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1) AcT trial protocol v2.0
2) Statistical analysis plan

Alteplase Compared to Tenecteplase in patients with Acute Ischemic Stroke: QuICR & OPTIMISE Registry based Pragmatic Randomized Controlled Trial

(The AcT RRCT)

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Sponsor
Governors of the University of Calgary

PROTOCOL AcT 02.0 ORIGINAL PROTOCOL

ISSUE DATE: May 23, 2021

Nair R, *et al. Stroke Vasc Neurol* 2024; 9:e002828. doi: 10.1136/svn-2023-002828

GENERAL INFORMATION

Signatures of Approval

Protocol No. AcT-02.0 Original Protocol (Draft)

Study Title: Alteplase Compared to Tenecteplase in patients with Acute Ischemic Stroke: QuICR & OPTIMISE Registry based Pragmatic Randomized Controlled Trial

My signature below confirms that I have read and approved this protocol and assures that this clinical study will be conducted according to all requirements of this protocol, the Declaration of Helsinki, International Conference on Harmonization (ICH) Guideline for Good Clinical Practice (GCP), the Tri-Council Policy Statement (2), and any applicable regulatory requirements.

Date

Date

Date

Name of Clinical Site:

Site Qualified/Principal Investigator:

Name Date
Title

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List of Abbreviations

AE	Adverse Event
CRU	Clinical Research Unit
CSC	Comprehensive Stroke Centre
DSMC	Data and Safety Monitoring Committee
ED	Emergency Department
EVT	Endovascular treatment
HIPAA	Health Insurance Portability and Accountability Act
IA	Intra-arterial
ICA	Internal Carotid Artery
ICH	Intracranial Hemorrhage
IRB	Institutional Review Board
IV	Intravenous
LVO	Large Vessel Occlusion
MCA	Middle Cerebral Artery
MI	Myocardial Infarction
MOP	Manual of Operating Procedures
mRS	Modified Rankin Scale
NIHSS	National Institutes of Health Stroke Scale
NINDS	National Institute of Neurological Disorders and Stroke
PI	Principal Investigator
RCT	Randomised Controlled Trial
REB	Research Ethics Board
SAE	Serious Adverse Event
SUADR	Serious Unexpected Adverse Drug Reaction
SAP	Statistical Analysis Plan
TICI	Thrombolysis in Cerebral Infarction Score
tPA	Tissue Plasminogen Activator
tNK	Tenecteplase

RESEARCH REFERENCES

Clinical trials.gov Number:	**NCT03889249**

FUNDING AND SUPPORT IN KIND

FUNDER(S)	**FINANCIAL AND NON-FINANCIAL SUPPORT GIVEN**

2. Study Synopsis

Trial Title	**Alteplase Compared to Tenecteplase: QuICR & OPTIMISE Registry based Randomized Controlled Trial**
Short Title	**ACT-**QuICR & OPTIMISE
Clinical Phase	PHASE III
Trial Design	Randomized Open label Registry based trial with blinded end-point assessment
Trial Participants	Acute ischemic stroke patients eligible to receive IV alteplase as per current guidelines
Planned Sample Size	1600
Treatment duration	Single dose administration
Follow up duration	90 days
Planned Trial Period	3 years
Outcome Measures	
Primary	modified Rankin Scale (mRS) 0 or 1 at 90-120 days
Secondary	• Discharge destination (home, early supported discharge, rehabilitation facility, long term care, death) • Home time • Actual 90-120 day mRS score • Door to needle time • Door-in-door-out (DIDO) times at the Primary Stroke Centre • Recanalization status (mTICI score) at first angiographic acquisition in patients taken to the angio-suite for the purpose of administering EVT • Proportion of patients administered EVT • Door-to-groin puncture time in patients undergoing EVT • CT-to-puncture time in patients undergoing EVT • Return to baseline level of functioning
Safety Outcomes	• Death within 90 days • Symptomatic ICH post-acute stroke treatment defined as per QuICR and OPTIMISE registries
Medicinal Products being tested	Tenecteplase (TNK-tPA) Alteplase (tPA)
Formulation, Dose, Route of Administration	IV tenecteplase 0.25 mg/kg (single bolus, maximum dose 25 mg) or IV alteplase 0.9 mg/kg (10% bolus & 90% as IV infusion over 1 hour, maximum 90 mg).

3. Coordinating Centre, Sponsor, Investigators and Facilities

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4. Background

4.1 The Problem

There are two established therapies for acute ischemic stroke, namely intravenous alteplase and endovascular thrombectomy (EVT). The guiding principles behind these therapies are fast, effective and safe reperfusion of ischemic brain.[1] Patients with acute ischemic stroke presenting within 4.5 hours from symptom onset are administered intravenous alteplase.[2] If there is evidence of large vessel occlusion (LVO), these patients are transferred to the nearest comprehensive stroke center (CSC) for EVT.[3, 4] Physicians, hospitals and health systems are focused on implementing efficient triaging systems and workflow processes to improve speed and efficacy of administration of these life-saving therapies.[5, 6] Although efforts over the years with intravenous alteplase administration has resulted in improvement in efficiency metrics like door to needle time (DTN) and door-in-door-out (DIDO) time, these metrics are still not optimal, and the therapy is underutilized.[7-9] Physicians continue to have concerns about low early reperfusion rates, increased risk of symptomatic intracerebral hemorrhage and challenges with drug administration (bolus + 60-minute infusion) with intravenous alteplase.

Recent phase II trials have shown that intravenous tenecteplase is potentially safer and may achieve higher early reperfusion rates than alteplase in patients with acute ischemic stroke.[10-12] Bolus administration makes tenecteplase easier to administer than alteplase (which requires infusion pumps).[13] Transfer of patients from primary stroke centers (PSC) to comprehensive stroke centers (CSCs) is potentially easier without infusion pumps. Moreover, depending on the province, tenecteplase either costs the same, or even less, than alteplase. It is therefore possible that the use of intravenous tenecteplase in patients with acute ischemic stroke otherwise eligible for intravenous alteplase may result in faster administration of thrombolysis and more efficient transport to CSCs, thus saving time, reducing adverse events (intracranial hemorrhage) and potentially improving patient outcomes, while saving the health system costs.[13] For these various reasons, robust evidence that tenecteplase is non-inferior to alteplase as an intravenous thrombolytic agent in patients with acute ischemic stroke will change current clinical practice as it did in patients with myocardial infarction.[14]

4.2 The principal research question

In patients with acute ischemic stroke eligible for intravenous thrombolysis, is tenecteplase non-inferior to alteplase for 90-day functional independence assessed using the modified Rankin Scale?

To answer this question, we propose a pragmatic, registry based, prospective, randomized, open label, blinded end point, controlled trial of intravenous tenecteplase vs. intravenous alteplase in patients with acute ischemic stroke otherwise eligible for intravenous thrombolysis as per current guidelines.

4.3 Why is a trial needed now?

The key arguments for the proposed trial are:

1) Intravenous alteplase (0.9 mg/kg body weight) has limited early efficacy in acute ischemic stroke patients with LVO and a known risk of intracranial hemorrhage.[15] Many patients are not thrombolysed or still do not have good outcomes after thrombolysis.
2) Intravenous tenecteplase is used for myocardial infarction.[14] Superior pharmacological properties suggest that it may have greater efficacy, a better safety profile and a substantially easier and more pragmatic mode of administration (bolus) compared to alteplase (bolus+infusion).[13]
3) Dosing of tenecteplase has been studied. The optimum dose in patients with ischemic stroke may be 0.25 mg/kg body weight.[12]
4) Safety of tenecteplase has been studied in phase II trials and one phase III trial.[10-12, 16] Phase II trials suggest that tenecteplase at a dose of 0.25 mg/kg body weight is potentially safer than alteplase.
5) Efficacy of tenecteplase has been studied for the intermediary outcome of early recanalization. A Phase II trial suggests that tenecteplase (0.25 mg/kg body weight) is superior to alteplase.[17]
6) Registries are now in place to capture ischemic stroke patients treated with intravenous thrombolysis. A registry embedded randomized controlled trial (RRCT) is now possible, and the registries can be used pragmatically to test one thrombolytic agent against another.[18]

4.3.1 The evidence for intravenous alteplase in patients with acute ischemic stroke

Intravenous (IV) thrombolysis with alteplase (single chain human recombinant tissue plasminogen activator) is the most widely used therapy in acute ischemic stroke patients presenting within the first 4.5 hours from symptom onset. The NINDS trial was the first RCT to show superiority of IV alteplase over placebo in improving clinical outcome when given within the first three hours of symptom onset.[19] The ECASS III study further extended the treatment window up to 4.5 hours from symptom onset.[20] The effect size was lower in patients treated beyond 3 hours with a Number Needed to Treat (NNT) increase by 1 for every 20 minutes delay in initiating alteplase. The value of faster thrombolysis was further emphasized in a patient level meta-analysis of all intravenous alteplase trials.[2] This study demonstrated the time-dependent effectiveness of alteplase administration up to 4.5 hours from symptom onset. There was evidence of benefit in patients aged over 80, those with minor stroke deficits, and those with or without imaging documented intracranial occlusion. The rate of intracranial hemorrhage varied from 2.7-15.7% across the alteplase trials, depending on the definitions used for symptomatic hemorrhages in these different studies. The risk of alteplase related hemorrhage was higher among patients who had more severe strokes. The magnitude of benefit was dependent on the site of arterial occlusion with better recanalization seen in patients with distal occlusions.

4.3.2 Issues around utilization of intravenous alteplase

Despite evidence for efficacy of intravenous alteplase, extensive public and professional education campaigns, and enhanced quality of acute stroke facilities, the rates of alteplase use remain relatively low.[21] Stroke symptoms may not always be easily recognizable, thus introducing delays in patients presenting to hospitals. Logistical issues including calculating separate bolus and infusion doses and arranging an infusion pump contribute to delay. Pharmacodynamic studies show that even a small delay between administration of bolus and start of infusion of intravenous alteplase reduces drug

efficacy.[22] The risk of intracranial hemorrhage with intravenous alteplase is small but real and can lead to significant morbidity or mortality.[23] Many emergency physicians continue to remain skeptical about the safety of intravenous alteplase in patients with acute ischemic stroke, further leading to underutilization of this therapy.

Recent studies suggest that early recanalization rates with intravenous alteplase in patients with proximal arterial occlusions are very low.[15] This relative lack of efficacy early in patients with more severe stroke together with an increased risk of intracranial hemorrhage has put pressure on health systems to bypass primary stroke centers (PSCs) en-route to comprehensive stroke centers (CSCs) for endovascular thrombectomy (EVT). Moreover, the specific logistics of administering alteplase (bolus and infusion) is a major reason for longer door in door out (DIDO) times in many primary stroke centers, further contributing to slower triage and transport and poorer outcomes in patients with disabling acute stroke.[9]

4.3.3 What is Tenecteplase?

Tenecteplase is a recombinant fibrin-specific plasminogen activator derived from native t-PA by modifications at three sites of the protein structure.[13, 24] It binds to the fibrin component of the thrombus (blood clot) and selectively converts thrombus-bound plasminogen to plasmin, which degrades the fibrin matrix of the thrombus.

4.3.4 The current evidence regarding efficacy and safety of intravenous tenecteplase

In-vitro studies demonstrate that tenecteplase has superior thrombolytic ability when compared to alteplase.[24] This could be because tenecteplase has higher fibrin specificity and greater resistance to inactivation by its endogenous inhibitor (PAI-1) when compared to alteplase. The initial evidence supporting the use of tenecteplase in patients with acute ischemic stroke comes from a dose-escalation safety study of 88 patients in which a dose of 0.5 mg/kg body weight resulted in a significantly higher rate of symptomatic intracranial hemorrhage (15%, 2/13 patients).[25] A subsequent adaptive dose-finding study found that a dose of 0.4 mg/kg body weight also resulted in a higher incidence of symptomatic intracranial hemorrhage (16%, 3/19 patients) and a lower rate of 24-hour major neurological improvement.[10] A phase II trial compared two doses of tenecteplase (0.1 mg/kg or 0.25 mg/kg body weight) with intravenous alteplase (0.9 mg/kg body weight) in patients with acute ischemic stroke presenting within 6 hours of stroke symptom onset. Patients were selected using advanced imaging and an explanatory framework.[11] Tenecteplase in both dose tiers was found to be superior to alteplase in achieving the co-primary outcome of reperfusion and mean NIHSS score improvement at 24 hours. Interestingly, the 0.25 mg/kg body weight dose was superior to the 0.1 mg/kg dose for all outcomes without increasing the risk of hemorrhage.

The single-arm TEMPO-1 trial that we led enrolled patients with minor non-disabling stroke symptoms and imaging documented evidence of intracranial occlusions to receive escalating doses of intravenous tenecteplase (0.1 and 0.25 mg/kg body weight).[26] The rate of symptomatic intracranial hemorrhage in this study was 2% (1/50 patients).

The ATTEST trial was a single-center phase II trial that randomized 104 patients to receive intravenous tenecteplase (0·25 mg/kg body weight) vs. standard dose intravenous alteplase.[12] The primary outcome was the proportion of penumbra salvaged (CT perfusion-defined penumbra volume at baseline minus CT infarct volume at 24–48 h). Despite prognostic imbalances at baseline with more negative imaging features in the tenecteplase group (a higher proportion of patients with large artery occlusion 75% vs. 61% and 33% larger ischemic core volume), there were trends towards earlier major neurological improvement and lower risk of intracranial hemorrhage in patients treated with tenecteplase.

