference 1%, 95%CI -10% to 12%, p=0·81), or of secondary endpoints, either for imaging or for clinical outcomes (Table 2, Figure 3).

Adding core volume and occlusion site to the regression models as a post-hoc analysis, did not affect either the primary outcome (mean difference 2·5%, 95%CI -8%,13%, p=0·65) or recanalisation rates (OR 0·7, 95%CI 0·2-2·2, p=0·65) (details of recanalisation by occlusion site given in Supplementary material Figure).

Safety outcomes

The safety population included 52 treated with tenecteplase and 51 treated with alteplase. Intracerebral haemorrhage (ICH) of any kind was seen in eight patients in the tenecteplase group and 14 in the alteplase group (OR

0·4, 95%CI 0·2-1·2, p=0·09); only one Parenchymal Haemorrhage (PH)[14] occurred in the tenecteplase group, compared to five in the alteplase group. Symptomatic ICH incidence, using either SITS-MOST definition or ECASS II definition, did not differ (Table 2).

There were 32 Serious Adverse Events (SAEs) in 22 (42%) patients given tenecteplase and 16 SAEs in 16 (31%) patients given alteplase, including ICH events fulfilling criteria for seriousness (Table 3). Details of non-serious AEs are given in the supplementary material.

Discussion

Neither radiological nor clinical outcomes differed significantly with IV tenecteplase 0·25 mg/kg compared to alteplase 0·9 mg/kg, the current standard of care. We found no difference in our primary end-point of penumbral salvage, an exploratory measure selected due to its established relationship to early clinical change[26]. Safety outcomes did not differ, notably intracerebral haemorrhages of all kinds, and parenchymal haematomas, the complication most strongly associated with treatment-related neurological deterioration[27].

A combined randomisation/minimisation approach using clinical variables produced groups balanced for clinical factors but advanced imaging identified potential baseline imbalances, such that the tenecteplase group included a higher proportion of patients with large artery occlusion (therefore lower probability of favourable response to treatment[1]) and larger ischaemic core (therefore higher risk of haemorrhagic complications[28]). While it is possible that a larger sample size would even out such differences in phase 3 trials, this finding argues strongly for inclusion of advanced imaging to fully characterise patients in phase 2 studies. Identifying the baseline imbalance in important prognostic variables is potentially relevant to the interpretation of our neutral results with respect to efficacy and safety.

The study population in ATTEST differed from previous trials of alteplase with respect to age, comorbidities and stroke severity, reflecting wider use of alteplase in current clinical practice.[13] Safety outcomes in the alteplase arm were comparable to previous experience with respect to mortality and SICH incidence.[23] We observed fewer very favourable outcomes than in studies with similar onset to treatment time (mRS 0-1 was achieved in 52% in ECASS-3 and in 41% in SITS-ISTR, compared to 20% in our study). This likely reflects a population with greater age at baseline in our sample (mean age 65 years in both of these studies compared to 71 years in ours) and a higher proportion with prognostically important age-related comorbidities such as atrial fibrillation; in addition we had a notably higher proportion of severe stroke syndromes even compared to more recent trials (57% TACS in our study compared to 42-44% TACS in IST-3).

We selected a dose of tenecteplase of 0·25 mg/kg based on available data[7, 8, 24, 29] although numbers of randomised subjects in previous studies were small. One previous study[24] terminated investigation of a higher dose (0·4 mg/kg) due to possible increased SICH incidence (3 in 19 patients). The same study suggested more frequent good outcomes with 0·25 mg/kg compared to 0·1 mg/kg, and Parsons and colleagues[29] also found superior efficacy of 0·25 mg/kg compared to 0·1 mg/kg, with no additional haemorrhagic risk.

We used advanced CT imaging for outcome analysis to offer insights into biological efficacy with modest sample size, but did not use imaging for patient selection. This differs from the approach of Parsons and colleagues[29], who demonstrated superiority of tenecteplase over alteplase for both reperfusion and clinical outcomes in a small randomised controlled trial that compared two different tenecteplase doses to standard alteplase. Their study used multimodal CT imaging to identify what is proposed to be a "responder" population in acute stroke, with favourable core:penumbra "mismatch" ratios, small ischaemic cores, and intracranial vessel occlusion, a strategy that has been expected to reduce sample size in phase two trials[30], but which limits generalisability. Parsons' imaging selection criteria

resulted in exclusion of 79% of IV rtPA-eligible patients, and ultimately only 12% of treatment-eligible patients were randomised, compared to 66% of patients in our study. Less restrictive inclusion criteria resulted in our patients having larger core (median [IQR] 14 ml [0-41] compared to 10 ml [5-17] in Parsons' study, and smaller penumbra volumes (median [IQR] 53 ml [0-110] compared to 79 ml [56-100], with only 64% (67/104) baseline vessel occlusion. In our study, 19% of patients had either acute ICA occlusion or a thrombus involving the terminal ICA extending to the proximal MCA segment, an arterial target lesion that responds poorly to intravenous thrombolysis[31], while these patients were excluded from their study.

The application of advanced imaging for outcome assessment in acute stroke trials has strengths and limitations. Limitations of this study included use of non-contrast CT to quantify final infarct, since the extent of the ischaemic lesion often remains poorly defined at 24h on this modality, restricted brain coverage for CT perfusion in most hospitals, and reliance on probabilistic thresholds to define core and penumbra that have only limited validation[32, 33]. MRI has high sensitivity in defining the extent of infarction[34], and greater lesion conspicuity at early time points than CT, but this is at the expense of poorer availability, and some additional bias in patient selection due to exclusion of patients with ferromagnetic implants including pacemakers, and difficulties in managing acutely unwell patients in the MRI environment[32]. Imaging selection criteria inevitably introduce some delay in treatment initiation for acquisition, processing and interpretation of additional scans, and thus may compromise treatment efficacy.When used as a biomarker for relevant outcomes (reperfusion, recanalisation, and tissue salvage) additional imaging offers valuable insights in phase two evaluation. Our selected imaging end-points used penumbral salvage as the primary end-point due to its close correlation with clinical change[18, 19]. Recanalisation is a powerful predictor of outcome[35] but early recanalisation and reperfusion are more relevant than late, and repeat imaging studies are often less practical when required within the first few hours of treatment. The optimal imaging biomarker remains uncertain. With respect to phase 3

trial design, additional imaging analyses may lead to significant loss of evaluable subjects from a trial. Even with a single centre study familiar with the techniques, 30% of recruited patients did not contribute to imaging analyses, the great majority of these due to absence of an initial perfusion lesion or vessel occlusion. In a multicentre trial, the proportion of non-evaluable subjects is likely to be increased further. While baseline imaging characteristics offered additional insight into stroke severity beyond clinical criteria, delaying treatment initiation in order to review imaging findings for patient selection pre-randomisation may be detrimental. Standardisation of image processing remains poor, and has compromised previous multicentre trials[36] although software to enable rapid and standardised processing of both MRI and CT perfusion imaging has been developed.[37 37]A phase 3 trial of conventional intention-to-treat approach should include allowance for recruitment of stroke mimics. Since we did not use imaging to select patients in the current study, we are also able to estimate the likely proportion of mimics.

Even if the efficacy of tenecteplase does not differ from alteplase, greater ease of administration alone may offer a significant advantage for tenecteplase over alteplase, for which delays between initial bolus and initiation of maintenance infusion are common and may compromise effectiveness; further investigation of tenecteplase for acute ischaemic stroke is merited.

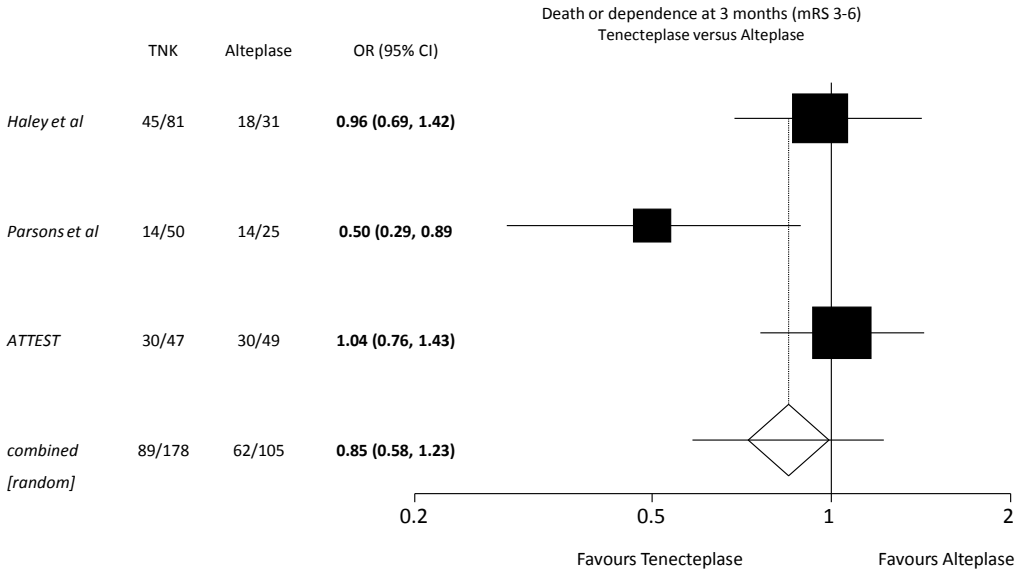
Research in Context

Systematic Review

We searched Medline from inception to December 8th 2014 for articles published in any language with the search terms "tenecteplase", "ischaemic stroke", and "clinical trial or study ", excluding articles on pulmonary embolism, acute myocardial infarction or cardiac failure, to identify

publications reporting the use of tenecteplase for treatment of acute stroke. The search identified two randomised, controlled trials comparing alteplase with different doses of tenecteplase [24, 29], a non-randomised ascending dose safety study of tenecteplase [7], a single-centre case series of low dose tenecteplase (0.1mg/kg) in late-presenting patients selected by multimodal CT imaging [8] and one case series of intra-arterial tenecteplase treatment [38].