The EXTEND-IA tNK trial (published in early 2018) was another phase II trial with an explanatory framework that enrolled 202 patients with CT-perfusion mismatch and proximal anterior circulation occlusions to receive intravenous tenecteplase (0.25 mg/kg body weight) vs. standard dose intravenous alteplase before being administered standard of care endovascular thrombectomy.[17] The primary outcome (reperfusion of greater than 50% of the ischemic territory or an absence of retrievable thrombus assessed on initial catheter angiopathy assessment before endovascular thrombectomy) occurred in 22% of patients receiving intravenous tenecteplase compared to 10% of patients receiving standard dose intravenous alteplase (rate ratio 2.2; 95% CI, 1.1 to 4.4; P=0.002 for noninferiority, p=0.03 for superiority). In secondary analysis, patients receiving intravenous tenecteplase fared better clinically compared to patients receiving alteplase (median mRS 2 vs. 3, respectively at 90 days, common odds ratio, 1.7; 95% CI, 1.0 to 2.8; p=0.04). The rate of symptomatic intracranial hemorrhage was 1% in both groups. The trial results suggest that by achieving higher earlier recanalization rates, intravenous tenecteplase is potentially capable of achieving better clinical outcomes in patients who are otherwise candidates for endovascular thrombectomy.

Finally, the only phase III trial that compared intravenous tenecteplase to standard dose alteplase was the Norwegian Tenecteplase Stroke Trial (NOR-TEST) trial.[16] This trial randomized 1100 patients using standard thrombolysis eligibility criteria to test if intravenous tenecteplase (at a dose of 0.4 mg/kg body weight) was superior to standard dose alteplase. The trial failed to meet its primary outcome with 64% achieving mRS 0-1 at 90 days in the tenecteplase group vs. 63% in the alteplase group (odds ratio 1.08, 95% CI 0.84 to 1.38). The trial was critiqued for a) including a large proportion of patients with mild stroke symptoms and stroke mimics (median NIHSS 4 suggesting that almost 50% of the enrolled sample may not have been the ideal target population for thrombolysis) and b) being powered only to detect superiority but not non-inferiority of tenecteplase vs. standard dose alteplase.[27] Moreover, the rate of symptomatic intracranial hemorrhage in the tenecteplase arm was slightly higher than that in the alteplase arm (3% vs. 2%), likely due to the higher dose of tenecteplase (0.4 mg/kg body weight) used in that trial. The AcT trial we propose is powered to detect non-inferiority. It will also include a more representative group of acute stroke patients. Data from the QuICR registry shows that the median NIHSS in all patients (n=592) who received thrombolysis within that registry in the year 2018 was 11 (significantly more severe strokes than in the NORTEST trial); moreover, the ongoing TEMPO 2 trial and the routine use of vascular imaging to help guide care in Canada will also limit enrolment of patients with mild non-disabling stroke symptoms and stroke mimics in the proposed trial.[13]

4.4 Risks to the safety of participants involved in the trial

Although recent phase II clinical trials have shown that intravenous tenecteplase at a dose of 0.25 mg/kg body weight is potentially safer than the current standard of care i.e. intravenous alteplase, the risk of hemorrhage, both intracranial and systemic exists (approximately 4 to 5% with either drug).[28, 29] Angioedema occurs after both alteplase and tenecteplase in a small proportion of patients. The incidence of severe angioedema in alteplase-treated patients is approximately 1%.[16, 29] Other adverse events associated with both alteplase and tenecteplase are extra-cranial hemorrhage (<1%) and transient hypotension (~0.1%).[16, 29] Although there is less empiric experience with the use of tenecteplase in ischemic stroke, these risks are thought to be either identical or less with tenecteplase.[16, 29] It is unlikely therefore that there may be any additional risks to the safety of participants involved in the trial.

4.5 Systematic Reviews and Meta-analysis

4.5.1 Systematic Reviews

Four small phase II trials and one phase III trial have been completed to date.[11, 12, 16, 17, 25] These trials have been heterogenous in design and patient selection strategies. Some of the trials have been dose escalation studies, others have used advanced CT Perfusion imaging criteria for patient selection or enrolled patients with mild strokes and stroke mimics predominantly. The bulk of the evidence comes from one large phase III trial (NORTEST; 75% of all patients enrolled to date in any trial) that used tNK at a dose of 0.4 mg/kg body weight.[16] A recent trial level meta-analysis showed that patients enrolled in these trials, on average, had significantly less severe strokes (mean baseline NIHSS ~ 6.8) when compared to patients enrolled in all the alteplase vs. placebo trials to date (median baseline NIHSS 12).[2, 29] Moreover, 15% of patients enrolled in the tNK vs. alteplase trials were stroke mimics.[29] Other differences in baseline characteristics like under-representation of females (40% in the tNK trials overall vs. 45% in the alteplase trials) and lower incidence of atrial fibrillation (14% in the tNK trials vs. 24% in the alteplase trials) all suggest that on average, the sampling frame of patients included in the tNK vs. alteplase trial level meta-analysis is not representative of patients with acute stroke treated with intravenous alteplase. Acknowledging these limitations, when analysing mRS 0-1 at 90 days as outcome, a meta-analysis of the five trials shows unadjusted rates of tNK vs. alteplase of 57.9% vs. 55.4%; random-effects meta-analysis risk difference of 4% (95% CI minus 1% to + 8%).[30] Interestingly, the rate of mRS 0-1 in the alteplase arm (55.4%) is significantly higher than that seen in the alteplase arm (~35%) of the patient level meta-analysis of alteplase vs. control trials; attesting to the significantly lower risk patients included in the tNK vs. alteplase trials to date.[30] When assessing safety, the same trial level meta-analysis shows unadjusted rates of symptomatic ICH with tNK of 3.3% alteplase 3.2% with an adjusted risk difference of 0% (95% CI minus 1% to + 2%). Overall therefore, current AHA Stroke guidelines assign a Class (Strength) of recommendation IIb and Level (Quality) of Evidence B-R (moderate quality from meta-analyses of moderate quality RCTs) for intravenous tNK in patients with acute ischemic stroke.[31] A recent editorial therefore concludes *"We look forward to and encourage the stroke community to participate in active and future stroke trials with tenecteplase"* [32]

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4.5.2 Ongoing trials

The ATTEST 2 phase III trial (NCT02814409) based in the UK has less rigid inclusion criteria but also excludes patients who in the opinion of the treating physician may benefit from endovascular thrombectomy. The trial plans to recruit 1800 patients.

The Tenecteplase versus Alteplase for Stroke Thrombolysis Evaluation (TASTE) trial (ACTRN12613000243718) is a randomized controlled phase III trial that uses advanced perfusion-imaging based selection criteria to test if tenecteplase at a dose of 0.25mg/kg body weight is superior to standard dose alteplase. The trial excludes patients with large vessel occlusions eligible for EVT and only includes patients who fulfill strict tissue-based imaging criteria.

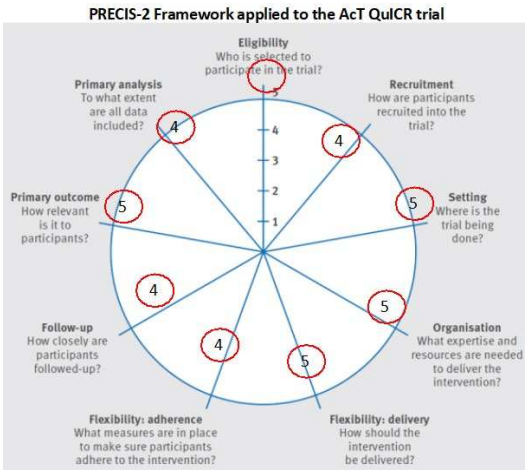
The EXTEND IA-tNK 2 trial (NCT03340493) is an Australian phase II trial that is testing efficacy and safety of higher dose tenecteplase (0.4 mg/kg body weight) vs. tenecteplase (0.25 mg/kg body weight) in patients who are candidates for endovascular thrombectomy.

The TEMPO-2 trial (NCT02398656; CIHR funded and led by our group) is a multicenter, randomized, open-label, controlled trial of intravenous tenecteplase (0.25 mg/kg body weight) versus standard of care antiplatelet therapy in patients with TIA or minor strokes and imaging documented intracranial occlusion. The trial plans to recruit 1274 patients.

Finally, the TWIST trial (NCT03181360) is a phase III trial testing the efficacy of intravenous tenecteplase (0.25 mg/kg body weight) vs. standard care in patients waking up with stroke symptoms who are otherwise not eligible for intravenous alteplase.

None of the ongoing trials are addressing the primary question whether intravenous tenecteplase can replace intravenous alteplase as standard thrombolytic therapy in all patients who are currently eligible for intravenous thrombolysis.

The results of the proposed AcT trial are also more likely to be externally valid and therefore generalizable to Canadian current clinical practice than these other trials.[33] An assessment of the AcT trial using the PRECIS-2 framework (https://www.precis-2.org; see adjacent figure) attests to external validity and generalizability.



4.6 How will the results of this trial be used?

Results from the proposed trial are likely to provide real life confirmatory evidence about the efficacy of intravenous tenecteplase in patients with acute ischemic stroke eligible for intravenous thrombolysis as per current guidelines. If the proposed trial demonstrates non-inferiority of intravenous tenecteplase when compared to alteplase, it

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is likely to change clinical practice. Given relative ease of administration of tenecteplase (bolus only) vs. alteplase (bolus + infusion), workflow metrics in acute stroke like door-in-door-out (DIDO) time, door to needle (DTN) and door to reperfusion time can all potentially improve, thus improving clinical outcomes. In addition, better reperfusion rates and potentially better safety data is likely to increase utilization of thrombolytic therapy overall while at the same time potentially decreasing the need for resource intensive endovascular thrombectomy in many patients.

5. The Proposed Trial

5.1 Trial design

The proposed trial is a pragmatic, registry based, prospective, randomized (1:1) controlled, open-label parallel group clinical trial with blinded endpoint assessment of 1600 patients to test if intravenous tenecteplase (0.25 mg/kg body weight, max dose 25 mg) is non-inferior to intravenous alteplase (0.9 mg/kg body weight, max dose 90 mg) in patients with acute ischemic stroke otherwise eligible for intravenous thrombolysis as per standard care.

The trial will be **embedded within the QuICR and the OPTIMISE registries**. It will use linkages to administrative datasets to collect relevant data not available through the registries.

QuICR (https://www.ucalgary.ca/quicr/) is a cloud-based registry that collects data on all ischemic stroke patients receiving thrombolysis and/or endovascular treatment in Alberta. Currently, all stroke centres in Alberta and one primary stroke center (PSC) in Saskatchewan are contributing to the registry. The registry includes 15 PSCs that provide intravenous thrombolysis treatment only, and 2 Comprehensive Stroke Centres (CSCs) that provide both thrombolysis treatment and endovascular treatment. If possible, we will also utilize the Stroke Ambulance centred in Edmonton at the University of Alberta Hospital (16th PSC) and the Northern Alberta tele-stroke network (currently participating in QuICR)

OPTIMISE is a national registry supported by the Canadian Stroke Consortium to support the implementation and quality control for endovascular therapy (and more recently intravenous thrombolysis) among patients with acute ischemic stroke. The data collection fields in OPTIMISE were designed to mirror the QuICR registry. Data is captured using a web-based electronic data capture and reporting system and housed on a secure server at the Population Health Research Institute (PHRI) at McMaster University.

All sites participating in QuICR will participate in the trial. Sites participating in OPTIMISE and collecting data on all thrombolysis patients will also participate in the trial. In addition, other CSCs or PSCs in Canada that agree to participate in either the QuICR or the OPTIMISE registries or have similar infrastructure will also be eligible to participate in the trial.

5.2 Planned trial interventions

The intervention group will receive intravenous tenecteplase as a single bolus at a dose of 0.25 mg/kg body weight (maximum dose 25 mg) as soon as possible after randomization.

The control group will receive standard of care dosing of intravenous alteplase (0.9 mg/kg body weight, 10% bolus and 90% infusion as per standard care, maximum dose 90 mg).

All patients will have standard of care medical management on an acute stroke unit. There are no additional trial specific management recommendations.

5.2.1 Study Drugs

Intravenous tenecteplase: The trade name for tenecteplase is TNKase™ in Canada.

TNKase™ is available as 50mg vials. Each 50mg vial of TNKase™ is packaged with one 10ml vial of sterile water for injection for reconstitution. Reconstitution of 50mg of tenecteplase in 10 ml of sterile water results in a solution concentration of 5mg/ml.

Intravenous alteplase: The trade name for alteplase is ACTIVASE™ in Canada. This thrombolytic agent is the current standard care.

ACTIVASE™ is reconstituted by aseptically adding to the vial the appropriate volume of sterile Water for Injection, (100 mL for 100 mg vials) resulting in a solution concentration of 1mg/ml.

5.2.1.1. Drug Labelling

The trial is testing a standard, off-the-shelf drug (alteplase) vs. another off-the-shelf drug (tenecteplase) currently used for another indication i.e. acute myocardial infarction. The drug packages describe the expiration date of the drug, the recommended storage conditions, the drug reconstitution method and the LOT number. . An Investigational Status Assessment (ISA) has been completed for use of alteplase; therefore, no labeling is required for alteplase drug packages. The sponsor will provide labels and the site PI will ensure that the tenecteplase drug packages are labelled as investigational with the current protocol version. The labels will also have the drug dosage as per current protocol and the name and address of the sponsor (See Appendix for Drug labels). To facilitate labelling and integration with clinical workflow, the sponsor can help sites make a “stroke kit” available, stocked with labelled tenecteplase and alteplase In addition, copies of the mixing and dosing chart can be made available to sites as posters. Finally, the randomizer (text or web based) will also provide instructions on mixing and dosing.

5.2.1.2 Drug Traceability

Sites will be collecting LOT numbers and expiry date of the vial of drug administered to a patient and entering these numbers into the study database by study ID number. Pharmacy Drug Recall records are able to track LOT Numbers, expiry, vendor and patient name using a logic match.

5.2.1.3 Drug Storage/Temperature Monitoring

Both drugs are kept in environmentally controlled conditions i.e. a hospital, by hospital pharmacies, according to the information on the current drug labels, and made available for use as required. The current label on storage requirement for tenecteplase mentions that the drug may be stored at room temperature not to exceed 30°C. Environmentally controlled conditions in the participating hospitals ensure such storage conditions for current clinical use of tenecteplase.

5.2.2. Clinical and Imaging Evaluations

There are no trial specific clinical and imaging evaluations except as per standard care. 90-120 day outcome using the modified Rankin Scale and EQ-5D-5L will be collected by the trial in co-ordination with the site. All other data will be collected either through the QuICR and OPTIMISE registries or through administrative data linkages. (See sections 5.11, 7.4 and Appendix)

5.2.3 Laboratory Evaluations

There are no trial specific laboratory evaluations and no central laboratory. All laboratory evaluations are as per standard care only.

5.2.4 Concomitant medications

Standards of care applicable to any patient receiving intravenous alteplase apply to patients in both arms of the trial. There are no prohibited medications except those considered as such based on best practices.

5.3 Proposed practical arrangements for allocating participants to trial groups

Eligible patients will be randomized in a 1:1 ratio. Randomization will use a validated minimal sufficient balance (MSB) algorithm, to assure balance by site. The algorithm will kick in after the site has enrolled 5 subjects. The standard distribution is 50-50, but when an imbalance is detected with a p-value less than 0.3, the distribution changes to 65-35 in the direction against the imbalance. Randomization will be centralized, secure and concealed using a real-time web-based server, thus eliminating the possibility of confounding due to allocation bias. The central server will be linked to a randomization mechanism and will only be able to allocate one patient to either arm of the trial at one time. Investigators will be able to rapidly access the randomizer through a secure internet web browser or secure automated phone number or text message. Investigators will have to enter a site-specific ID number followed by an Investigator specific ID number and the weight of the patient in kilograms. They will then receive the drug allocation (tenecteplase vs. alteplase) along with unique study subject ID and the calculated drug dosage. They will later be prompted to enter if a) the correct drug was administered b) the correct dose was administered and c) the vial LOT number and expiry date of the administered drug.

Emails will be sent from the central trial server to site investigators and trial personnel with the site ID, unique study subject ID and drug allocation (study enrolment form). A copy of this email can be

printed and included in the patient chart. In addition, this email will also have a PDF checklist reminding site co-ordinators to ensure the following

1. The study enrolment form is placed in the clinical patient chart
2. The patient and surrogate contact information is documented in the checklist
3. Consent is obtained (see section 5.4) and documented in the study database
4. The unique study subject ID and registry ID are entered in the study database
5. Report any serious adverse events occurring within the first 24 hours of randomization. This information will be documented on paper and/or in the study database

Each site will maintain a patient log that will include the above checklist, a copy of the patient enrolment form and patient or surrogate consent forms (if not obtained electronically; see section 5.4 Consent). The central trial coordinator and/or the automated study database application will contact the site registry coordinator within 7-10 days of enrolment to ensure the above steps are complete. A manual of procedures (MOP) will be made available to each participating site detailing all the steps above.

5.4 Consent

Where approved by the local research ethics board (REB), to reduce time to treatment, patients will be randomized using a deferral of consent procedure. The responsible treating physician will determine patient eligibility for the trial and initiate randomization if the patient is deemed eligible for the trial.

All patients will be enrolled by deferred consent. This study design respects TCPS2 guidelines and reflects the imperative to treat patients quickly, so as not to bias results or disadvantage enrolled patients compared to patients not enrolled in the trial. Because of this design, we will institute the following patient protections: 1. Identify a specific ethics lead (Dr. Michel Shamy, a stroke neurologist and ethics researcher from the University of Ottawa), a patient engagement expert (Dr. Job McIntosh, Alberta Health Services) and a patient/caregiver lead; 2. Develop a patient-oriented, 1-page information sheet for patients and their families at the time of enrollment (the information sheet); 3. Develop a patient-oriented, 2-page consent form for patients and their families regarding ongoing participation in the trial; 4. Develop a questionnaire to be administered to a sample of enrolled patients about their experiences with and attitudes towards deferred consent; 5. Hold a focus group with stroke patients to review the trial protocol and above documents prior to initiation of the trial. Past precedence with the ESCAPE trial (Shamy MCF et al; Deferral of Consent in Acute Stroke Trials: Lessons from the ESCAPE Trial. Stroke 2019; in press) suggest that deferral of consent is practical at all sites.

A detailed document outlining justification for deferred consent based on the Declaration of Helsinki Article 26 and Article 3.8 of the Tri-council Policy Statement-2 is available for enrolling sites. It is recommended that consent be obtained from subjects or their surrogates within 7 days of randomization or before discharge, whichever is earlier. In patients who die, and consent has yet to be obtained, reasonable efforts should be made to obtain consent from their surrogates. If consent could not be obtained in spite of best efforts (no surrogate or surrogate cannot be contacted), the patient will continue

in the trial. Subjects or, if incapacitated, their legal representatives, will have the right to withdraw from further participation (including long-term follow-up by record linkage). Subjects may withdraw consent for further contact but continue follow-up via record linkage. In the event that consent is withdrawn, data collected up until the point of withdrawal will be used for analysis.

We will provide an option for a site-specific electronic consent application with electronic signature support that will be linked to the randomization tool. This solution shall be made available via a secure web-application. Alternatively, sites can print the consent form from the site and upload a scanned signed paper version. The deferred consent capture process shall consist of the following steps:

1) An electronic or paper physician script will be made available to the physician prior to enrolling a patient in the trial. This script will contain information that the physician can use as guideline to help inform the patient/shared decision maker (SDM) about the trial. The use of this physician script is not mandatory;
2) An electronic or paper information sheet will also be made available to the physician to help him/her inform the patient or shared decision maker (SDM) about the trial.
3) If and when the patient is capable of communicating and/or the SDM is available, the physician will provide them with the information in the sheet above. It is recognized that sharing of the information sheet with patient/SDM may happen before, during or after randomization;
4) After randomization has been completed for a patient, a unique identifier (study ID) will be assigned to each patient by the application, shown on-screen to the physician or site-coordinator and sent via e-mail to the site co-ordinator;
5) After treatment has been administered, as an added quality assurance step, a randomization validation process will be initiated. This process will confirm whether the drug and dosage used match the randomization values and, if the trial information sheet was shown to the patient and/or SDM;
6) Once the randomization process has been completed, in conjunction with the validation exercise, the site co-ordinator shall connect to the electronic consent capture application via a PC, tablet, or web-enabled mobile phone;
7) The site co-ordinator will be asked to provide the unique patient identifier and their own user identifier; additionally, the site co-ordinator will select whether consent will be provided by the patient or a surrogate, and whether electronic or paper-based consent will be provided;
8) The system will check to ensure the validity of the identifiers and then will show the relevant (site-specific) consent information to the participant;
    a. If electronic consent was selected, the participant will then be asked to review the information and, if they consent, validate/check the acknowledgement box, enter their name, date, month and year of birth, signature, and submit the form.
        i. The date of birth option can be disabled, if required/request by the REB review process
    b. As a secondary consent capture method, the patient may choose to instead use a paper form, email or phone consent as approved by local ethics boards. If a patient chooses this option, the qualified site personnel will present the patient with a paper form with the same information as the electronic one. Once the patient fills-out this form, they will then enter the patient's unique consent id and then scan and upload the form to the electronic consent application. Either consent method shall be deemed to sufficiently satisfy the consent requirements.

9) Once submitted, the application will securely store the consent information and associated signature in a secure database and file store.
10) This information can then be reviewed by auditors or authorized personnel by requesting it of the Clinical Research Unit.
11) If subjects or, if incapacitated, their legal representatives, withdraw from further participation (including long-term follow-up by record linkage), sites will inform the sponsor about this within 7 days of this happening. Subjects may withdraw consent for further contact but continue follow-up via record linkage. In the event that consent is withdrawn, data collected up until the point of withdrawal will be used for analysis. The site will update the patient consent log and inform the sponsor of the same.

The policies and procedures will receive prior review and approval by the IRB/REB of all hospitals to which prospective trial subjects may be admitted. These IRB/REBs will provide ongoing oversight of the trial.

During the conduct of the trial, a publicly accessible website will be available to provide information about the trial; and after the trial's completion, the community will be notified of its outcome. The trial is also registered at www.clinicaltrials.gov.

5.5 Proposed methods for protecting against sources of bias

The trial will have allocation concealment and blinded endpoint assessment. Given the time sensitive nature of acute stroke treatment, blinding the enrolling health personnel and patient to treatment allocation is not practical. Primary clinical outcome data (assessed using the modified Rankin Scale) will be determined by the Rankin Focused Assessment (RFA) method[34] using centralized telephone interviews conducted by central trial personnel blinded to treatment allocation. The trial coordinator may contact the site to help schedule phone follow-ups. Follow-ups will be scheduled 90 to 120 days after randomization. The manual of procedures (MOP) will describe in detail the Rankin Focused Assessment method for use by central trial personnel.

5.6 Inclusion/exclusion criteria

The proposed trial will recruit patients from the emergency departments of the hospitals participating in the QuICR and OPTIMISE registries.

Inclusion Criteria
Inclusion criteria is pragmatic. All patients with acute ischemic stroke eligible to receive intravenous alteplase as per standard care will be eligible for enrolment in the proposed trial. Patients eligible for endovascular thrombectomy in addition to intravenous thrombolysis are eligible for enrolment.

Exclusion Criteria
Contra-indications to intravenous thrombolysis as used by treating physicians as current standard of care apply.

The benefits of thrombolysis with intravenous alteplase in the pediatric population is unknown. Any patient < 18 years of age may therefore not be enrolled.

Women with pregnancy known to the investigator by history or examination, without requiring pregnancy testing, may only be enrolled in consultation with an expert stroke physician (either in person or through tele-stroke)[35]

5.7 Proposed duration of treatment period

All eligible patients will receive the allocated intravenous thrombolytic therapy (tenecteplase or alteplase) acutely as a one-time treatment. Intravenous tenecteplase is administered as a single bolus injection over 5 to 10 seconds while intravenous alteplase is administered as a bolus over 1 minute followed by an infusion over approximately 60 minutes. There are no additional planned co-interventions throughout the duration of the trial.

5.8 Proposed frequency and duration of follow up

Duration of follow-up will be 90 days, up to 120 days. The primary outcome (mRS) will be collected at the end of follow-up (ideally at 90 days but definitely within 120 days).

5.9 Primary and secondary outcome measures

Primary Outcome: modified Rankin Scale 0-1 at 90 - 120 days

Secondary Outcomes:

1. Discharge destination (home, early supported discharge, rehabilitation facility, long term care, death) * #
2. Home time (defined as number of days subject spends at home after index stroke event) [36]
3. Actual 90-120 day mRS score*#
4. Door to needle time*#
5. Door-in-door-out (DIDO) times at Primary Stroke Centres*#
6. Recanalization status (mTICI score) at first angiographic acquisition in patients taken to the angio-suite for the purpose of administering EVT*#
7. Proportion of patients administered EVT *#
8. Door-to-groin puncture time in patients undergoing EVT*#
9. CT-to-puncture time in patients undergoing EVT*#
10. Return to baseline level of functioning
11. Cognition assessed via a brief, on-line cognitive assessment tool.

Safety Outcomes:

1. Death within 90 days*#

2. Symptomatic ICH post-acute stroke treatment defined (As defined in the MOP)*Indicates data collected in the QuICR registry # Indicates data collected in OPTIMISE registry

5.10 How will the outcomes be measured at follow up?

The primary outcome (assessed using the modified Rankin Scale) will be determined by the Rankin Focused Assessment (RFA) method using centralized telephone interview by trained study personnel blinded to treatment allocation. The Rankin Focused Assessment consists of a 4-page form, accompanied by a 5-page Instructions. Details will be provided in the MOP.

Secondary outcome measures described above are all available through the QuICR and OPTIMISE registries and will be collected from those data sources. The home time outcome will be determined through linkage with administrative data to calculate the total time in the first 90 days after index event that a stroke patient is not an inpatient. Processes for administrative linkages within Alberta, Ontario and potentially other participating sites will be different based on local resources and policies. Return to baseline level of functioning will be determined using the same centralized telephone interview used to determine the primary outcome. Cognition will be assessed using an on-line assessment platform, with a login by Trial ID (no personal ID provided to the cognitive platform) and the cognitive data output to the trial database by Trial ID. These strategies and methods will be detailed in a manual of operations.

Primary safety outcomes death and symptomatic ICH will be collected through the trial database

5.11 Adverse event and Serious Adverse Events Reporting and Management

Definitions

An adverse event (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Adverse events occur after enrolment and are defined as not being present prior to enrolment.

Serious adverse events (SAEs) are a subset of 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 results 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. A SAE is also an event that results in a congenital anomaly or birth defect.