The ascending dose safety study had no active comparator and tested doses from 0.1mg/kg to 0.5mg/kg, with SICH as the main end-point.[7] Both RCTs evaluated more than one dose of tenecteplase: 31 [24] and 25 patients[29] were treated at the 0.25mg/kg dose. Imaging selection criteria in the trial of Parsons and colleagues resulted in exclusion of 79% of IV rtPA-eligible patients, a further 9% of whom were not randomised due to open IV thrombolytic or endovascular treatment. A 2013 Cochrane review[39] included summary data from these trials. The trial of Haley and colleagues included a higher dose tenecteplase group (0.4mg/kg) to which recruitment was discontinued due to safety concerns about SICH incidence at that dose, therefore summary data including all dose levels may exaggerate SICH incidence. Key characteristics of these trials compared with ATTEST are given in the supplementary material Table, and the effects on death and disability (mRS 3-6) compared below:



Interpretation

No previous study has compared a single dose of tenecteplase against alteplase with current clinical selection criteria in a 4.5h time window. Our results inform the limitations of imaging-based end-points in phase 2 clinical trials where imaging has not also been used to select patients, and the potential importance of extended imaging to ensure biological characterisation in phase 2 trials, where clinical features alone may disguise important prognostic differences. Clinical outcomes give more representative data on which to base a phase 3 trial.

Source of funding

The Stroke Association (TSA 2010/04).

Declaration of Interests

Prof. Muir received a personal fee for speaking at a sponsored satellite meeting at European Stroke Conference 2013 on acute stroke treatment. Boehringer Ingelheim manufactures both drugs used in this trial.

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Other authors have no conflicts of interest.

Acknowledgements

Trial steering committee: Prof. Gary Ford (Chair, Newcastle University), Dr. M. J. MacLeod (University of Aberdeen);

Data & safety monitoring committee: Prof. Kennedy Lees (Chair, University of Glasgow), Dr. Mark Parsons (University of Newcastle, New South Wales), Dr. Christopher Weir (University of Edinburgh);

External adjudicators: Prof. Michael Hill, Dr. Andrew Demchuk, University of Calgary.

Contributors:

KM was the chief investigator who designed and managed the whole study. XH was the co-investigator who recruited patients, collected and analysed

all data, wrote the first draft and subsequent versions with input and key revisions by all authors. BC was the co-investigator who recruited patients, analysed imaging data and edited the manuscript. DK and FM were co-investigators who recruited patients and edited the manuscript. IF and SML calculated the sample size, developed the statistic plan and performed the statistical analysis. AS was in-charge of clinical reporting of images. All authors reviewed and approved the final report.

Table 1. Demography, risk factors and stroke characteristics in the per protocol population.

		Tenecteplase (n=47)	Alteplase (n=49)
Clinical			
Age years mean(SD*)		71 (13)	71 (12)
Male (n, %)		30, 64%	31, 63%
Dominant hemisphere stroke (n, %)		24, 51%	26, 53%
Baseline NIHSS (Median, IQR)[min-max]		12 (9-18)[2-26]	11 (8-16)[3-27]
Onset to Treatment Time min	mean(SD)	184 (44)	192 (45)
	Median (IQR)	180 (156, 215)	200 (160, 220)
Time between Initial and Follow-up Imaging (hours) mean (SD)		28·5 (7·1)	27·3 (7·5)
Door to Needle Time min mean(SD)		42(17)	38 (19)
Previous stroke/TIA (n, %)		12, 26%	11, 22%
Hypertension (n, %)		20, 43%	28, 57%
Diabetes (n, %)		7, 15%	7, 14%
Blood glucose mmol/L mean (SD)		7 (1)	7 (2)
Atrial Fibrillation (n, %)		19, 40%	15,31%
Hyperlipidaemia (n, %)		4, 9%	7, 14%
Smoker (n,%)		13, 28%	10,20%
Stroke clinical syndrome			
TACS (n, %)		27, 57%	28, 57%
PACS (n, %)		16, 34%	16, 33%
LACS (n, %)		2, 4%	3, 6%
POCS (n, %)		2, 4%	2, 4%
ASPECT score mean (SD)		7 (2)	7 (2)
Imaging			
Penumbra Volume ml	Median (IQR)	40 (4-62)	37 (9-69)
	Mean (SD)	53 (31)	49 (30)
Core Volume ml	Median (IQR)	20 (2-55)	15 (3-40)
	Mean (SD)	32 (36)	24 (29)
Occlusion (n,%)		35/47, 74%	38/49, 78%
Tandem/ICA		10/35,29%	8/38, 21%
M1§		16/35, 46%	15/38, 40%
M2§		6/35, 17%	11/38, 29%
M3§		1/35, 3%	3/38, 8%
ACA/PCA		2/35, 6%	1/38, 3%

*SD, standard deviation; NIHSS: National Institute of Health Stroke Scale; IQR: Interquartile Range; TIA: Transient Ischaemic Attack; TACS; Total Anterior Circulation Syndrome; PACS: Partial Anterior Circulation Syndrome; LACS: Lacunar Syndrome; POCS: Posterior Circulation Syndrome; ASPECT score: Alberta Stroke Program Early CT score; ICA: Internal Carotid Artery; § Middle Cerebral Artery M1, M2, M3 segment; ACA: Anterior Cerebral Artery; PCA:

Posterior Cerebral Artery.