The pharmacology and risks of alteplase in patients with acute ischemic stroke are well known; in addition, there are multiple recent phase II and one recent phase III trial attesting to the safety of intravenous tenecteplase in patients with acute ischemic stroke. Of note, in particular is the Norwegian Tenecteplase Stroke Trial (NOR-TEST) trial. This Phase III trial randomized 1100 patients using standard thrombolysis eligibility criteria to test if intravenous tenecteplase (at a dose of 0.4 mg/kg body weight) was superior to standard dose alteplase. The dose of intravenous tenecteplase

used in the NORTEST trial was higher than that proposed in this trial (0.25 mg/kg body weight, maximum dose 25 mg) while the dose of intravenous alteplase was the same as in this trial. The table below lists SAEs noted in the NORTEST trial in patients administered intravenous alteplase or intravenous tenecteplase. There was no difference in incidence of SAEs up until day 90 or patients affected by at least one SAE up until day 7. The NORTEST trialists reported the following SAEs, namely, any intracerebral hemorrhage, symptomatic intracerebral hemorrhage, death, angioedema, recurrence of ischemic stroke, epistaxis, chest pain, gastrointestinal bleeding, other extracranial bleeding and readmission as possibly or probably related to drug administration (see Table below). There were no differences in any of these expected SAEs between patients administered intravenous tenecteplase vs. alteplase.

Based on data from the NORTEST phase III trial and other recent phase II trials in acute ischemic stroke patients and based on substantial literature attesting to the safety of intravenous tenecteplase in patients with acute myocardial infarction for over 15 years, unexpected serious adverse drug reactions or events are unlikely in the trial. (See Table below for a list of SAEs from the NORTEST trial and Appendix, Table 1 for a list of expected adverse drug reactions that have been well described associated with stroke thrombolysis). The natural history of acute stroke is such that high morbidity is expected (See Appendix Table 1). Since both thrombolytic agents have a biological half-life measured in hours, treatment-related morbidity and AEs will occur within a short interval after administration.

	Tenecteplase (n=549)	Alteplase (n=551)	p value
All SAEs to day 90	145 (26%)	141 (26%)	0·74
Patients with at least one SAE up to day 90	127 (23%)	120 (22%)	0·59
Patients with at least one SAE up to day 7	61 (11%)	56 (10%)	0·62*
Possibly or probably related to study drug	51 (9%)	53 (10%)	0·85*
Any ICH†	47 (9%)	50 (9%)	0·82*
Symptomatic ICH‡	15 (3%)	13 (2%)	0·70*
Death	15 (3%)	14 (3%)	0·84*
Angio-oedema	1 (<1%)	2 (<1%)	1·00*
Recurrence of ischaemic stroke	9 (2%)	5 (<1%)	0·30*
Recurrence of transient ischaemic attack	1 (<1%)	0	0·50*
Epistaxis	0	1 (<1%)	1·0*
Chest pain	3 (<1%)	5 (<1%)	0·73*
Gastrointestinal bleeding	1 (<1%)	0	0·50*
Other extracranial bleeding	4 (<1%)	9 (2%)	0·26*
Readmission	14 (3%)	22 (4%)	0·24*
Patients with at least one SAE, days 8–90	74 (13%)	75 (14%)	0·95
Possibly or probably drug related	6 (1%)	8 (1%)	0·79*
ICH§	6 (1%)	7 (1%)	0·79*
Death	14 (3%)	12 (2%)	0·68*
Angio-oedema	0	0	NA
Recurrence of ischaemic stroke	4 (<1%)	6 (1%)	0·75*
Recurrence of transient ischaemic attack	4 (<1%)	1 (<1%)	0·21*
Epistaxis	0	0	NA
Chest pain	4 (<1%)	7 (1%)	0·55*
Gastrointestinal bleeding	2 (<1%)	0	0·25*
Other extracranial bleeding	1 (<1%)	4 (<1%)	0·37*
Readmission	63 (11%)	68 (12%)	0·71

Data are n (%). Deaths within 3 months and intracranial haemorrhages (ICHs) within 24–48 h are reported in the analysis of secondary outcomes. ECASS=European Cooperative Acute Stroke Study. NA=not applicable. SAE=serious adverse event. *Fisher's exact test. †Any intracranial haemorrhage defined as any haemorrhagic transformations or parenchymal haematoma according to ECASS I criteria.[13] ‡Symptomatic intracranial haemorrhage defined according to ECASS III criteria.[14] §Any type of ICH leading to readmission after discharge.

***Table 3*: Serious adverse events**

Table above shows a list of SAEs in the intravenous tenecteplase vs. intravenous alteplase arms of the NORTEST trial.

Investigators are directed to the following product monographs for expected adverse drug reactions of alteplase and tenecteplase.

1. Alteplase (Activase)

   http://www.rochecanada.com/content/dam/roche_canada/en_CA/documents/Research/ClinicalTrialsForms/Products/ConsumerInformation/MonographsandPublicAdvisories/Activase/Activase_AIS_PM_E.pdf

2. Tenecteplase (TNKase™)

http://www.rochecanada.com/content/dam/roche_canada/en_CA/documents/Research/ClinicalTrialsForms/Products/ConsumerInformation/MonographsandPublicAdvisories/TNKase/TNKase_PM_E.pdf

The trial intends to obtain AE and SAE data through a combination of a) the study database b) the ongoing QuICR and OPTIMISE registries and c) through linkages to administrative data sources. The following AEs/SAEs that occur during hospital stay are captured in the QuICR* and OPTIMISE# registries.

- Urinary tract infection*
- Pneumonia*
- Deep venous thrombosis*
- External ventricular drain placement*
- Recurrent stroke*
- Hemicraniectomy*
- Sub-occipital craniectomy*
- Symptomatic intracerebral hemorrhage*#
- Intracerebral Hemorrhage*
- Groin or arterial access site hematoma#
- Endovascular procedure-related vessel perforation#
- Endovascular procedure-related vessel dissection#

*Indicates data collected in the QuICR registry
# Indicates data collected in OPTIMISE registry

Table 1 in the Appendix represents an empiric last of AEs and SAEs derived from the pooled database of multiple clinical trials. These are published, known adverse events associated with stroke because these are control arm patients only. The combined list above and the list in the Appendix will all be considered EXPECTED adverse events for the trial.

The following steps will be used to collect data on the above adverse events:

1. Given the short half-life of both thrombolytic agents (tenecteplase and alteplase) and known safety profile, adverse events occurring within the first 24 hours attributable (related) to either agent that correspond to known effects of these drugs or of hyperacute stroke (Appendix, Table 1) will be considered expected. Adverse events that occur beyond 24 hours will be considered unrelated to study drug.
2. Data on SAEs occurring within the first 24 hours including SAEs of special interest for the study, namely, a) symptomatic intracerebral hemorrhage (post-acute stroke treatment defined as per QuICR and OPTIMISE registries) b) angioedema and c) peripheral bleeding requiring blood transfusion will be collected through the trial database.
3. AEs measured routinely by the QuICR and OPTIMISE registries (highlighted as * or # above) will be collected from those registries.
4. Administrative data linkages will be used to collect data on other AEs. The Canadian Discharge Abstract Database (DAD) captures all hospital separations for all Canadian provinces and

territories, with the exception of the province of Quebec. Each hospital discharge record includes up to 25 diagnosis codes, recorded using the ICD coding system, along with other clinical and demographic information. In addition, each diagnosis field in the database has an accompanying single digit field for the diagnosis type that is recorded whenever a diagnosis is recorded. The diagnosis type codes are as follows: type M: most responsible diagnosis; type 1: preexisting conditions (comorbidities) that influence care, or the hospital stay; type 2: conditions that arose after admission and that may thus represent complications of care; and type 3: preexisting conditions (comorbidities) that do not influence care, or the hospital stay. Type 2 diagnosis type codes will be used primarily to identify any AEs. Other data elements as relevant (interventions offered during admission; Group 11 or Blood transfusion given; Group 17) will also be collected. It will therefore not be possible to classify seriousness when data are obtained through administrative data linkages. Details on the data elements collected through DAD in each province will be made available in the MOP. The nature of the specific data linkages will be different in different jurisdictions (Alberta vs. Ontario). Data will be queried and obtained at regular intervals through the duration of the trial from the above data sources.

5. Although we predict that there will be no adverse events that will be classified as serious, unexpected and related, we will maintain constant contact with investigators to manage this rare possibility. Investigators are under obligation under the *Protecting Canadians from Unsafe Drugs Act (Vanessa's Law)* (Canada), to report potential Serious Unexpected Adverse Drug Reactions (SUADR) and we will utilize our relationship with the sites to identify any event that fits this definition.

The Trial Safety Committee will review unblinded safety data throughout the trial to ensure that events are being reported and they fall within accepted norms for routine stroke care. The DSMC will periodically review unblinded overall safety data to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual case basis. Details of the DSMC review are outlined in the DSMC Charter. Management of AEs will be as per local jurisdictions.

6. Statistics

6.1 The proposed sample size and justification

The primary endpoint is the proportion of subjects that achieve a 90-day score of 0 or 1 on the mRS scale in both arms. The choice of this dichotomous outcome is based on clinical acceptance, an assessment of the distribution of mRS in the individual patient level meta-analysis and the choice of similar outcome in most ongoing trials.

A total of 1600 subjects will be randomly assigned to receive either intravenous tenecteplase or alteplase in a 1:1 ratio. Based on prior literature, we assume that the incidence of primary outcome (mRS 0-1) 90 days after randomization will be 38% and 35% respectively.[2, 17, 29, 30] Assuming a one-side non-inferiority margin of 5%, a one-sided significance Type I error of 2.5% and 90% power to show that tenecteplase is non-inferior to alteplase, we will need 759 subjects in each arm of the trial. The choice of 5% as a non-inferiority margin represents 50% of the estimate of effect size (10%) for intravenous alteplase administered within 3 hours of stroke symptom onset vs. control for the outcome mRS 0-1 measured at 90 days.[2] This data from Emberson et al is the largest patient level

pooling of data from all intravenous alteplase vs. control trials to date.[2] The choice of 5% as the non-inferiority margin in this trial means that at least half of the point estimate of effect for intravenous alteplase vs. control will be preserved. Additionally, the choice of 5% is strictly less than the lower CI bound of ~6% on the same point estimate in data from Emberson et al.[2] Hence the non-inferiority margin is guaranteed to be less than the lowest reasonable estimate of alteplase vs. control (placebo) effect size.

As always with non-inferiority designs, the 5% non-inferiority margin is the lower boundary on the 95% confidence interval surrounding the point estimate obtained from the proposed trial.[37] As a specific example, if the rate of excellent functional outcome in the alteplase group is actually 35% as postulated, the worst corresponding rate in the tNK group which will meet the non-inferiority test is 34.7%, for which the lower confidence bound on the difference is - 4.96%. In the ESCAPE trial that we led, loss to follow-up for primary outcome was 1.3%.[3] Similar rates are seen in the ongoing trials that we lead (ESCAPE NA 1 and TEMPO 2 trials; approximately 2 to 4%). We therefore estimate a loss to follow-up rate <5%, thus resulting in a sample size of 1600 subjects.

Missing data rate in the QuICR registry in 2017 was < 5% for all data fields. The OPTIMISE registry is achieving similar metrics in 2018. Data linkages with administrative data sources and central telephone assessment of primary outcome by trial personnel in co-ordination with registry personnel will also ensure that loss to follow-up is minimal.

6.2 The planned recruitment rate

Data from the QuICR registry (years 2016 - 2018) suggests that 550 to 600 patients with acute ischemic stroke receive thrombolysis every year within Alberta. The proposed trial has pragmatic eligibility criteria; any patient who is eligible for thrombolysis as per current guidelines and treating physician discretion is eligible for recruitment into the trial. Trial recruitment will use the existing registry infrastructure and processes to recruit subjects into the trial. Quick and easy randomization will be readily available to frontline physicians and allied health personnel at the emergency room. Information about the trial will be repeatedly reinforced as part of the QuiCR and OPTIMISE registries' ongoing quality improvement collaborative (see attached letters of support from both registries). Although ideally, we would like to enroll every eligible patient in the trial, after accounting for other ongoing acute stroke trials and being pragmatic about potential difficulties in trial enrolment early on, we will target at least 50% of all current thrombolysis eligible patients for recruitment in the first year at each enrolling site. This would mean approximately 300 subjects recruited in the first year into the trial from sites participating in the QuICR. OPTIMISE sites will also be participating. These sites average over 1200 thrombolysis cases per year in total, so again if conservatively, only 50% of all current thrombolysis eligible patients are recruited in the first year, this would mean 600 patients per year once all sites are running. Even if only half the sites in both registries are actively enrolling in the first year, this would still allow us to recruit at least 450 patients in the first year and 600 or more patients from year two onwards. Between the QuICR and OPTIMISE sites therefore, even accounting for variability in site initiation, contracts and REB approvals, we expect to be able to complete enrollment in 3 years.

6.3 Participant Compliance Monitoring

Because the treatment is a one-time dose of an intravenous thrombolytic agent, given immediately after randomization, no problems with compliance are anticipated in the trial.

6.4 Rate of loss to follow up and Missing data

We are estimating a loss to follow up rate of < 5%. Central assessment of primary outcome by trial personnel in co-ordination with registry personnel will ensure that loss to follow-up is minimal. In the ESCAPE trial, loss to follow-up was 1.3%. Similar rates are seen in the ongoing trials that we lead (ESCAPE NA 1 and TEMPO 2). Sensitivity analyses using various imputation techniques (worst case, best case, multiple imputation, hot-decking and nearest neighbor) will be specified prospectively in the Statistical Analysis Plan (SAP) if more than 5% of patients randomized are missing the primary endpoint.

6.5 Centers involved

The QuiCR registry currently includes 15 Primary Stroke Centers that provide thrombolysis treatment only, and 2 Comprehensive Stroke Centers that provide both thrombolysis treatment and endovascular treatment. OPTIMISE registry sites will also be participating. All QuICR and OPTIMISE sites, as well as additional sites, will be eligible to participate in this study if they a) are actively participating or agree to participate in one of the two registries or b) have regular institutional reporting/contribution of data to administrative datasets (e.g. Discharge Abstract Database) that can be accessed and linked to trial outcomes as per statistical analysis plan, and c) obtain local REB/IRB approval.

6.6 Proposed types of analyses

Primary efficacy analysis will be intention to treat. Given pragmatic eligibility criteria and up-front one-time treatment, we expect no cross-overs and very few protocol violations. A protected hierarchical analysis plan will be performed. First, non-inferiority will be established if the lower boundary of the 95% confidence interval of the % difference in subjects achieving good outcome (mRS 0-1) in the tenecteplase versus the alteplase arm is greater than – 5% (the non-inferiority margin). If non-inferiority is demonstrated, then a test of superiority will be performed as part of secondary analysis. The primary analysis for efficacy will be a stratified Cochran-Mantel-Haenszel (CMH) test where registry (QuICR vs. OPTIMISE) will be used as a stratification variable. If non-inferiority is not demonstrated, all subsequent analyses will be considered hypothesis-generating. As sensitivity analysis, logistic regression will be used to provide an adjusted estimate of effect size of the effectiveness of tenecteplase over alteplase. The odds ratio of good 90-day outcome associated with the treatment groups will be estimated using a logistic regression model after adjusting for registry, age, sex, baseline stroke severity and onset-to-needle time. For secondary analyses, frequency tables will be used to summarize binary variables by treatment group. Descriptive statistics will be used to summarize continuous data variables by treatment group. Similar to the primary efficacy variable, binary secondary outcomes will be analyzed using logistic regression analysis. The continuous secondary outcomes will be analyzed using analysis of covariance (ANCOVA) and mixed-effects regression with the registry (QuICR vs. OPTIMISE) variable treated as a random-effect. If non-inferiority of the primary outcome is demonstrated, a hierarchical multiplicity

adjustment will be used to determine the Type I error for testing the secondary outcomes in order to control the overall familywise type I error rate for the study. Sex will be a unit of analysis. In addition, given previous literature suggesting underutilization of thrombolysis in females, sex-based differences in recruitment, workflow and processes and outcomes will constitute important pre-specified subgroup analyses.[38] Details will be provided in the statistical analysis plan that will be developed and reviewed by the trial steering committee.

6.7 Proposed frequency of analyses

Schedule for interim analyses (at every 1/3rd of total patients enrolled; see Table) will be finalized in consultation with the DSMC. The overall principle of interim analyses will be to determine early if tenecteplase causes more mortality OR is significantly inferior than alteplase at interim. The safety stopping rule pertains to a substantial mortality difference favoring alteplase at interim. This rule is met if the observed p-value for mortality comparing randomized groups is below a threshold defined using a power family approach to alpha-spending using φ=1, and if the numeric rate of mortality favors alteplase (e.g., if it is found that tenecteplase is substantially and significantly inferior to alteplase in terms of mortality at interim). For inferiority, the stopping rule is defined in terms of absolute difference between the tenecteplase and alteplase rates of mRS 0-1; if at interim, the difference Δ for mRS 0-1 in the tenecteplase vs. alteplase group is lower (worse) than the indicated value, the trial will be stopped for significant inferiority of tenecteplase as it will be unlikely that the effectiveness endpoint can be met. Final analyses will be conducted on completion of the trial.

Evaluable Sample Size	Stopping for Safety	Stopping for Futility
	Two-Sided Alpha for Mortality	Effect size Δ for mRS 0-1
533 (one-third)	0.0167	-10.0%
1067 (two-thirds)	0.0218	-5.0%
1600 (final)	n/a	n/a

6.8 Pre-specified subgroup analyses

Subgroup analyses will be performed by registry (QuICR vs. OPTIMISE), type of enrolling hospital (PSCs vs. CSCs), age (continuous and as < 80 years vs. >= 80 years), sex and baseline stroke severity (NIHSS; < 8, 8-15 and > 15). Subgroup analyses will help to determine if there is overwhelming efficacy or futility in any pre-specified subgroup. Subgroup analyses will remain exploratory.

7. Trial Management

7.1 Arrangements for day-to-day management of the trial

The study will be managed by the AcT/OPTIMISE Trial Management committee and will be overseen by the QuiCR and OPTIMISE executives. The trial will also have a co-ordinator who will be responsible for the day-to-day management of the trial. The co-ordinator will work closely with the QuICR and OPTIMISE registry personnel to conduct regular trial quality improvement initiatives and to ensure blinded assessment of outcomes. The Clinical Research Unit (CRU) at the University of Calgary and another data management vendor will develop the trial database and Randomization tool

the data management vendor will host the trial database and randomization tool. Subject data will be captured and managed by study sites on a Web-based electronic data capture tool (eCRF) configured by the sponsor and hosted by the electronic data capture vendor. Interpretation of results, manuscript submission for publication, and presentation of results at national and international conferences, will be performed by the Trial Executive.

7.2 Data Confidentiality

The QuICR registry data is entered by registry/Alberta Health Service (AHS) personnel in a secure database at the Clinical Research Unit (CRU) of the University of Calgary. The QuICR registry is operating under a legal agreement (Information Management Agreement – IMA) with AHS, such that the University of Calgary is acting as a manager of identifiable health information for Alberta Health Services. The custodian of the data is the Alberta Health Services. The Alberta Health Service has completed a Privacy Impact Assessment for the QuICR Registry through the Office of the Information and Privacy Commissioner of Alberta that protects confidentiality of data.

OPTIMISE is managed by the Canadian Stroke Consortium. OPTIMISE registry data is entered by site personnel using a simple web-based electronic case report form, held in a REDCap database and stored on a secure server at the Population Health Research Institute at McMaster University. Data entry is handled at each site and quality assurance processes managed at the central data management site. Patient identifiers are not collected centrally. Each patient is assigned a unique OPTIMISE ID #; sites maintain patient logs that link patient identifiers to the unique OPTIMISE ID # in a secure platform.

The electronic data capture vendor will maintain a secure trial database that will link study subject ID with the registry ID. This database will also record SAEs occurring within 24 hours, 90-120 day outcomes and patient contact information. The registry ID in this secure database will be used to link to administrative data sources (e.g. DAD). Details will be provided in the MOP.

The randomization and electronic consent applications will be maintained by the electronic data capture vendor in a secure -managed environment. Both the application and the environment will adhere to industry-standard security practices such as data encryption, regular backups, etc.

The secure final study database will have deidentified data (by Trial ID) from a) the QuICR or the OPTIMISE registry, b) the trial database, c) the electronic consent application and d) the administrative data sources. This data will be stored on password protected and encrypted computers at the University of Calgary, in a statistical program data set.

Paper records will be kept in locked file cabinets or in a secure office. Clinical information will not be 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 information of study participants is maintained at all times. Province specific privacy regulations where applicable, must be followed. Only properly authorized persons will have access to any records. Personal medical information is always treated as confidential.

7.3 Ethics

Where approved by the local ethics review board, to reduce time to treatment, patients will be randomized using a deferral of consent procedure. The study protocol, deferred consent documents (patient and surrogate) and any subsequent modifications will be 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 will be documented in a letter to the Sponsor (and any Participating Site Investigators).

The deferred consent process is described in Section 5.4 and will include an e-consent process or a paper-based consent process. Where permitted, for subjects who cannot provide consent themselves, a legally authorized representative, or person with power of attorney, may sign the consent form. A copy of the consent form will be given to the subject, the legally authorized representative, or the person with power of attorney; and this fact will be documented in the subject's clinical 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 treated) will be informed of the new information.

7.4 Monitoring

The AcT trial is a pragmatic registry embedded trial testing if intravenous tenecteplase (an intravenous thrombolytic drug tested and found safe in multiple phase 2 and one phase 3 trial is patients with acute ischemic stroke) can replace intravenous alteplase (the current standard care) in patients who are otherwise eligible to receive the latter in routine care. The emphasis in trial execution will therefore be on making sure that the "right" patient receives the appropriate intervention (i.e., correct randomization, treatment assignment) with adequate assessment of primary outcome (i.e., complete, correct, and timely blinded event ascertainment).

To align the requirements of good clinical practice with the considerations in a pragmatic randomized clinical trial, a risk-based approach to monitoring will be used. The registries will use their existing infrastructure and mandate to focus on quality patient care. Central monitoring will be the focus with limited on-site risk-based monitoring (if required) in co-ordination with the registry coordinators.

The following steps will be in place and monitored:

1. Randomization: The randomization module is built by the CRU at the University of Calgary and the electronic data capture vendor. The module and the mechanisms to maintain allocation concealment throughout the trail will be written down, signed by the sponsor and the electronic data capture vendor will be available for assessment. The randomization module will be tested at a frequency determined by the electronic data capture vendor current quality control practices to ensure that the above requirements are met.

2. Consent: Where approved by the local ethics review board, to reduce time to treatment, patients will be randomized using a deferral of consent procedure. Sites will receive a check list and automated emails from the sponsor reminding them to obtain consent. Upon request, the

electronic data capture vendor shall make available detailed consent information, including patient signatures to authorized study personnel. Sites will document in a checklist (see section 5.3 above) if consent has been obtained, from whom and reasons if consent could not be obtained. If consent could not be obtained in spite of best efforts (no surrogate or surrogate cannot be contacted), the patient will continue in the trial. Where approved by local ethics committees and if electronic consent was not possible, the last page of the signed paper consent form for each subject will be sent for central review.

With the exception of patients who died within 7 days or who are unable to provide consent and do not have any legal representatives, sites are expected to obtain consent within 7 days of subject enrolment or before discharge, whichever is earlier. In subjects who die, and consent has yet to be obtained, sites are expected to make reasonable efforts to obtain consent from legal representatives. A letter of bereavement may be provided to sites to send to the next of kin that provides information on the patient's enrolment. If subjects or, if incapacitated, their legal representatives, withdraw from further participation (including long-term follow-up by record linkage), sites will inform the sponsor about this within 7 days of this happening. Subjects may withdraw consent for further contact but continue follow-up via record linkage. In the event that consent is withdrawn, data collected up until the point of withdrawal will be used for analysis. The site will update the patient consent log and inform the sponsor of the same.

3. Conduct of the trial: This trial is a pragmatic randomized controlled trial embedded within registries (QuICR and OPTIMISE) that are focused on improving quality of health care delivery in patients receiving acute stroke treatments such as thrombolysis and EVT. The sponsor will therefore help support the efforts of the registries in educating and implementing best clinical practices in all participating sites.

   Delegation Logs: The site qualified investigator (QI) will have primary responsibility for screening and enrolling patients within the trial. The site QI will, along with the sponsor, inform and train local qualified physicians and co-ordinators to screen, randomize and administer allocated drug. A delegation of authority log will document the personnel who have completed this training and can enrol patients into the trial. The delegation log will also document key trial-specific tasks that may be delegated to other members of the team, including site co-ordinators. Assessment of primary outcome is done centrally using phone call and is the sponsor's responsibility. The sponsor will keep a delegation log of personnel trained in this task.

   Training Logs: Through sponsor-initiated study start up meetings, site QI initiated meetings or through emails/webinars, local qualified physicians treating acute stroke patients as per routine care and site co-ordinators will be informed and trained to screen, randomize and administer allocated drug (as described in the Delegation of Authority Log section above). A training log for these training sessions will be maintained. We will require all randomizing investigators and site co-ordinators to undertake an online training procedure for the trial. This training will be required prior to activation of an individual's randomizer access. We will log their successful training centrally and will provide sites with lists of trained personnel. In addition, the sponsor will periodically provide the sites with posters, checklists and other educational material to help

with the conduct of the trial. Finally, the sponsor will train personnel centrally for assessment of 90-120 day outcome. A training log for the above sessions will be maintained centrally.

Documentation to support qualification of individuals: The Qualified Investigator at the site, and the site lead research co-ordinator CV's and documentation supporting current training in GCP and Health Canada Division 5 will be collected.

Since physicians administering intravenous thrombolysis to patients with acute stroke are qualified to do so as part of routine standard of care, the site QI will ensure that physicians enrolling patients continue to remain qualified to screen and treat acute stroke patients annually. This will be done by ensuring that all physicians with randomizer access continue to be actively licenced to practice medicine in their jurisdiction (e.g. CPSO, CPSA) by collecting annual print-outs from those regulatory bodies confirming their active license.

4. Interventions: The trial will capture if a) the correct drug b) the correct dose was administered c) and c) the vial LOT number and expiry date of the administered drug in the secure database maintained by the data management vendor. (see section 5.3).

5. Data linkages and flow: The data flow is designed to
   a) Ensure patient confidentiality. The sponsor will not have access to the patient's Health Care Number that could reidentify individual patients.
   b) Collate all data from multiple existing data sources to develop a final de-identified trial database that only has the unique Trial ID.

   To fulfil the above objectives, the data flow shown in Figure below will be followed. The Trial database is developed by the Clinical Research Unit (CRU) at the University of Calgary and maintained by another data management vendor. ..

   Each subject enrolled in the trial gets a unique Trial ID. The Trial ID is stored in the secure Trial Database. Each patient in the QuICR or OPTIMISE registries also has a registry identifier (herewith called Registry ID). The sites will enter the Registry ID in the secure Trial Database. An automated email from a secure server will go to the site reminding them to enter the Registry ID into the Trial Database. The sponsor will be able to check if the Registry ID is entered into the Trial Database but will not have "read access" to individual Registry IDs.