Table 2. Study outcomes in the protocol-defined population.

	Tenecteplase (n=47)	Alteplase (n=49)	p value¢	Mean difference (95%CI)	Odds Ratio (95%CI)
Primary outcome					
Percent penumbral salvage at 24-48 h mean (SD)*	68% (28)	68% (23)	0·81	1·3% (-10-12)	-
Secondary imaging outcomes					
Co-registered final infarct volume at 24-48 h ml mean (SD)#	50 (62)	47 (62)	1.00	0·1 (-20, 20)	-
Total infarct volume at 24-48 h ml mean (SD)Q	75 (101)	66 (91)	0·75	5 (-26, 35)	-
Recanalisation at 24-48 h (n, %)§	21/32, 66%¤	26/35, 74%¤	0·38	-	0·6 (0·2-1·8)
Secondary clinical outcomes					
Early neurological improvement at 24 h (n, %) ¶	19/47, 40%	12/49, 24%	0·10	-	2.1 (0.9-5.2)
Improvement in NIHSS between baseline and 24 h mean (SD)	3 (6)	2 (6)	0·74	-0·4 (-3-2)	-
mRS at 30 Days (n, %)					
mRS 0-1	7/47, 15%	7/49, 15%	0·89	-	1·1 (0·3-3·5)
mRS 2-3	20/47, 43%	21/49, 44%			
mRS 4-5	15/47, 32%	14/49, 29%			
mRS 6	5/47, 11%	6/49, 13%			
mRS 0-1 at 90 days (n, %)	13/47, 28%	10/49, 20%	0·28	-	1·8 (0·6-5·5)

Home by 90 days (n, %)	30/47, 64%	36/49, 73%	0·36	-	0·6 (0·2-1·8)
Days at home by 90 days mean (SD)	45 (39)	50 (36)	0·64	-3·1 (-16-10)	-
Mortality at 90 days (n, %)	8/47, 17%	6/49, 12%	0·51	-	1·3 (0·4-3·7)
Safety outcomes (A=51, T=52)					
Any ICH (n, %)	8/52, 15%	14/51, 27%	0·09	-	0·4 (0·2-1·2)
Any parenchymal haemorrhage (n, %)	1/52, 2%	5/51, 10%	0·12		
Parenchymal haemorrhage type 2 (n, %)	0/52, 0%	3/51, 6%	0·94		
Symptomatic ICH (ECASS II[14] definition) (n, %)	3/52, 6%	4/51, 8%	0·59	-	0·6 (0·1-3·2)
Symptomatic ICH (SITS-MOST[23] definition) (n, %)	1/52, 2%	2/51, 4%	0·50	-	0·4 (0·04-5·1)
*SD, standard deviation; NIHSS: National Institute of Health Stroke Scale; IQR: Interquartile Range; ICH: Intracerebral haemorrhage; mRS: Modified Rankin Scale; ¢ p-values were calculated from linear of logistic regression models that adjust for stratification variables and are a test for difference between groups. # Co-registered infarct volume was defined as infarct volume measured on 24-48 h CT slices co-registered to baseline CT perfusion; QTotal infarct volume was defined as total infarct volume measured on follow-up CT at 24-48 h; § Recanalisation was defined as TIMI[21] 2-3; ¤ The percentages for recanalisation were derived from the number of subjects with an occlusion. ¶ Early neurological improvement at 24 h was defined as NIHSS reduction e 8 points or 24 h NIHSS 0-1. Odds ratios for PH or PH2 ICH were not calculated.					

Table 3: Serious Adverse Events during the trial period:

	Tenecteplase N=52	Alteplase N=51
Serious Adverse Events		
All SAEs to day 90	32	16
All SAEs within 7 days	8	9
Probably or definitely related to study drug	3	5

	n	
Up to day 7	**8**	**9**
Angioedema	1	0
New ischaemic stroke	2	0
Epistaxis	1	0
Pneumonia	2	2
ICH	1	5
Other		
Chest pain	1	0
Malignant glioma	0	1
General deterioration	0	1

Days 7-90	**24**	**7**
GI Bleeding	2	0
New ischaemic stroke	4	2
Other extracranial bleeding	2	0
Pneumonia	2	0
Venous thromboembolism	1	0
Other		
Atrial fibrillation	3	1
Abdominal pain, constipation , diarrhoea	3	0
Chest pain	3	0
Gastroenteritis	0	1
Fall	1	1
Planned medical procedures	5	1
Dehydration	1	0
Depression	0	1
Renal impairment	3	0
Hypotension	2	0