   The designated Alberta Health Service analyst for QuICR (privacy assessed) and the site co-ordinators for OPTIMISE trial sites have access to the Registry ID and patient Health Care Numbers. Through their "read access" of the Trial ID and the Registry ID in the trial database, they will be able to then provide the Trial ID and the linked Health Care Numbers to the Canadian Institute for Health Information (CIHI).

   CIHI and the QuICR and OPTIMISE registries will all then provide data labelled with the Trial ID only back to the sponsors. This final database with Trial ID as the only identifier will be the data available to the Investigators for analysis. Quality of the data linkages will be ascertained by qualified personnel with expertise in health service data linkages.

Version 2.0

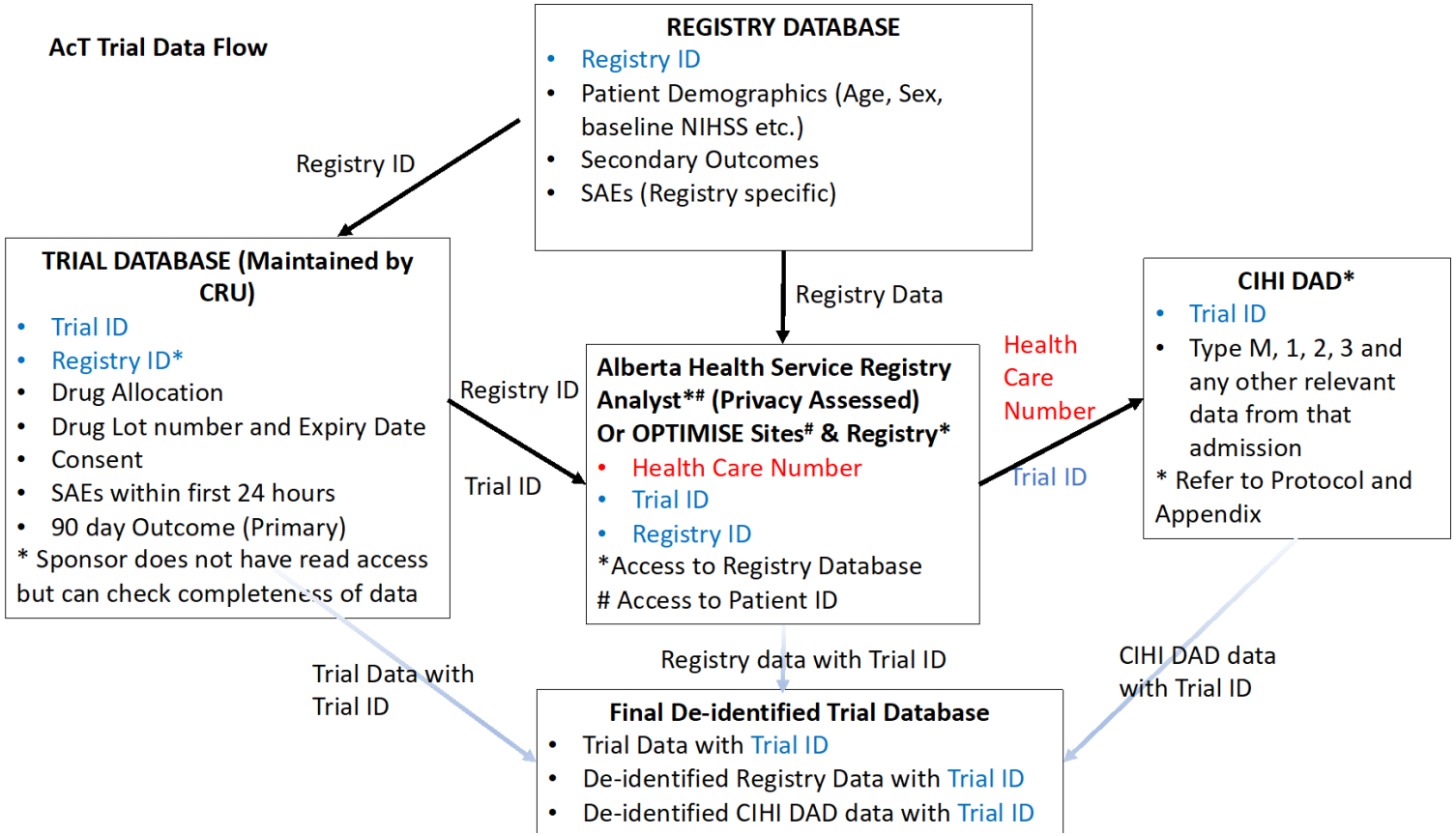

Figure showing data workflow within the AcT Trial.
SAE, Serious Adverse Events; CIHI, Canadian Institute for Health Information; DAD, Discharge Abstract Database.

Nair R, *et al. Stroke Vasc Neurol* 2024; 9:e002828. doi: 10.1136/svn-2023-002828

6. Blinded Primary Outcome Assessment: A blinded assessor trained in administering the Rankin Focused Assessment method and DQ-5D-5L will perform the 90 to 120-day outcome assessments. The definition of a blinded assessor is that they are blinded to the treatment allocation and not involved in the patient's care. This person will sign a form that attests to the blinding as described above for performing all outcome assessments. Protocol deviations will be reported for any 90-120-day assessments that were performed by an "unblinded" assessor. Every effort will be made to collect an outcome assessment on each subject and have minimal "lost to follow-up assessments". Occasionally, the local site staff may be requested to contact the subject for this information...Cognitive assessments will be completed directly by consenting participants using an online, automated assessment platform, with login via Trial ID only, so no bias is possible and identifiable data will not be captured.

7. Safety data: Details on mechanisms to be utilized in the trial to collect safety data and data on AEs is described in section 5.11. The sponsor will work the sites and the registries to ensure that all secondary and safety outcomes for the trial that are available through the registries (see Section 5.9) are entered within 90 days of subject enrolment.

8. Change in Study Personnel: When there are any changes to study personnel during the trial, it is the site's responsibility to notify the sponsor. Personnel who are no longer active at the site will be assigned an "inactive status" and their data privileges modified appropriately. The sponsor will maintain a log of any and all such changes. In co-ordination with the site co-ordinator, the sponsor will ensure access to all trial specific educational material to any new study personnel listed by the site.

7.5 Study Documentation and Record Keeping

7.5.1 Retention of Documents

The sponsor and the participating site investigators should maintain appropriate records as described in the protocol. At the site level, this will include the study protocol and any amendments, ethics correspondence and approval, patient log with a duly filled in checklist, a copy of the patient enrolment form and patient or surrogate consent forms (see section 5.3) They will also maintain their part of the trial database. At the sponsor/central level, this will include the study protocol and any amendments, ethics approval documents from sites, trial database along with the last page of the signed consent forms, copies of any communication with Health Canada and site initiation and training logs including logs of all educational efforts described in section 7.4. Any or all of these files may be stored electronically. The sponsor (and any participating site investigators) must keep these documents on file for 25 years after completion or discontinuation of the study or as deemed by data sources such as CIHI After that period of time the documents may be destroyed, subject to local regulations.

7.5.2 Source Documents

Any participating site investigators shall supply the sponsor on request with any required data from the study documentation or clinic records. 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.

7.5.3 Inspections

The sponsor understands that documents for this trial should be made available to appropriately qualified personnel or to health authority inspectors after appropriate notification.

7.6 Data Safety Monitoring Committee

The independent Data Safety and Monitoring Committee (DSMC) will be constituted in consultation with the steering committee and include clinicians, a nurse representative, a statistician and a patient representative. The DSMC will meet by teleconference to review safety data after each cohort of approximately 500 participants is enrolled. To prevent bias at the trial and investigator level, all interim results on safety will only be available to the DSMC. Stopping rules based on safety alone will be specified in the DSMC charter. Mechanisms to ensure this will be detailed in the DSMC Charter. The Data Safety and Monitoring Committee will be constituted based on the DAMOCLES charter format. The DSMC will meet by teleconference to review safety data after each 1/3rd cohort of subjects are enrolled.

7.7 End of trial

End of trial is defined as when data on the last patient recruited into the trial is completed. The trial will end when the trial steering committee agrees that one or more of the following situations applies:

1. The planned sample size has been achieved
2. The DSMC advises discontinuation because of safety concerns
3. New information makes it inappropriate to continue to randomise patients to one or other arm of the trial
4. Recruitment is so poor that completion of the trial is considered difficult

7.8 Ancillary Studies, Co-Enrollment and Publication Policy

The trial steering committee will constitute a publication committee that will include site principal investigators and the lead statistician. Policies will be detailed in a Publication Policy Charter. Ancillary studies will also be vetted by the trial steering committee and a decision made on merits of the proposed study.

Patients enrolled in the AcT RRCT are eligible to be enrolled into the EASI-TOC RCT. This study targets a subgroup of patients with ischemic stroke who have what are called tandem occlusions. EASI-TOC aims to determine whether, in addition to thrombectomy to recanalize intracranial occlusion (standard of care); patients with a tandem occlusion should also be acutely treated with a stent to revascularize their cervical internal carotid artery during the thrombectomy procedure. Inclusion in the AcT and EASI-TOC trials do not subject patients to increased risk. No multiplicative biological interaction between the interventions studied in both studies is expected. Patients are randomized just before or at the start of the thrombectomy procedure, once the carotid lesion is

confirmed by angiography. The allocation is 1:1 (stent versus no stent). Both approaches lesion is confirmed by angiography. The allocation is 1:1 (stent versus no stent). Both approaches used in EASI-TOC are considered standard care and used in daily clinical practice. Neither approach is therefore experimental. The pragmatic design of the two studies prevents patients from being subjected to additional study specific investigations, procedures or clinical follow-up beyond usual care.

It is estimated that < 5% of patients eligible for enrolment into the AcT RRCT will also be candidates for EASI-TOC (approximately 60-80 patients out of a planned 1600 patients to be included in AcT). Approximately 1/3rd of subjects eligible for AcT will likely undergo endovascular thrombectomy i.e. 533 subjects.

Statistical simulations with different possible interaction scenarios suggest limited effect on outcomes within both studies. Outcome ascertainment in EASI-TOC does not influence or affect outcome ascertainment in AcT. There are precedents in medicine for including patients in more than one randomized trial, especially in the fields of oncology and critical care and more recently with COVID-19.

On request, the trial steering committee will follow the same processes above to determine if co-enrolment will be allowed for other trials. The process will involve consultation with statiscians, ethics boards, patient focus groups and with Health Canada.

7.9 Data sharing plan

The Trial Steering Committee will follow the CIHR guidelines on public access to trial results. Upon completion of trial, a public use database will be prepared by stripping any and all personal identifiers. The data files will be 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.

8. References

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APPENDIX

Table 1: List of Adverse Events. (Stroke. 2014; 45: 2677-2682)

Adverse Event (AE)	Incidence (AEs/	Serious Adverse Event (SAE)	Incidence (AEs/

	1,000 patients)		1,000 patients)
Pyrexia	160.69	Stroke in Evolution	11.2
Headache	137.32	Pneumonia	16.8
Urinary Tract Infection	87.45	Ischemic Cerebral Infarction	21.7
Anxiety	90.04	Brain Edema	26.0
Constipation	88.48	Cardiac Failure	29.4
Hypertension	75.67	Myocardial Infarction	32.3
Pneumonia	65.63	Cerebral Hemorrhage	34.8
Nausea	68.92	Aspiration Pneumonia	37.3
Vomiting	67.88	Respiratory Failure	39.5
Stroke in Evolution	60.95	Hemorrhagic Transformation	41.7
Hypokalemia	57.32	Pulmonary Embolism	43.7
Atrial Fibrillation	52.81	Atrial Fibrillation	45.5
Depression	44.16	Urinary Tract Infection	47.2
Somnolence	32.38	Pulmonary Edema	48.7
Insomnia	39.31	Cerebrovascular Disorder	50.2
Cardiac Failure	33.94	Cardiac Arrest	51.7
Hypotension	36.36	Carotid Artery Disease	53.1
Hematuria	35.15	Sepsis	54.3
Brain Edema	30.13	Angina Pectoris	55.5
Pain	25.97	Hypotension	56.6
Hemorrhagic Transformation	28.92	Coma	57.7
Edema Peripheral	25.80	Pyrexia	58.8
Bradycardia	30.48	Renal Failure	59.8
Ecchymosis	25.97	Headache	60.7
Diarrhea	29.61	ICP increased	61.6
Ischemic Cerebral Infarction	28.57	Respiratory Tract Infection	62.5
Cerebral Hemorrhage	24.59	Bradycardia	63.3
Angina Pectoris	26.32	Transient Ischemic Attack	64.1
Aspiration Pneumonia	24.76	Gastrointestinal Hemorrhage	64.9
Cough	23.72	Hypertension	65.6
Dyspnea	24.94	Hemorrhagic Cerebral Infarction	66.3
Hyperglycemia	24.76	Somnolence	67.1
Tachycardia	24.24	Cerebral Incarceration	67.6
Musculoskeletal Pain	23.72	Deep Vein Thrombosis	68.2
Back Pain	24.07	Syncope	68.8
Confusional State	20.61	Depression	69.3
Respiratory Tract Infection	19.05	Hematuria	69.7
Rash	18.18	Vomiting	70.2
Purpura	11.77	Cerebrovascular Accident	70.6