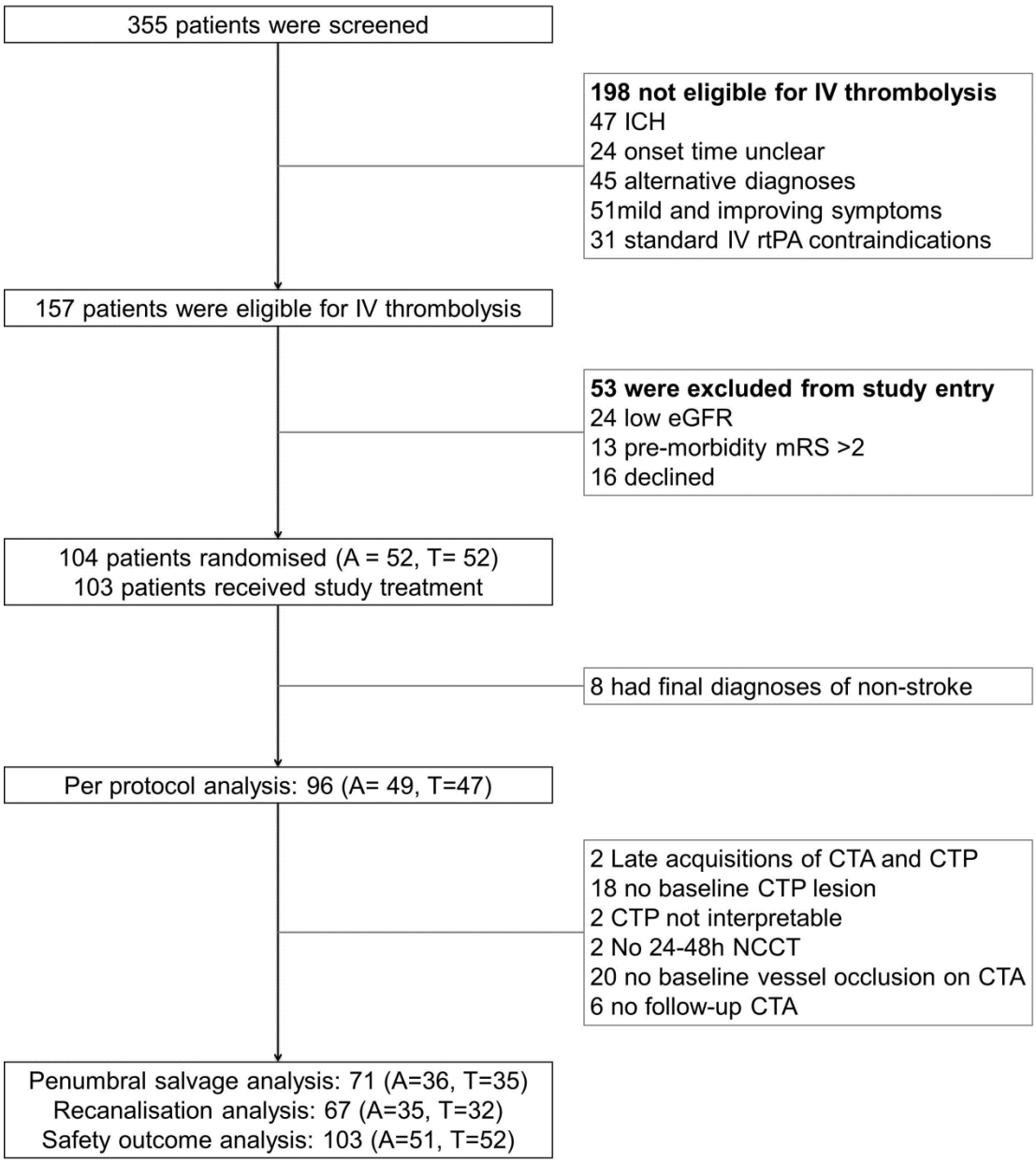
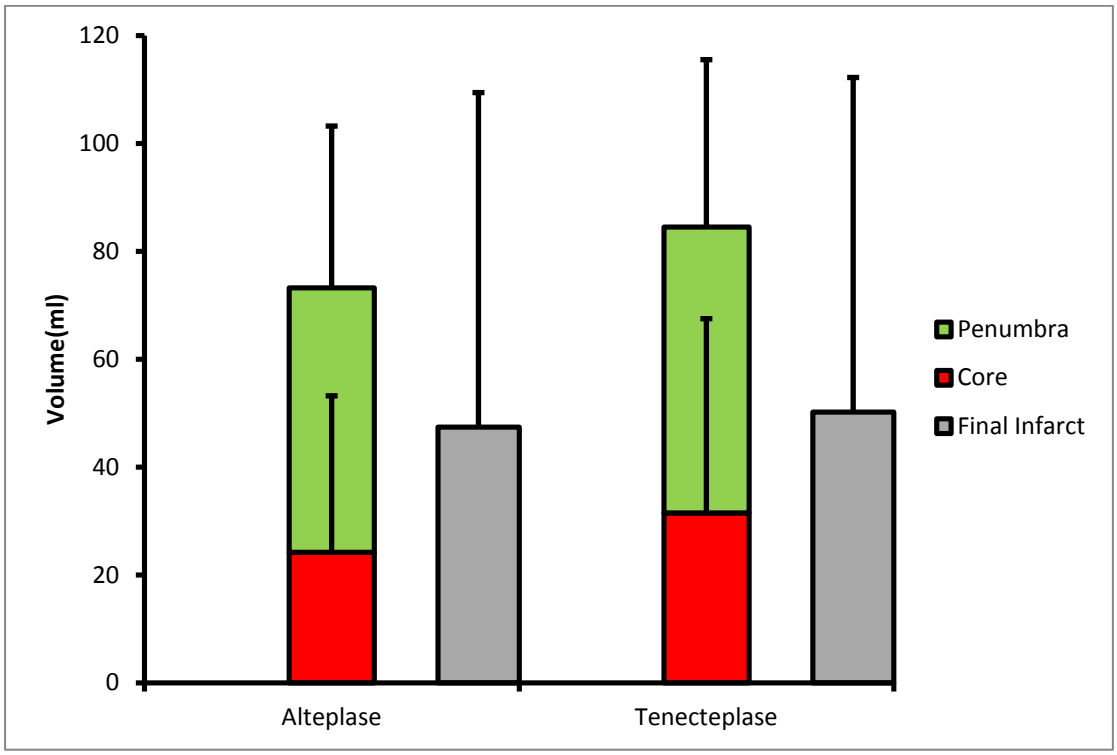


Figure 1. Study CONSORT chart

IV=Intravenous; ICH=Intracerebral Haemorrhage; rtPA: Recombinant tissue plasminogen activator; eGFR=Estimated glomerular filtration rate; mRS=Modified Rankin scale; A=Alteplase; T=Tenecteplase; CTA=CT angiography; CTP=CT perfusion; NCCT=Non-contrast CT.

Figure 2. Baseline CT perfusion lesion segmented into core and penumbra and co-registered final infarct volumes at 24-48 hours. Error bars show standard deviation.



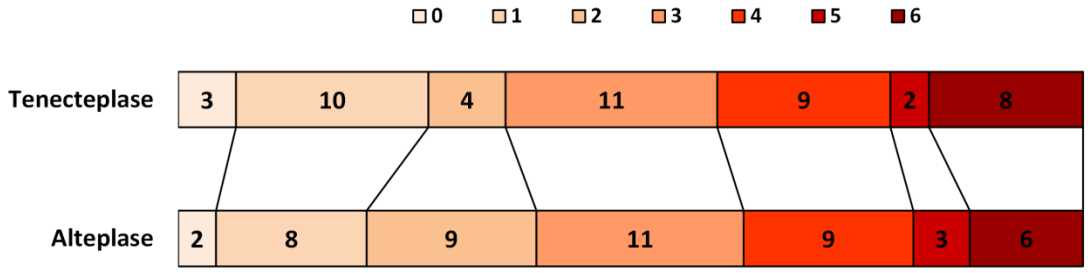


Figure 3. Distribution of Modified Rankin scale at 90 days.

The number in each category is the absolute number.Definitions of scores: 0=no symptoms at all. 1=no significant disability despite symptoms; able to carry out all usual duties and activities. 2=slight disability; unable to carry out all previous activities but able to look after own affairs without assistance. 3=moderate disability; requiring some help, but able to walk without assistance. 4=moderately severe disability; unable to walk without assistance, and unable to attend to own bodily needs without assistance. 5=severe disability; bedridden, incontinent, and requiring constant nursing care and attention. 6=dead.

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Alteplase-Tenecteplase Trial Evaluation for Stroke Thrombolysis (ATTEST) – Phase 2 Randomised Clinical Trial

Supplementary material

Full Inclusion and Exclusion Criteria

Main inclusion criteria

- Clinical diagnosis of supratentorial acute ischaemic stroke with score of $\geq$1 on the NIH Stroke Scale
- Male or non-pregnant female ≥18 years of age
- Within 4.5 hours of onset as defined by time since last known well
- CTP and CTA examinations acquired prior to treatment

Main exclusion criteria

- Contraindications to thrombolytic drug treatment for stroke
    - Evidence of intracranial haemorrhage or significant non-stroke intracranial pathology (including CNS neoplasm, aneurysm or AVM) on pre-treatment CT
    - Established hypodensity on pre-treatment brain CT of more than one third of the MCA territory or ASPECT score <4 (sulcal effacement or loss of grey-white differentiation in cortical territories alone are not counted towards ASPECT score)
    - Hypodensity consistent with recent cerebral ischaemia other than the presenting event
    - Very severe stroke (eg NIHSS>25)
    - systolic blood pressure> 185 or diastolic BP> 110 mm Hg, or aggressive management (intravenous pharmacotherapy) necessary to reduce BP to these limits
    - If on warfarin, INR <1.4
    - Current prescription of non-warfarin oral anticoagulant drugs
    - Significant abnormality of coagulation parameters pre-treatment (prolonged INR or APTT, or platelet count <100,000/mm^3)
    - administration of heparin within the previous 48 hours and a thromboplastin time exceeding the upper limit of normal for laboratory, or use of therapeutic dose low molecular weight heparin within 48h
    - Clinical history suggestive of subarachnoid haemorrhage even if no blood is evident on CT
    - Risk of bleeding (Major surgery within previous 1 month; intracranial or spinal surgery; recent trauma to the head or cranium; prolonged cardiopulmonary resuscitation (> 2minutes) within the past 2 weeks; acute pericarditis and/or subacute bacterial endocarditis; acute pancreatitis; severe hepatic dysfunction, including hepatic failure, cirrhosis, portal hypertension (oesophageal varices) and active hepatitis; active peptic ulceration; any known history of haemorrhagic stroke or stroke of unknown origin; arterial aneurysm and known arteriovenous malformation)
    - Dependent (mRS 3-5) pre-stroke
    - Blood glucose <2 mmol/l or >18 mmol/l