Pulmonary Edema	17.66	Convulsion	71.0
Urinary Incontinence	13.16	COPD	71.4
Myocardial Infarction	16.80	Dyspnea	71.8
Respiratory Failure	15.24	Anemia	72.2
Anemia	14.72	Aspiration	72.5
Abdominal Tenderness	17.49	Bronchitis Acute	72.9
Rhonchi	12.64	Cardiopulmonary Failure	73.2
Lung Crepitation	12.47	Constipation	73.6
Extrasystoles	13.16	Epilepsy	73.9
Restlessness	16.10	Abdominal Tenderness	74.2
Bronchitis Acute	14.89	Dehydration	74.6
Pulmonary Congestion	11.77	Ileus	74.9
Urinary Retention	13.51	Septic Shock	75.2
Renal Failure	12.29	Colon Neoplasm	75.5
Conjunctivitis	11.43	Rectal Hemorrhage	75.8
Carotid Artery Disease	11.43	Tachycardia	76.1
Dizziness	12.81	Thrombosis	76.4
Phlebitis	12.47	Ventricular Tachycardia	76.7
Edema	11.77	Arrhythmia	76.9
Accident and/or Injury	7.45	Coronary Artery Disease	77.2
Hematoma	11.77	Multi-organ Failure	77.4
Erythema	12.47	Neurological Symptom	77.7
Sleep Disorder	10.91	Respiratory Arrest	77.9
Pulmonary Embolism	10.91	Shock	78.2
Ventricular Tachycardia	11.60	Urosepsis	78.5
Cerebrovascular Disorder	6.75	Cardiogenic Shock	78.7
Hypomagnesemia	11.26	Hip Fracture	78.9
Bronchospasm	9.52	Melena	79.1
Body Temperature Increased	11.60	Nausea	79.4
Dysphagia	8.48		
Convulsion	9.52		
Pain in Extremity	10.74		
Coma	7.62		
Sweating Increased	9.52		
Sepsis	8.83		
Breath Sounds Decreased	7.62		
Arthralgia	8.31		
Urine Output Decreased	9.35		
Leucocytosis	6.58		
Apnea	9.00		
Cardiac Arrest	8.31		
Syncope	9.18		
Hemorrhagic Cerebral	7.79		

Infarction			
Pleural Effusion	7.27		
Fatigue	9.00		
Neck Pain	8.66		
Rash Erythematous	5.71		
Hyponatremia	8.48		
Shoulder Pain	7.79		
Hypercholesterolemia	7.45		
Dehydration	6.93		
Procedural Complication	7.62		
Diabetes Mellitus	7.97		
Arrhythmia	6.93		
Edema Legs	6.23		
Fecal Incontinence	6.93		
Hypoxia	5.89		
Hiccups	6.41		
Neurological Symptom	6.23		
Lung Disorder	5.54		
Epistaxis	7.45		
Decubitus Ulcer	7.27		
Sick Sinus Syndrome	6.06		
Hyperlipidemia	6.06		
Gastrointestinal Hemorrhage	6.23		
Infusion Site Reaction	6.41		
Hypocalcemia	6.06		
Epigastric Pain	5.54		
Muscle Spasms	6.41		
Tachycardia Supraventricular	6.23		
Pruritus	5.89		
Deep Vein Thrombosis	5.54		
Candidiasis	4.68		
Atrial Flutter	5.54		
ICP Increased	6.06		
Transient Ischemic Attack	5.19		
Epilepsy	5.71		
Atelectasis	4.50		
Wheezing	5.89		
Melena	3.98		
Hyperthermia	4.85		
Hepatic Enzyme Increased	5.19		
Fall	5.71		
Protein C Increased	5.54		

Injection Site Reaction	3.64		
ALT Increased	4.50		
Speech Disorder	3.29		
COPD	4.16		
Sensory Disturbance	4.16		
Hyperkalemia	4.85		
Bacterial Infection	3.46		
Oral Candidiasis	4.68		
Excoriation	4.33		

ICP indicates intracranial pressure; COPD: chronic obstructive pulmonary disease; ALT: alanine transaminase.
Underlined SAEs are events not found on the original adverse event list. ICP indicates intracranial pressure; COPD: chronic obstructive pulmonary disease.

Table 2: Summary Characteristics of the 4 phase II trials and the one phase III trial to date comparing intravenous tenecteplase to intravenous alteplase in patients with acute ischemic stroke

	TNK-S2B	Australian TNK	ATTEST	Nor-Test	EXTEND-IA TNK
Countries	United States	Australia	Scotland	Norway	Australia and New Zealand
Number of sites	10	3	1	13	13
Patients, n	112	75	96	1100	202
TNK Dose(s)	0.1 mg/kg | 0.25 mg/kg | 0.4 mg/kg	0.1 mg/kg | 0.25 mg/kg	0.25 mg/kg	0.4 mg/kg	0.25 mg/kg
Age, mean (SD)	69.1 (16.6)	70 (8.23)	71 (12.5)	71 (13.8)	71.1 (14.4)
Sex, male	58 (51.8%)	39 (52%)	30.5 (31.8%)	660 (60%)	110 (54.5%)
Severity (NIHSS), mean (SD) or median (IQR)	TNK 0.1: 8 (5-11) TNK 0.25: 10 (6-15) TNK 0.4: 9-5-17) ALT 13 (5-17)	14.4 (2.3)	TNK: 12 (9-18) ALT: 11 (8-16)	5.7 (5.3)	TNK: 17 (12-22) ALT: 17 (12-22)
Permitted time window	≤ 3h	≤ 6h	≤ 4.5h	≤ 4.5h	≤ 4.5h
Onset to treatment, mins, median (IQR) or mean (SD)	--	176 (48) TNK 0.1 3.1+0.9 TNK 0.25 3.0+0.7 ALT 2.7+0.8	188 (44.5) TNK: 180 (156-215) ALT: 200 (160-220)	TNK: 118 (79-180) ALT: 111 (80-174)*	TNK: 125 (102-156) ALT: 134 (104-176)
Atrial fibrillation	--	28 (37.3%)	34 (35.4%)	119 (10.8%)	--
Hypertension	89 (79.5)	47 (62.7%)	48 (50%)	482 (43.8%)	--
Dyslipidemia	56 (50%)	37 (49.3%)	11 (11.5%)	126 (11.5%)	--
Diabetes	21 (18.8%)	15 (20%)	14 (14.6%)	144 (13.1%)	--
Current smoker	16 (14.2%)	15 (20%)	23 (24%)	346 (31.5%)	--
Large vessel occlusion	--	77%	47%	--	100%

Table 3: Baseline characteristics of patients enrolled in the intravenous alteplase vs. control trials (top panel) vs. patients included in the intravenous tenecteplase vs. alteplase trials (bottom panel). Patients enrolled in the tenecteplase vs. alteplase trials had significantly less severe strokes (baseline NIHSS 6 vs. 12 on average), significantly more stroke mimics (15%), under-representation of females (40% in the tNK trials overall vs. 45% in the alteplase trials) and

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lower incidence of atrial fibrillation (14% in the tenecteplase trials vs. 24% in the alteplase trials), thus suggesting that tenecteplase vs. alteplase trial level meta-analysis does not represent well patients with acute stroke treated with intravenous alteplase.

Webtable 1: Baseline characteristics of IST-3 and 8 previous trials, overall and separately according to time to treatment

	≤3 hours			>3, ≤4.5 hours			>4.5 hours			Total		
Baseline variable	**8 previous trials**	**IST-3**	**p value**	**8 previous trials**	**IST-3**	**p value**	**8 previous trials**	**IST-3**	**p value**	**8 previous trials**	**IST-3**	**p value**
Number randomised	929	620		1620	1148		1128	1266		3721	3035	
Treatment delay (hours)	2.2 (0.6)	2.4 (0.4)	<0.0001	3.9 (0.4)	3.8 (0.4)	<0.0001	5.2 (0.6)	5.4 (0.5)	<0.0001	3.9 (1.2)	4.2 (1.2)	<0.0001
Age (years)	67 (11)	83 (8)	<0.0001	65 (12)	79 (11)	<0.0001	66 (12)	73 (13)	<0.0001	66 (12)	77 (12)	<0.0001
≤80	869 (94%)	114 (18%)	<0.0001	1589 (98%)	474 (41%)	<0.0001	1106 (98%)	830 (66%)	<0.0001	3606 (97%)	1418 (47%)	<0.0001
>80	60 (6%)	506 (82%)		31 (2%)	674 (59%)		21 (2%)	436 (34%)		112 (3%)	1617 (53%)	
Stroke severity (NIHSS)	14 (7)	14 (7)	0.789	11 (6)	13 (7)	<0.0001	11 (6)	11 (7)	0.216	12 (6)	12 (7)	0.216
0-4	50 (5%)	54 (9%)	0.097	129 (8%)	139 (12%)	<0.0001	82 (7%)	207 (16%)	<0.0001	266 (7%)	400 (13%)	<0.0001
5-10	284 (31%)	187 (30%)		687 (42%)	370 (32%)		483 (43%)	507 (40%)		1469 (39%)	1064 (35%)	
11-15	211 (23%)	124 (20%)		394 (24%)	237 (21%)		281 (25%)	240 (19%)		887 (24%)	601 (20%)	
16-21	219 (24%)	156 (25%)		300 (19%)	244 (21%)		190 (17%)	217 (17%)		715 (19%)	618 (20%)	
≥22	135 (15%)	99 (16%)		72 (4%)	158 (14%)		52 (5%)	95 (8%)		270 (7%)	352 (12%)	
Female	373 (40%)	368 (59%)	<0.0001	637 (39%)	609 (53%)	<0.0001	462 (41%)	592 (47%)	0.004	1487 (40%)	1570 (52%)	<0.0001
History of hypertension	559 (60%)	430 (69%)	0.0002	912 (56%)	723 (63%)	0.0003	622 (55%)	800 (63%)	<0.0001	2114 (57%)	1954 (64%)	<0.0001
History of stroke	123 (13%)	142 (23%)	<0.0001	222 (14%)	291 (25%)	<0.0001	195 (17%)	266 (21%)	0.024	544 (15%)	699 (23%)	<0.0001
History of diabetes mellitus	186 (20%)	49 (8%)	<0.0001	275 (17%)	133 (12%)	<0.0001	217 (19%)	206 (16%)	0.061	690 (19%)	388 (13%)	<0.0001
History of atrial fibrillation	189 (20%)	237 (38%)	<0.0001	279 (17%)	360 (31%)	<0.0001	224 (20%)	316 (25%)	0.003	700 (19%)	914 (30%)	<0.0001
Aspirin use	262 (28%)	320 (52%)	<0.0001	411 (25%)	460 (40%)	<0.0001	276 (24%)	526 (42%)	<0.0001	955 (26%)	1306 (43%)	<0.0001
Weight (kg)	77.6 (16.5)	68.2 (13.5)	<0.0001	77.2 (15.1)	71.0 (14.5)	<0.0001	75.9 (16.5)	74.8 (15.3)	0.080	76.9 (15.9)	72.0 (14.9)	<0.0001
Systolic blood pressure (mmHg)	154 (22)	157 (24)	0.022	152 (20)	155 (24)	0.001	153 (21)	155 (24)	0.046	153 (21)	155 (24)	<0.0001
Diastolic blood pressure (mmHg)	85 (14)	81 (15)	<0.0001	84 (13)	82 (14)	0.0001	84 (13)	83 (15)	0.039	84 (13)	82 (15)	<0.0001

Categorical data presented as n (%), continuous data presented as mean (SD). The p-values can be used to identify the statistically, but not necessarily clinically, significant differences between patients in IST-3 and patients in the 8 earlier trials.

Table 2 Key baseline characteristics of the studies included in the meta-analysis

Baseline characteristics	Haley et al. [8]	Parsons et al. [9]	Huang et al. [12]	Logallo et al. [13]	Combined		*p* value*
					Tenecteplase	Alteplase	
Age, mean (SD)	69.9 (15.7)	69 (8.9)	71 (12.5)	71 (13.8)	70.5 (14.2)	71.1 (13)	0.423
Male, *n* (%)	46 (56.7)	25 (50)	61 (63.5)	660 (60)	393 (58.5)	399 (60.8)	0.433
Hypertension, *n* (%)	64 (79)	31 (62)	48 (50)	482 (43)	324 (48.2)	301 (45.8)	0.409
Diabetes, *n* (%)	15 (18.5)	7 (14)	14 (14.5)	146 (13.2)	91 (13.5)	91 (13.8)	0.874
Hyperlipidemia, *n* (%)	30 (37)	24 (48)	11 (11.4)	126 (11.4)	103 (15.3)	98 (14.9)	0.878
Active smoker, *n* (%)	14 (17.2)	6 (12)	23 (23.9)	346 (31.4)	194 (28.9)	195 (29.7)	0.763
Atrial fibrillation, *n* (%)	–	19 (38)	34 (35.4)	119 (10.8)	82 (12.5)	90 (14.4)	0.368
Previous stroke/TIA, *n* (%)	19 (23.4)	–	23 (23.9)	239 (21.7)	146 (21.7)	135 (20.5)	0.638
Premorbid mRS ≥ 3, *n* (%)	–	0	0	62 (5.6)	27 (4.1)	35 (5.6)	0.243
Baseline NIHSS, mean (SD)	10.7 (8.5)	14.3 (2.3)	12.5 (5.9)	5.7 (5.3)	6.82 (6.2)	6.85 (5.9)	0.928
Onset to treatment time, mean (SD)	–	171 (45.9)	193 (45)	124 (72.4)	132.5 (74.2)	129 (70.3)	0.388
Stroke mimics, *n* (%)	2 (2.4)	0	7 (7.2)	190 (17.2)	99 (14.7)	93 (14.1)	0.815