    - Seizure at onset of symptoms unless brain imaging identifies positive evidence of significant brain ischaemia (eg CTA confirmed arterial occlusion, early ischaemic change on plain CT, hypoperfusion on CTP)
    - Pregnancy
- Known impaired renal function (eGFR <30 ml/min) precluding contrast CT
- Known allergy to radiological contrast
- History of allergies to active substances in either trial medication, or to excipients including gentamicin
- Severe concurrent medical condition that would prevent participation in study procedures (e.g. cardiac failure with severe pulmonary oedema) or with life expectancy ≤ 3 months.

Image Analysis Methods: Additional Details

The contralesional anterior cerebral artery and superior sagittal sinus were selected for Arterial Input Function (AIF) and Venous Output Function (VOF) respectively to obtain the tissue enhancement curve. Deconvolution of the tissue enhancement curve and AIF was performed using a modified singular value decomposition (SVD) with compensation for the effects of arterial delay and dispersion[1]. A series of delay time values, DTi ranging from zero to Tmax were applied and for each delay time value a modelled arterial transport function was convolved with the measured global AIF to produce AIFi which is used for SVD deconvolution of the tissue curve to produce an impulse residue function IRFi with its maximum appearing at Tmax (i). Delay time (DT) was determined as the minimal DTi value which produces Tmax (i) =0. Cerebral Blood Flow (CBF) and Cerebral Blood Volume (CBV) were calculated from the peak height and area under the curve of the tissue enhancement curves respectively with Mean Transit Time (MTT) = CBV/CBF. Motion correction was applied automatically. A noise elimination technique "clustering analysis" of < 10 mm^2 was used to minimise small artifactual pixels induced by noise[2]. After AIF and VOF selection a Hounsfield Unit filter was applied in order to mask cerebrospinal fluid (CSF) and bone, leaving only brain parenchyma as far as possible. Voxels with CBV values > 90 cm^3/100 g were then masked to improve the accuracy of CTP maps[3]. Ischaemic core was defined as tissue with reduced CBF (relative CBF < 40% that of contralesional hemisphere) and prolonged delay time (DT; relative DT > 2 sec); penumbra volume was defined as tissue with prolonged DT only (relative DT > 2 sec) but relative CBF ≥ 40% of contralateral[4].

Follow-up NCCT was used to determine outcome infarct volume and presence of ICH. A rigid body 3-D transformation was used to register the follow up NCCT imaging to the baseline perfusion scan. Structural CT sequences were reformatted manually and visually verified to match the orientation of the original CTP. Reformatted structural CT slices resulting from co-registration were used to measure final infarct volume with manually drawn ROIs using visual inspection. Co-registered final infarct volume rather than total infarct volume was used to calculate penumbral salvage because of the limited anatomical coverage of CT perfusion acquired with a 4 cm detector.

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Table: Adverse events during the study period. *SAE listings exclude ICH events, which are described in Table 2 in detail. Total SAE numbers include ICH events meeting criteria for seriousness.