*Analysis of variance tests (ANOVA) were used to compare means for continuous variables and Chi square tests or fisher's exact test were used to compare proportions for categorical variables

SD standard deviation, *TIA* transient ischemic attack, *mRS* modified Rankin Scale, *NIHSS* National Institute of Health Stroke Scale

Figure 1: Exemplar labelling of intravenous tenecteplase and intravenous alteplase

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INVESTIGATIONAL DRUG/DROGUE DE RECHERCHE

TENECTEPLASE (TNK) 50mg Vial
**** DOSE 0.25mg/kg body weight****

Sponsor: University of Calgary,
Rm C1050-1403-29th St NW, Calgary, AB. T2N 2T9
AcT Trial Protocol V1.5

CAUTION:To be used by qualified investigators only/
Reservee uniquement a l'usage de chercheurs competent

INVESTIGATIONAL DRUG/DROGUE DE RECHERCHE

ALTEPLASE (TPA) 100mg Vial
**** DOSE 0.9 mg/kg body weight****

Sponsor: University of Calgary,
Rm C1050-1403-29th St NW, Calgary, AB. T2N 2T9
AcT Trial Protocol V1.5

CAUTION:To be used by qualified investigators only/
Reservee uniquement a l'usage de chercheurs competent

Linkage rates for the QuICR Registry to Administrative Datasets

Based on November 2018 QuICR Extract

	2015/16	2016/17	2017/18	2018/19 Q1
QuICR - DAD	97.7%	97.8%	96.7%	96.7%
QuICR – NACRS *Community stroke presentations only*	94.3%	97.3%	96.0%	94.7%

Linkage rates depend on the quality of QuICR and administrative data. If linkage rates do not meet acceptable standards, Analytics can supply project sponsors with an account of lost records for chart review. Some examples of relevant QuICR data quality issues are provided in the following table:

	2015/16	2016/17	2017/18	2018/19 Q1
Null PHN	0.96%	0.0%	1.26%	0.55%
Invalid DOB	0.19%	0.0%	1.95%	0.0%

Source: QuICR Extract as of November 2018 (VW_QUICR_EXTRACT)

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AcT trial: Statistical Analysis Plan (SAP)

Version and Date	Version 1.0, 12-April-2022
Protocol Title	Alteplase Compared to Tenecteplase in patients with Acute Ischemic Stroke: QuICR & OPTIMISE Registry linked Pragmatic Randomized Controlled Trial (The AcT RRCT)

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APPROVAL SIGNATURES

Type text here April 2022

Statistician**: Tolulope Sajobi** Date

April 2022

Principal Investigator: **Dr. Bijoy K. Menon** Date

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1. Introduction

This Statistical Analysis Plan (SAP) is for the Alteplase Compared to Tenecteplase in patients with Acute Ischemic Stroke (AcT) Trial. Intravenous thrombolysis with alteplase is widely used in patients with acute ischemic stroke presenting early after symptom onset. Recent phase II trials have suggested that intravenous tenecteplase may be safer and potentially achieves higher early reperfusion rates than alteplase. This study investigates whether intravenous tenecteplase is non-inferior to intravenous alteplase with respect to clinical outcomes.

2. Trial Objectives

The Alteplase compared to Tenecteplase (AcT) trial will therefore seek to demonstrate the non-inferiority of intravenous tenecteplase compared to intravenous alteplase in terms of 90-day functional outcome assessed using the modified Rankin Score. The secondary objectives of this study are to compare intravenous tenecteplase to alteplase in terms of safety and relevant secondary outcomes.

3. Study Design

The AcT trial is a pragmatic, registry linked, prospective, randomized (1:1) controlled, open-label parallel group clinical trial with blinded endpoint assessment of 1600 patients to test if intravenous tenecteplase (0.25 mg/kg body weight, max dose 25 mg) is non-inferior to intravenous alteplase (0.9 mg/kg body weight, max dose 90 mg) in patients with acute ischemic stroke otherwise eligible for intravenous thrombolysis as per standard care. The trial will recruit patients from the emergency departments of participating primary or comprehensive stroke centers across Canada. Study outcomes will be collected through the trial and/or through linkage to ongoing registries and national administrative databases.

4. Randomization

Randomization will be centralized, secure and concealed using a real-time web-based server, to prevent confounding due to allocation bias. Investigators can access the randomizer either through the internet, secure text or through a local telephone. The trial will have allocation concealment and blinded endpoint assessment. Given the pragmatic design of the trial and the time sensitive nature of acute stroke, blinding the enrolling health personnel to treatment allocation is not practical. A 1:1 randomization will be used to allocate patients to intravenous tenecteplase (0.25 mg/kg body weight, max dose 25 mg) or intravenous alteplase (0.9 mg/kg body weight, max dose 90 mg). Randomization will use a validated minimal sufficient balance (MSB) algorithm to assure balance by site.

5. Sample size

The primary outcome will be 90-day mRS score which will be determined by the Rankin Focused Assessment (RFA-A) method using centralized telephone interview by trained study personnel blinded to treatment allocation.

A total of 1600 subjects will be randomly assigned to receive either intravenous tenecteplase or

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alteplase in a 1:1 ratio, assuming a missingness of primary outcome data/loss to follow-up rate <5%. Based on prior literature, the incidence of primary outcome (mRS 0-1) 90 days after randomization is assumed to be 38% and 35% respectively for tenecteplase vs. alteplase. Assuming a one-sided non-inferiority margin of 5%, a one-sided significance Type I error of 2.5% and 90% power to show that tenecteplase is non-inferior to alteplase, 759 subjects are needed in each arm of the trial. The choice of 5% as a non-inferiority margin represents 50% of the estimate of effect size (10%) for intravenous alteplase administered within 3 hours of stroke symptom onset vs. control for the outcome mRS 0-1 measured at 90 days obtained from the largest patient level pooled meta-analysis of such data. The choice of 5% as the non-inferiority margin in this trial means that at least half of the point estimate of effect for intravenous alteplase vs. control will be preserved. Hence the non-inferiority margin is guaranteed to be less than the lowest reasonable estimate of alteplase vs. control (placebo) effect size.

6. Interim Monitoring

Schedule for interim analyses (at every 1/3rd of total patients enrolled) will be finalized in consultation with the Data Safety Monitoring Committee (DSMC). The overall principle of interim analyses is to determine early if tenecteplase causes more mortality or is significantly inferior to alteplase at interim. Early stopping of the trial for efficacy is generally to be avoided. The guidance on stopping for safety pertains to a substantial mortality difference favoring alteplase at interim. This may be met if the observed p-value for mortality comparing the two randomized groups is below a threshold defined using a power family approach to alpha-spending using ϕ=1, and if the numeric rate of mortality favors alteplase (e.g., if it is found that tenecteplase is substantially and significantly inferior to alteplase in terms of mortality at interim). For inferiority of tenecteplase, the stopping may be defined in terms of absolute difference between the tenecteplase and alteplase rates of mRS 0-1 at 90-120 days. As an example, if at interim, the difference Δ for mRS 0-1 at 90-120 days in the tenecteplase vs. alteplase group is lower (worse) than an indicated value (see table in DSMC Charter for some suggested thresholds), the trial may be stopped for significant inferiority of tenecteplase. Details are provided in the AcT trial DSMC charter.

7. Definition of the target populations

7.1. Intention to Treat population

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

7.2. Per-protocol population

All patients enrolled in the trial who received any dose of study drug and met all the inclusion and exclusion criteria per current Canadian Stroke Best Practices Recommendations. Since the trial has pragmatic eligibility criteria, patients who may have been inadvertently enrolled and received thrombolysis beyond 4.5 hours from stroke onset and any treatment crossovers are defined as protocol deviations for analysis.

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8. Blinding

Treatment assignment is open label. Blinding of the outcome assessment at 90-120 days will be ensured by having central personnel trained on administration of the Rankin Focused Assessment, blinded to treatment allocation, and not involved in the acute treatment period conduct the assessment via telephone.

9. Statistical Analysis

Primary analysis of the trial data to establish non-inferiority will be conducted using risk difference analysis. First, non-inferiority will be established if the lower boundary of the 95% confidence interval of the percentage difference in subjects achieving excellent outcome (mRS 0-1) in the tenecteplase versus the alteplase arm is greater than – 5% (the non-inferiority margin). If non-inferiority is demonstrated, then a test of superiority of tenecteplase vs. alteplase will be performed as part of secondary analysis. In addition, logistic regression will be used to provide an adjusted estimate of the effectiveness of tenecteplase over alteplase for the primary outcome. The risk ratio of good 90-day outcome (mRS01) associated with the treatment groups will be estimated using a mixed-effects logistic regression model that adjusts for age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.

Secondary analyses will evaluate key safety (mortality and symptomatic intracerebral hemorrhage as defined in the AcT trial MOP) and secondary outcomes using relevant tests of association. Frequency tables will be used to summarize categorical variables by treatment group. Descriptive statistics will be used to summarize continuous data variables by treatment group.

The secondary outcomes and the corresponding analyses are described as follows. Both unadjusted (not described in Table below) and adjusted (described in table below) will be reported. Unadjusted analysis will be tests of difference in proportions, means or medians or regression analysis as appropriate. All analyses will be conducted secondary analyses are conducted at α = 0.05.

Outcome	Analysis
mRS 0-1 i.e., excellent functional outcome (blinded)	Efficacy secondary analysis of the mRS 0-1. Logistic mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
mRS 0-2 i.e., good functional outcome (blinded)	Risk difference and the corresponding 95%CI to assess non-inferiority. Risk ratio and the 95%CI to evaluate efficacy

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	Adjusted logistic mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Ordinal mRS (blinded)	Ordinal logistic mixed-effects regression with treatment (Tenecteplase vs Alteplase) as exposure; for age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Return to pre-stroke status (pragmatic outcome) (blinded)	Risk difference and the corresponding 95%CI to assess non-inferiority. Risk ratio and the 95%CI to evaluate efficacy Adjusted logistic regression analysis with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Euroqol 5-D Visual Analogue Scale (EQ5D-VAS) (blinded)	A linear mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
EQ5D-5L (blinded)	Adjusted and unadjusted ordinal logistic regression analyses will be conducted for each EQ5D item (mobility, self-care, usual activities, anxiety, and depression) as the outcome variable. Health utility index derived from the EQ5D-5L items. Linear regression analysis with robust standard errors with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects,

	and site, and registry (QuiCR vs. OPTIMISE) as explanatory variables.
Home time*	A generalized linear mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects. In addition to home time, similar exploratory analysis will be conducted for other registry and administrative data outcomes such as length of hospital stay until discharge and discharge destination (home, home with home care, home with early supportive discharge, rehabilitation hospital, long term care and hospice) that will use appropriate regression analysis.
Mortality	Risk difference and the corresponding 95%CI A Kaplan-Meier survival distribution. Patients alive after 90/120 days were censored. Logistic mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Symptomatic intracerebral hemorrhage	Risk difference and the corresponding 95%CI Logistic mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Intracranial Hemorrhage on follow-up Imaging (Blinded)	Difference in proportion of ICH categories and the corresponding 95%CI

• Parenchymal hemorrhage (hemorrhagic infarction type 1, hemorrhagic infarction type 2, parenchymal hematoma type 1, parenchymal hematoma type 2, remote hemorrhagic infarction type 1, remote hemorrhagic infarction type 2, remote parenchymal hematoma type 1, remote parenchymal hematoma type 2) • Subdural hemorrhage • Subarachnoid hemorrhage • Intraventricular hemorrhage	
Peripheral Bleeding requiring Blood Transfusion	Risk difference and the corresponding 95%CI
Angioedema	Risk difference and the corresponding 95%CI
Proportion of patients receiving EVT	Risk difference and the corresponding 95%CI. Logistic mixed-effects regression model with treatment (Tenecteplase vs Alteplase) as exposure; age, sex, baseline stroke severity, and stroke onset-to-needle time as fixed effects, and site, and registry (QuiCR vs. OPTIMISE) as random effects.
Other SAEs and SUSARs	Risk difference and the corresponding 95%CI

10. Subgroup Analyses

Heterogeneity in treatment effects will be explored via subgroup analyses of pre-specified prognostic variables in the ITT population primarily and in the per-protocol population secondarily. These will include analysis of primary outcome (mRS01) and key safety outcomes by

a. age (continuous and as < 80 years vs. >= 80 years),
b. sex (male vs. female),
c. baseline stroke severity as measured by the National Institute of Health Stroke Scale (NIHSS; continuous and < 8, 8-15 vs > 15),
d. presence of large vessel occlusion on baseline CTA
e. stroke onset-to-needle time (continuous and as <=180 minutes vs. > 180 minutes)
f. registry (QuiCR vs. OPTIMISE),
g. type of enrolling hospital (PSCs vs. CSCs),

Evidence of a treatment-by-sub-group variable interaction will be tested by including a multiplicative interaction term (treatment*subgroup variable) in the model. Subgroup analyses will help to determine if there is efficacy or futility in any pre-specified subgroup. Statistical significance for each subgroup analysis will be exploratory and conducted at α = 0.05

11. Missing data

Since the trial enrolls participants using a deferred consent approach, participants with missing mRS because of refusal of consent will be excluded completely from the analysis. Under the ITT principle, all remaining patients who are randomized are included in the analysis. Thus, every effort will be made to keep all missing data to a minimum i.e., < 5%. If, despite best efforts, there are missing data, then for the primary outcome analysis, data will be assumed to be missing completely at random (MCAR). Sensitivity analyses will be conducted to examine the impact of MCAR assumption on study conclusions using available case analysis and multiple imputation methods. Similarly, assumptions and missing data methods will be adopted for analysis of secondary outcomes.

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