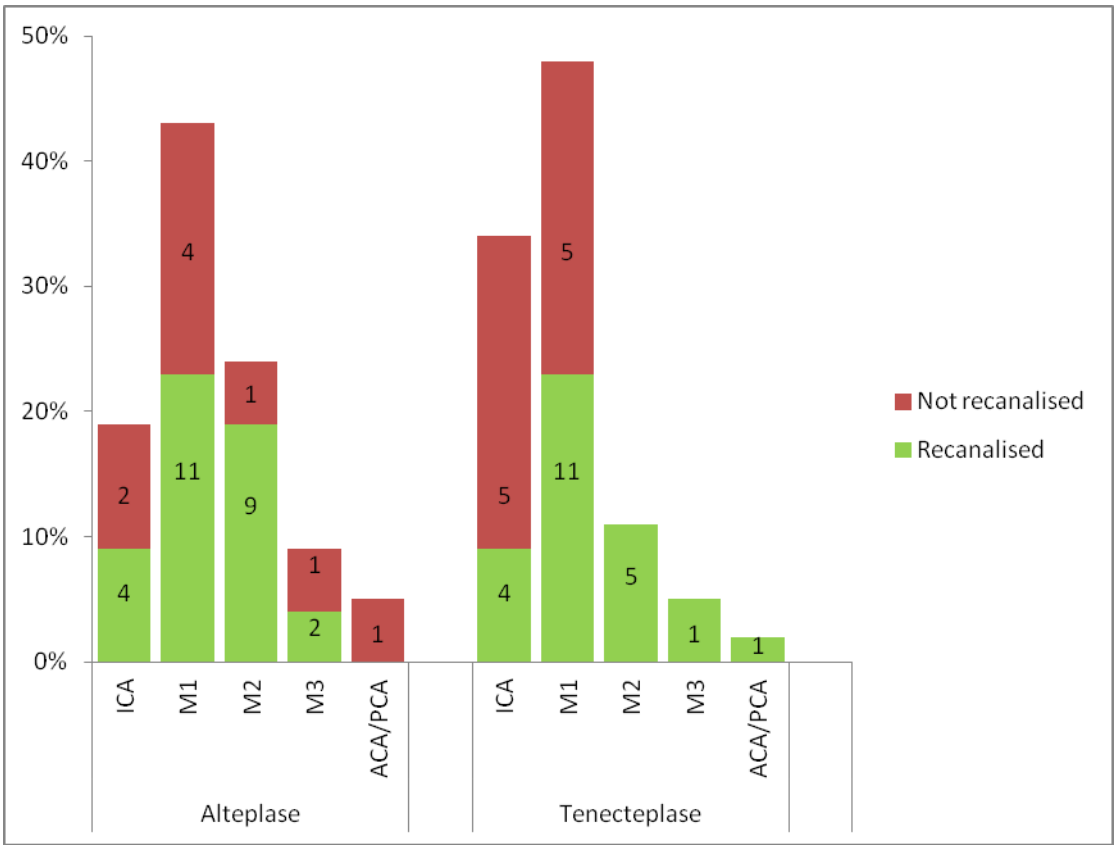
	Tenecteplase N=52	Alteplase N=51
Serious Adverse Events*		
All SAEs to day 90	32	16
All SAEs within 7 days	8	5
Probably or definitely related to study drug	3	5
Subjects with at least one AE *n (%)*	22 (42%)	16 (31%)
Category	n (latency of the event, days)	
Extracranial Bleeding		
Upper gastrointestinal haemorrhage	1 (60)	0
Rectal haemorrhage	1 (70)	0
Epistaxis	1 (0)	0
Post-menopausal bleeding	1 (53)	0
Other Possible Treatment-Related		
Anaphylactoid reaction	1 (0)	1 (0)
Stroke-Related		
Atrial fibrillation	4 (62, 86, 84, 86)	2 (62, 96)
Pneumonia	4 (0, 33, 21, 1)	2 (1, 1)
Pulmonary Embolism	1 (18)	0
Urinary sepsis	1 (50)	0
Further ischaemic stroke	3 (45, 14, 1)	2 (14, 44)
Significant Neurological deterioration	3 (0, 2, 0)	1 (1)
Other		
Dehydration	1 (24)	0
Decreased mobility	0	1 (28)
Malignant glioma	0	1 (0)
Non-cardiac chest pain	1 (63)	0
Viral gastroenteritis	0	1 (44)
Depression	0	1 (51)
Renal impairment	2(71, 24)	0
General physical health deterioration	0	1 (67)
Abdominal pain	1 (70)	0
Uterus biopsy	1 (53)	0
Carotid endarterectomy	1 (12)	1 (11)
Joint resurfacing surgery	1 (88)	0
Total Adverse Events (including serious)		
All AEs to day 90	110	76
Probably or definitely related to study drug	3	7
Subjects with at least one AE *n (%)*	42 (81%)	36 (71%)
MEDRA classification	Incidence (n AEs)	
Blood	1	0
Cardiac	6	6

Congenital (ASD)	1	0
Eye	0	1
GI	8	6
General Disorders	3	5
Hepatobiliary	0	1
Infections	13	7
Injury	5	5
Investigations	3	0
Metabolic/ Nutritional	3	0
Musculoskeletal	3	5
Neoplasms	0	1
CNS	17	17
Psychiatric	4	2
Renal & Urinary	5	1
Respiratory	11	9
Skin	1	1
Procedures	4	2
Vascular	7	2

Table: Characteristics of prior randomised studies comparing alteplase and tenecteplase in acute ischaemic stroke.

Study	Time Window	TNK Dose Levels	Death or Dependence (mRS 3-6)		SICH	
			TNK	Alt	TNK	Alt
Haley	3h	0.1mg/kg (n=31); 0.25mg/kg (n=31); 0.4mg/kg (n=19)	45/81	18/31	5/81	1/31
Parsons	6h	0.1mg/kg (n=25); 0.25mg/kg (n-25)	14/50	14/25	2/50	3/25
ATTEST	4.5h	0.25mg/kg (n=47)	30/47	30/49	1/52	2/51

Figure: Recanalisation rates according to treatment groups and occlusion sites (percentage within each treatment group shown).



ICA=Internal carotid artery; M1=Middle cerebral artery first segment; M2=Middle cerebral artery second segment; M3=Middle cerebral artery third segment; ACA=Anterior cerebral artery; PCA=Posterior cerebral artery. Numbers within each bar represent numbers of subjects.